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Inkjet printing based assembly of thermoresponsive core–shell polymer microcapsules for controlled drug release

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A controlled drug delivery system (DDS) was designed by integrating the thermoresponsive copolymer poly(*N*-isopropylacrylamide-co-methacrylic acid) (poly(NIPAAm-co-MAA)) with core-shell 1,6-hexanediol diacrylate (HDDA) microparticles. The monodisperse HDDA particles with hollow core and nanoporous shell were fabricated in a continuous manner by a initially proposed inkjet printing process combined with UV polymerization. The thermoresponsive poly(NIPAAm-co-MAA) copolymer was grafted onto the surface of HDDA microcapsules by free radical initiated polymerization. Particularly, the lower critical solution temperature (LCST) of the copolymer was adjusted to human physiological temperature by the optimal comonomer ratio of the MAA. With temperature changes around the LCST, the copolymer, which was modified on the internal nanopore, served as a "retractable gate" by virtue of its changes in conformation between swollen and collapsed structures. Thus, controlled drug release was achieved by the reversible "open-close" transition characteristics of the nanopores. Fluorescein as a hypothetical drug molecule was loaded in the microcapsules and used to investigate the controlled release of the material. The results confirmed that this system represents a promising candidate for use in preparing controlled DDSs.

Introduction

Controlled drug delivery systems (DDSs) or so-called smart DDSs are the on-demand delivery and release of drugs controlled by internal or external stimuli. Controlled DDSs have drawn considerable attention because of their ability to ultimately overcome the adverse properties associated with conventional drug delivery, and the improvement in therapeutic efficiency and safety via the use of a predetermined dose and the sustained therapeutic level of the drug.[1-4]

Micro/nano carriers incorporated/modified with stimuli polymers are generally used to fabricate various controlled DDSs. Stimuli utilized to control the behaviour and properties of DDSs include temperature, pH, light, redox, magnetic, ultrasonic, and so forth.[5,6] Among them, temperature appears to be a favored stimulus to trigger and regulate the release of a drug because the local temperature can be easily changed according to the physical and biological environments.[7] For instance, a pathological region usually shows distinct hyperthermia because of the enhanced metabolic activity in that area. Furthermore, there are various physical means to heat the required area, thus permitting the drug to be release at the desired site. In particular, in cancer therapy, tumor tissues are selectively heated to the temperatures in the range of 39 °C to 45 °C (or higher) by thermal irradiation or an ultrasound treatment.[8-10]

Poly(*N*-isopropylacrylamide) (PNIPAAm), with a lower critical solution temperature (LCST) in water of 32 ℃, is the most commonly used thermoresponsive polymer.[11-13] As the temperature changes around the LCST, the PNIPAAm undergoes a reversible transition between the “coil-hydrophilic” state and the “globule-hydrophobic” state, which can be attributed to a competition between inter- and intramolecular hydrogen bonding.[14] The excellent biocompatibility and approximately physiological temperature of LCST makes PNIPAAm an ideal material for use in controlled drug release.[15,16]

To date, a variety of versatile drug carriers, including liposomes, dendrimers, inorganic particles, and polymeric particles have been synthesized.[17-23] While, core-shell structured polymer microcapsules have been approved as a preferred candidate due to the advantage that both hydrophobic and hydrophilic therapeutic agents can be encapsulated, and the fact that they have a high loading capacity, and low production cost.[24-27] Classical approaches for producing core-shell polymer microcapsules include solid colloidal templating[28,29] and emulsion polymerization[30]. However, these methods are time-consuming and relatively need harsh conditions are required for solid colloidal templating, and the resulting capsules sometimes show a large size distribution in the case of emulsion polymerization. As expected, these approaches limit the application of core-shell polymer microparicles.

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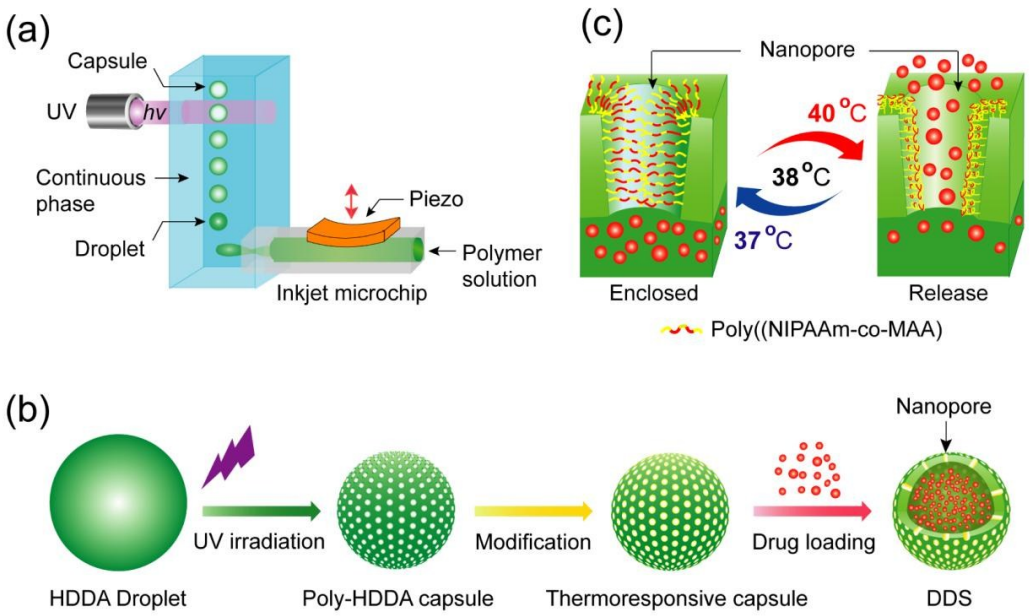

Scheme 1 (a) Schematic illustration of the experimental set-up used to produce HDDA microcapsules. HDDA solution is diluted by anisole to a concentration of 2.5 wt% containing 1.0 wt% of a photoinitiator (Photocure-1173), and then it is loaded in the inkjet microchip reservoir as dispersed phase for injection; Continuous phase is 10 mM of aqueous SDS solution and it is filled in the glass cell for polymer droplets collection and prevent them deformation. (b) Illustration of the preparation sequence for the thermoresponsive DDS. (c) Drug enclosing and releasing state with temperature changing around LCST.

Drop-on-demand (DOD) inkjet printing has recently attracted great interest in the field of particle fabrication. Using the method, droplets at the picoliter level manipulated and can be precisely dispersed with spatial and temporal control. With the mechano-electrical governed mechanism, droplet size and the velocity of a fluid can easily be controlled by changing the applied pulse waveform on the piezo actuator of the inkjet. Size and morphology controlled particles with a variety of sizes can be generated by combining the inkjet printing technique with a variety of solidification approaches, such as, calcination, freezing, and polymerization.[31-34] We previously developed a facile and robust method for the preparation of monodisperse polymer particles based on dipped inkjet printing technology.[35] However, the relatively low production efficiency (the maximum frequency was 10 Hz), and especially, only very few applicable objectives (the density of the polymer solution is required to be larger than the continuous phase) of the previous method, prompted us to exploit new approaches.

In this study, thermoresponsive core-shell polymer microcapsules were fabricated for controlled release by inkjet printing. As illustrated in Scheme 1, monodisperse 1,6-hexanediol diacrylate (HDDA) particles with hollow core-porous shell structures were prepared by bottom-up injection system combined with a UV polymerization approach. Herein, the bottom-up injection system was initially proposed. It could improve the production efficiency significantly without any pressure-assistance, and further enlarge the application scope of the inkjet printing. The thermoresponsive DDS was then fabricated by grafting the surface of HDDA microcapsules with the copolymer poly(N-isopropylacrylamide-co-methacrylic acid) (poly(NIPAAm-co-MAA)). The LCST of the poly(NIPAAm-co-MAA) was increased to human physiological temperature by the optimal comonomer ratio of MAA. With temperature changes around the LCST (38 °C), the copolymer, which was modified on the internal nanopore, served as a "retractable gate" by virtue of its changes in conformation between swollen and collapsed structures. Thus, controlled drug release was achieved by the reversible "open-close" transition characteristics of the nanopores.

Results and discussion

Preparation of hollow core-porous shell HDDA microcapsules

Cross-linked HDDA particles with hollow core-porous shell were produced by the initially proposed bottom-up injection system combined with UV polymerization. In conventional dipped inkjet printing, the nozzle of inkjet microchip was immersed at the top position of continuous phase. It requires the density of the polymer solution (dispersed phase) larger than continuous phase, so that the polymer droplet could sink to the bottom of the cell by gravity after injection. However, limited scope of low toxicity organic solvents can be used for drug delivery preparation, such as, ethanol, butanol, n-hexane, anisole, and ethyl acetate. Most of these solvents have a lower density than water, thus, it is not possible to use them as dispersed phase in conventional dipped inkjet printing. In other words, the conventional dipped inkjet printing is favourable for water-soluble polymer. On the contrary, the present bottom-up injection system requires the density of the polymer solution lower than continuous phase. Therefore, most of low toxicity organic solvents could be used as dispersed phase for injection, that would be enlarge the scope of the inkjet printing application.

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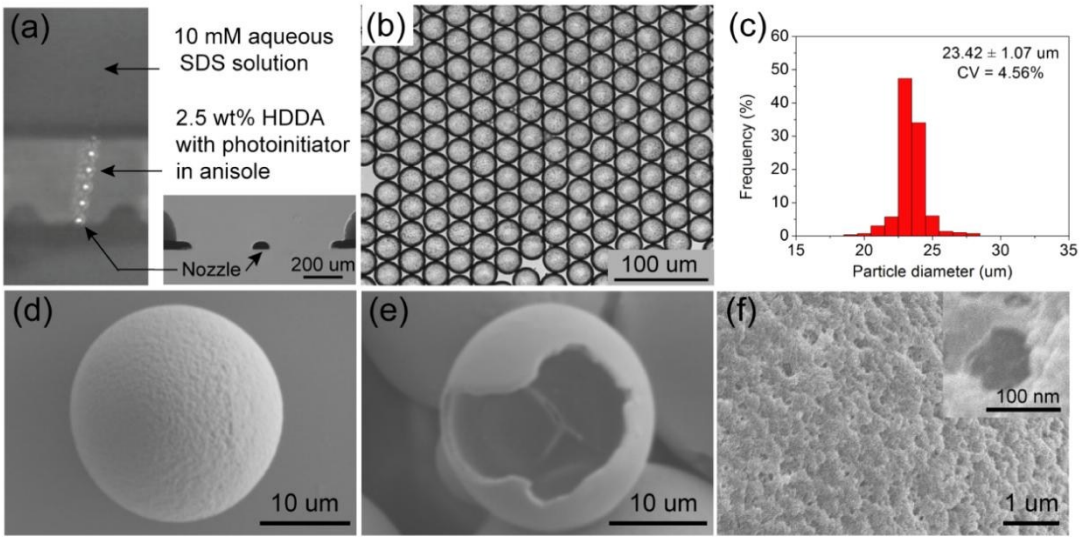

Fig. 1 (a) Photograph of typical monodisperse polymer solution droplets jetting into the continuous phase. The enlarged image is the nozzle of the inkjet microchip. (b) Optical micrograph of the HDDA particles. (c) Size distribution. SEM images of (d) morphology of single HDDA particle and (e) interior structure. (f) Typical FE-SEM image of the surface of HDDA particles.

The monodisperse polymer solution droplets were ejected from the inkjet microchip with an optimal driving waveform, see Fig. 1a. Herein, the conditions for injection were set as follows: driving voltage 30 V, pulse width 20 μs, frequency 20 Hz. Further research showed that the maximam frequency could be increased to 200 Hz. The polymerization occurs once the polymer droplet is exposed to UV irradiation. And the resulting HDDA particles sank to the bottom of the cell, making the collection of particles very convenient. Fig. 1b shows an optical micrograph of typical cross-linked HDDA particles, showing that the resulting HDDA particles have a uniform size distribution. The monodispersity of particles was evaluated by measuring the mean particle size and the diameter coefficient of variation (CV). As shown in Fig. 1c, monodisperse HDDA particles were prepared with a mean size of 23.42 μm and a CV value of 4.56%. The surface and internal morphologies of the particles were examined by SEM (Fig. S1, ESI†). Well-defined spherical particles with a hollow core structures were observed (Fig. 1d and e). The ratio of the diameter of a hollow core to the entire microsphere was about 95% and the corresponding volume ratio was about 85%, indicating that it has a high capacity for drug loading. The thin and porous shell was characterized by FE-SEM and a typical image is shown in Fig. 1f. Pores with sizes ranging from 50 nm to 150 nm were distributed on the shell.

The mechanism responsible for the formation of hollow cores likely involves the diffusion of polymer molecules, which was similar to the hollow core−shell structure of PSS particles described in our previous report.[35] Briefly, HDDA molecules in the internal droplets diffused to the interface of the droplets after it was ejected into the continuous phase. As these molecules gradually became stacked on the boundary of the polymer droplet and the continuous phase, a vacant area was formed in the centre of droplet. The polymerization was then triggered by UV irradiation. Finally, a cross-linked polymer shell with a hollow core structure was generated in the resulting particles. The nanoporous shell resulted from the extraction effect and the surfactant porogenic effect. The extraction effect arises the extraction of anisole that is present in the polymer solution by water (the solubility of anisole in water is 1.6 g/L). The nanopores were generated when the anisole was transported from the internal droplet to the continuous phase. On the other hand, the surfactant SDS that was added in the continuous phase not only prevents droplets or particles form coalescing, but also as functions as a porogenic agent.[36,37] During the formation of a hydrophobic polymer droplet in the continuous phase, the hydrophobic end of the SDS can become inserted into the polymer interface. After the polymerization is complete, the SDS and other residual reactants were removed, resulting in the formation of nanopores.

Synthesis of cross-linked poly(NIPAAm-co-MAA) copolymer

To shift the LCST of poly(NIPAAm-co-MAA) to human physiological temperature, the molar ratio of MAA in the copolymer was mainly investigated. The ingredients and reaction conditions for the synthesis of cross-linked poly(NIPAAm-co-MAA) are listed on Table S1, ESI†. The copolymer was prepared by radical-initiated precipitation polymerization in the presence of *N,N′*-methylenebisacrylamide (MBA) as a cross-linker with potassium persulfate (KPS) as the initiator. The resulting copolymer solution was dialyzed in PBS to remove residual reactants. A photograph of the resulting copolymer solutions, polymer a to polymer e, at room temperature (about 25 ℃) are displayed in Fig. S2, ESI†. The color of the polymer solutions changed from transparent to milky white with incresing concentrations of MAA.

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The clouding point (CP) measurement method was employed to measure the LCST of the poly(NIPAAm-co-MAA) solutions. The LCST values of the copolymer increased from 32 ℃ to 42 ℃ when the concentration of MAA was increased from 0 to 8 mM (Fig. 2a). An LCST of 38 ℃ was obtained when the molar ratio of NIPAAm to MAA was 100:10. The increased LCST value can be explained by the hydrophilicity of the MAA molecule which enhanced the extent of hydrogen bond formation between the copolymer with water. Therefore, the MAA incorporated copolymer requires a higher energy for dehydration so that realizes intramolecular hydrogen bonds. However, when the concentration of MAA was increased to 8 mM, in which NIPAAm was 20 mM, an irregular and minor change in transmittance was observed (Fig. S3, ESI†), indicating that the copolymer conformation change between swelling and collapsing became weak. Additionally, in order to obtain the response time of the copolymer to temperature, the real time CP measurement of copolymer (molar ratio is 100:10) in cooling from 50 ℃ to ~25 ℃ was investigated (Fig. 2b). The entire process for the copolymer transition from collapsing to swelling was 1-2 s. Meanwhile, the colour of the copolymer solution changed from a milky white to essentially transparent.

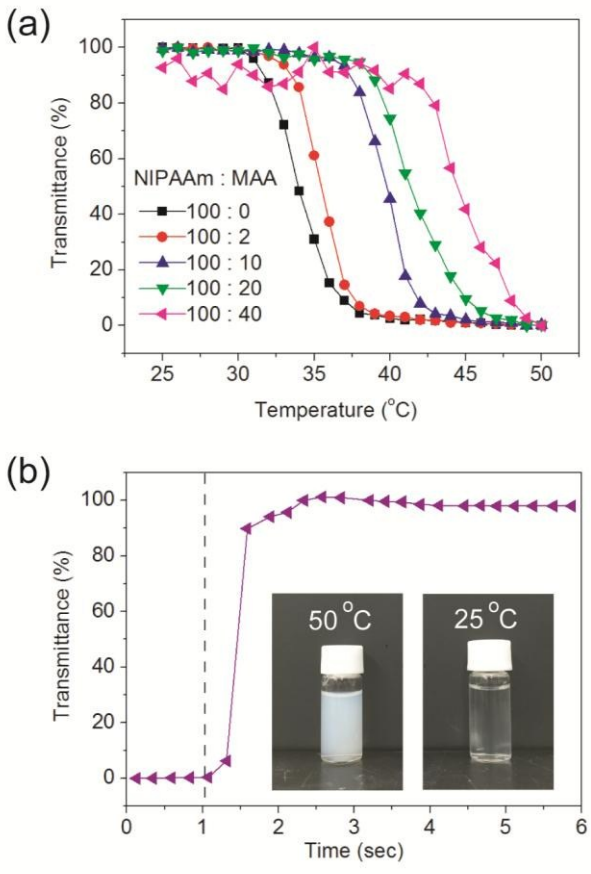

Fig. 2 (a) Typical CP measurement of various poly(NIPAAm-co-MAA) solution in PBS from 25 °C to 50 °C. (b) Real time CP measurement of cross-linked poly(NIPAAm-co-MAA) (molar ratio is 100:10) during suddenly cooling from 50 °C to ~25 °C. The cooling stage is beginning at the time of 1 sec. Insert photographs indicate the colour of copolymer solution at 50 °C and 25 °C, respectively.

Fabrication of thermoresponsive microcapsules

The thermoresponsive hollow microspheres were fabricated by grafting poly(NIPAAm-co-MAA) onto HDDA particles via the residual C=C bonds on the surface of the HDDA particles. As evidenced by data published in a previous study,[38] the concentration of unsaturated double bonds in the polymer was generally dependent on the polymerization time. Fig. 3a shows an FT-IR spectrum of the HDDA monomer and poly-HDDA particles, the double peaks at 1620 cm^{-1} are assigned to C=C bonds. Moreover, the Raman spectra analysis was further confirmed the existing of residual C=C bonds on poly-HDDA particles (Fig. S4, ESI†). The C=C bonds were mainly attributed to the shortly UV irradiation time. Thus, the poly(NIPAAm-co-MAA) was synthesized on the surface of HDDA particles via reactions with these C=C bonds.

Poly(NIPAAm-co-MAA)-HDDA particles were prepared by the free radical-initiated polymerization with NIPAAm and MAA monomers in molar ratios of 100:10. The morphology of the poly(NIPAAm-co-MAA)-HDDA particles was examined by FE-SEM. It can clearly be seen that nanopores with sizes in the range of 20 nm to 100 nm were still present on the shell of composite microspheres after the grafting of the copolymer (Fig. 3b). Meanwhile, the chemical composition of the resulting poly(NIPAAm-co-MAA)-HDDA particles was confirmed by FT-IR spectroscopy and EDX (Fig. S5 and S6, ESI†). The N-H amide bending frequency in FT-IR and the nitrogen peak in EDX indicated that the NIPAAm based copolymer has successfully synthesized on the HDDA particles.

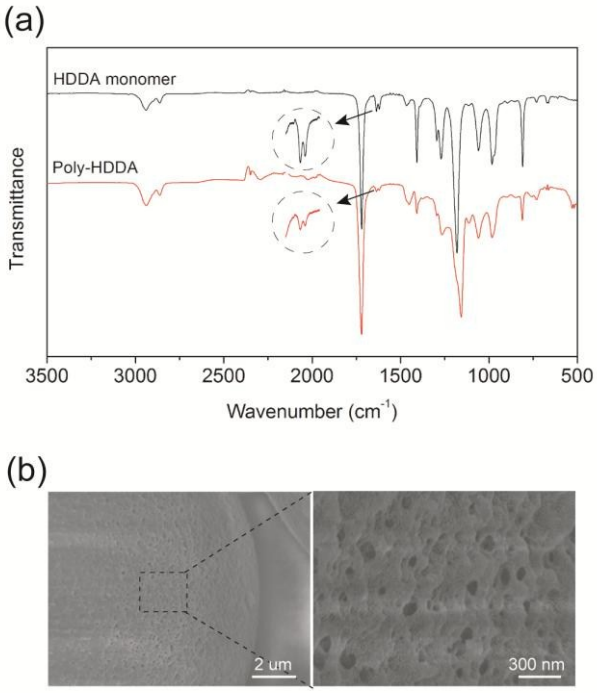

Fig. 3 (a) FT-IR spectrum of HDDA monomer and poly-HDDA particles. The double peak at 1620 cm^{-1} is assigned to unsaturated double carbon bonds. (b) FE-SEM images of the surface of a poly(NIPAAm-co-MAA)-HDDA particle.

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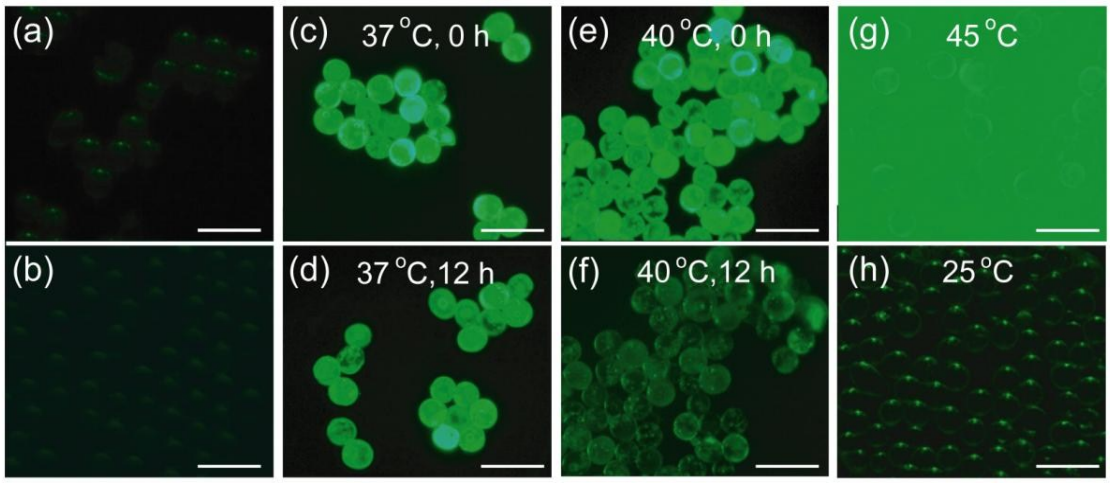

Fig. 4 Thermoresponsive microcapsules (a) and unmodified microcapsules (b) without loading Fluorescein. (c,d): Thermoresponsive microcapsules encapsulation Fluorescein at the initial point (b) and time after 12 h (c) under 37 °C. (e,f): The Fluorescein release from the thermoresponsive microcapsules under 40 °C at the time of 0 h (e) and 12 h (f). (g,h): Unmodified microcapsules without loaded Fluorescein incubated with 0.1 μg/mL Fluorescein (g) and after washing the microspheres with PBS (h). The scale bar in all of the images is 50 μm.

Drug loading and release investigation

The capability of the thermoresponsive microcapsules for use in controlled drug release was investigated using the Fluorescein disodium salt as hypothetical drug molecule. To mimic *in vivo* conditions, PBS solution was used as a solvent. By heating the mixture of the Fluorescein-microcapsules solution at 45 °C, the "gates" (pore channel) on the shell were opened as the result of the collapsed poly(NIPAAm-co-MAA). Consequently, the Fluorescein molecules were able to enter the hollow core of the microcapsules by molecular diffusion. The Fluorescein molecules then became encapsulated inside the microcapsules after the suspension was cooled to room temperature (below the LCST), in which the "gates" closed by the swollen copolymer.

Fluorescence images of microcapsules loaded with Fluorescein in PBS at 37 °C were recorded by fluorescence microscopy (Fig. 4). In comparison with naked microcapsules (Fig. 4a), a strong green fluorescence was observed in the Fluorescein-microcapsules (Fig. 4c). Meanwhile, the fluorescence intensity remained essentially unchanged after the Fluorescein-microcapsules were incubated in a PBS solution at 37 °C for 12 h, see Fig. 4d. A release study performed in PBS with the temperature maintained at 40 ℃ was carried out. A remarkable fluorescence drop was observed after a 12 h incubation (Fig. 4e and 4f). For comparison, the unmodified HDDA microcapsules (Fig. 4b) with Fluorescein loading were also examined. A strong fluorescence was observed for the mixture of HDDA microcapsules and Fluorescein (Fig. 4g). However, no obvious fluorescence on the microcapsules was detected after the Fluorescein loaded microcapsules were washed with PBS (Fig. 4h).

Fig. 5a shows release profiles for Fluorescein from the thermoresponsive hollow microcapsules at various temperatures. No significant release was detected at 28 °C and 37 °C within 24 h, where the Fluorescein molecules were fully enclosed inside the particles by the swelling of the copolymer. By contrast, a dramatic release was detected at 40 °C and 43 °C, temperatures that were above the LCST of the copolymer, thus the nanopores on the microcapsule shell were in an "open" state. In both cases, a sustained release was observed for periods of up to 10 h, and release rate at a temperature at 43 °C was slightly higher at the initial stage.

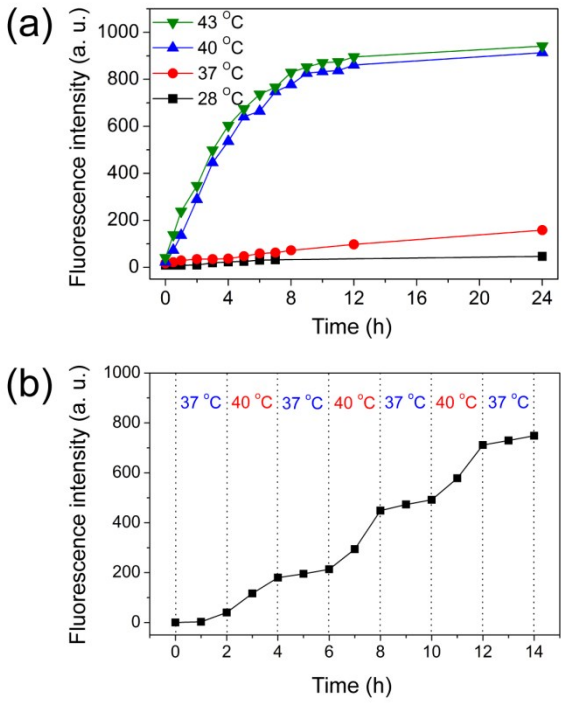

Fig. 5 Release profiles of Fluorescein from the thermoresponsive microcapsules at various temperatures (a) and with the temperature alternately at 37 °C and 40 °C (b).

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The reversibility release of Fluorescein from thermoresponsive hollow microcapsules was investigated by the temperature alternately at 37 ^{o}C and 40 ^{o}C. As shown in Fig. 5b, the Fluorescein release occurs when the temperature increased to 40 ^{o}C. Once the temperature set at 37 ^{o}C, the release rate was declined sharply. The obviously periodic variation of release was found in the results. Hence, the "open-close" transition characteristics of the "retractable gate" can be precisely controlled by the temperature around LCST changes. All these results confirm that the microcapsules can enclose and release the Fluorescein in a controlled manner. Additionally, the long time and sustained release characteristics eliminated the burst release that is commonly observed in the conventional DDSs.

Conclusions

In summary, thermoresponsive hollow polymer microcapsules with a monodisperse size distribution were prepared based on inkjet printing combined with a UV polymerization process for use in controlled drug release. The microcapsules were fabricated by grafting poly(NIPAAm-co-MAA) to the cross-linked HDDA particles. Compared with previously dipped inkjet printing technologies,[35,39,40] the efficiency of the present bottom-up injection system has significantly improved the production efficiency in this area, due to the increase in the maximum frequency of 200 Hz without any pressure-assistance. Meanwhile, the system can be general use in cases of an organic-soluble polymer, an attribute that enlarges the scope of the application. Predictably, smaller sized spheres and a higher yield could be achieved by using a finer nozzle and an arrayed inkjet setup.

The LCST of the thermoresponsive copolymer was increased to human physiological temperature by the copolymerization of an appropriate molar ratio of MAA. Based on the fact that the particles undergo reversible changes in conformation between swollen and collapsed states, the copolymer modified nanopore serves as a "retractable gate" on the microcapsules to control the release of encapsulated drug molecules. The results of Fluorescein loading and release as a function of temperature around the LCST confirm that this system is a promising candidate for use in controlled DDSs.

Experimental

Chemical and reagents

The *N*-isopropylacrylamide (NIPAAm) monomer obtained from Kohjin Co. Ltd. (Tokyo, Japan) was further purified by dissolution and recrystallization in *n*-hexane. Methacrylic acid (MAA), 1,6-hexanediol diacrylate (HDDA), 2-hydroxy-2-methylpropiophenone (Photocure-1173), *N,N'*-methylenebisacrylamide (MBA), dialysis tubing cellulose membrane (molecular weight cut-off of 12 kDa), and potassium persulfate (KPS), Fluorescein disodium salt, were purchased from Sigma Aldrich (St. Louis, Missouri, USA). Sodium dodecyl sulfate (SDS), anisole, *n*-hexane and ethanol were obtained from Wako Pure Chemical (Osaka, Japan). Deionized water was produced by a Direct-Q™5 Nihon Millipore ultrapure water system (Tokyo, Japan).

Preparation of core-shell polymer microcapsules

The equipment for core-shell polymer microcapsules formation was constructed as illustrated in Scheme 1a. It is composed of a piezoelectric DOD inkjet microchip (Fuji Electric, Tokyo, Japan), a continuous phase-filled glass cell, and a UV light source. The inkjet microchip was placed near the bottom of the glass cell, and the nozzle was immersed in the continuous phase. HDDA diluted by anisole to a concentration of 2.5 wt% containing 1.0 wt% of photoinitiator (Photocure-1173) was loaded in the inkjet microchip reservoir as polymer solutions for injection. The continuous phase was 10 mM of aqueous SDS solution. Herein, the density of the polymer solution was lower than the continuous phase. Therefore, after jetting the polymer solution into the continuous phase, the polymer droplet tended to float to the surface up be exposed to UV radiation. A 13.6-19.2 mW at 375 nm UV light source (NSPU510CS, Nichia Corporation, Anan, Japan) was used for UV-induced polymerization. Upon UV irradiation, the particles were polymerized within a few seconds. Afterwards, all particles were deposited to the bottom of the glass cell. Finally, the particles were transferred to a centrifuge tube and repeatedly washed with water and ethanol to remove the unreacted monomers from the sample surface. The resulting particles were stored in water until used.

Synthesis of poly(NIPAAm-co-MAA) grafted HDDA particles

The poly(NIPAAm-co-MAA) grafting HDDA particles were synthesized by free radical initiated polymerization at 70 ℃ using MBA as a cross-linker and KPS as the initiator. The polymerization was carried out in a 50 mL round-bottomed three-necked flask equipped with a reflux condenser. A typical run was as follows. 0.50 mmol of NIPAAm, 0.05 mmol of MAA and 0.05 mmol of MBA were dissolved in 25 mL boiled and deoxygenated water. The HDDA particle stock solution was then added and the mixture was heated to 70 ℃ and purged with nitrogen. After stirring the mixture at 200 rpm for 30 min, 1.25 mL of a 2 mg/mL KPS solution was rapidly injected. The polymerization was carried out for 6 h with stirring at 200 rpm and kept under a nitrogen atmosphere. Finally, the obtained particles were purified by repetitive cycles of centrifugation and washing with water and ethanol. The composite microsphere was denoted as poly(NIPAAm-co-MAA)-HDDA.

Regarding the copolymer synthesis for LCST measurements, the same procedures were followed except for adding HDDA particles. In order to replace the buffer solution, the resulting copolymer solutions were dialysed in dialysis tubing against a phosphate buffer solution (PBS, 50 mM, pH 7.4).

LCST of poly(NIPAAm-co-MAA) investigation

The cloud point (CP) measurement was performed on a UV-vis spectrometer at a wavelength of 520 nm. Polymer solutions were heated from 25 ℃ to 50 ℃ in 1 ℃ increments. At each step, the samples were stabilized for 10 min before detection. Values for the LCST of the polymer solutions were determined as the temperature at which the normalized transmittance was 90%. Real time CP measurements were carried out by cooling the polymer solution from 50 ℃ to ~25 ℃ within 1sec. In this process, 1 mL of polymer solution at 50 ℃ was added to the glass cell and transmittance measurements were made at the intervals of 0.25 sec. Subsequently,

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1 mL of polymer solution at 4 ℃ was added at once to the glass cell to cool the solution with the temperature below LCST. And the transmittance values were recorded until it became constant.

Particle characterization

The injection process was observed through a high speed camera (Keyence VW-9000, Osaka, Japan). Olympus system microscope BX53 equipped with a digital camera DP73 (Olympus Co., Tokyo, Japan) was used to investigate the size and size distribution of the particles. To determine the size distribution of the particles, at least 300 microspheres in each sample were measured using the software associated with the optical microscope. The monodispersity of particles was evaluated by measuring the mean particle size and the diameter coefficient of variation (CV).

The surface and internal morphologies of particles were examined by means of an SEM system (S-3400N, Hitachi Co., Tokyo, Japan). High resolution images were obtained by a field-emission scanning electron microscope (FE-SEM, JEOL JSM-7500F). Samples for SEM were prepared by placing a drop of washed and redispersion particle stock solution directly on a glass slip and drying at room temperature. All specimens for SEM measurements were sputtered with a layer of gold using a sputter coater (SC-701, Sanyu Electron Co. Ltd., Tokyo, Japan) at fixed conditions (time 150 s, current 10 mA). For the morphology of internal structures, the particles were split by squeezing between glass plates before SEM characterization.

The chemical compositions of the particles before and after copolymer modification were determined using an X-ray energy dispersive spectrometer (EDAX Genesis XM4). The FT-IR studies were carried out on a JASCO FT/IR-6100 spectrometer.

Fluorescein loading and release

Fluorescein loading was achieved by the dispersion of dried thermoresponsive microcapsules in PBS with a 0.1 μg/mL solution of Fluorescein. The solution was then heated to 45 ^{o}C and then allowed to stand for 2 h. Subsequently, the solution was cooled to room temperature and allowed to stand for 2 h. Finally, microspheres loaded with Fluorescein were collected and dialysed overnight in PBS under room temperature to remove any excess Fluorescein.

Fluorescein enclosed and release studies were performed on a labatory constructed setup as illustrated in Fig. S7. The set up was composed of a fluorescence microscope and a temperature control system. The microspheres loaded with Fluorescein were first dispersed in the PBS solution, and then transferred to a glass tube that was placed in the centre of the plastic box. The plastic box was filled with water and the temperature was maintained or adjusted by the continuous introduction of heated water. The Fluorescein release processes at 37 ^{o}C and 40 ^{o}C was recorded by the incident light fluorescence microscopy, which equipped with a external mercury lamp (Olympus BH2-RFL-T3) and a Olympus U-MNIBA2 filter set (excitation, 470–490 nm; emission, 510–550 nm; dichroic mirror, 505 nm).

The release profiles of Fluorescein from the thermoresponsive microcapsules under various and alternately temperatures were studied in PBS using a fluorescence photometer. The microcapsules (50 mg) loaded with Fluorescein were introduced into a quartz cuvette, and then incubated with 1 mL of PBS at desired temperature. The fluorescence intensity of the Fluorescein in the release medium was measured in real time by a Hitachi F-4500 fluorescence spectrophotometer (Hitachi Corporation, Tokyo, Japan). The exciting wavelength was set at 483 nm, and the emission wavelength was 512 nm.

Acknowledgements

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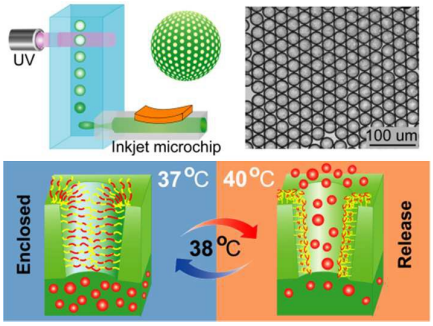

A thermoresponsive polymer microcapsule with hollow core-porous shell structure was fabricated based on inkjet printing, which can control drug release by temperature changing around 38 °C.

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