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Thermosensitive multilayer hydrogels of poly(N-vinylcaprolactam) as nanothin films and shaped capsules

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Abstract

We report on nanothin multilayer hydrogels of cross-linked poly(N-vinylcaprolactam) (PVCL) that exhibit distinctive and reversible thermoresponsive behavior. The single-component PVCL hydrogels were produced by selective cross-linking of PVCL in layer-by-layer films of PVCL-NH₂ copolymers assembled with poly(methacrylic acid) (PMAA) via hydrogen bonding. The degree of the PVCL hydrogel film shrinkage, defined as the ratio of wet thicknesses at 25°C to 50°C, was demonstrated to be 1.9 ± 0.1 and 1.3 ± 0.1 for the films made from PVCL-NH₂-7 and PVCL-NH₂-14 copolymers, respectively. No temperature-responsive behavior was observed for non-cross-linked two-component films due to the presence of PMAA. We also demonstrated that temperature-sensitive PVCL capsules of cubical and spherical shapes could be fabricated as hollow hydrogel replicas of inorganic templates. The cubical (PVCL)₇ capsules retained their cubical shape when temperature was elevated from 25°C to 50°C exhibiting $21 \pm 1\%$ decrease in the capsule size. Spherical hydrogel capsules demonstrated similar shrinkage of $23 \pm 1\%$. The temperature-triggered capsule size changes were completely reversible. Our work opens new prospects for developing biocompatible and nanothin hydrogel-based coatings and containers for temperate-regulating drug delivery, cellular uptake, sensing, and transport behavior in microfluidic devices.

Keywords

Poly(N-vinylcaprolactam); thermoresponsive films; multilayer hydrogels; cubical capsules

Introduction

Temperature-sensitive polymers, capable of demixing from aqueous solutions at a lower critical solution temperature (LCST) and remixing after cooling, have attracted significant attention due to their tremendous potential in controlled delivery, tissue engineering, and biosensing.^{1,2,3,4,5} Poly(N-isopropylacrylamide) (PNIPAM, LCST 32°C) and its copolymers have been widely used to build up a variety of temperature-responsive materials for drug delivery and chemical sensing, such as environmentally-responsive coatings and gel particles.^{6–14} However, PNIPAM is known for its cytotoxicity resulting in low cell viability, which imposes limitations on its applications.^{15,16}

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Supporting Information Available: Experimental details on synthesis of PVCL copolymers, FTIR and NMR spectra of the copolymers, as well as of hydrogen-bonded (PVCL-NH₂/PMAA) and (PVCL) hydrogel capsules. This information is available free of charge via the Internet at <http://pubs.acs.org/>.

In this regard, poly(N-vinylcaprolactam) (PVCL) has an advantage of possessing excellent biocompatibility.^{17,18} Unlike PNIPAM, hydrolysis of PVCL does not result in toxic amide compounds¹⁵ making it attractive for biomedical and pharmaceutical applications.^{16,19,20} PVCL-containing materials such as nano and microgels have been explored for cell immobilization and cancer drug delivery.^{21–31} Moreover, in contrast to PNIPAM, PVCL possesses a “classical” Flory–Huggins thermoresponsive phase diagram.^{32,33} Indeed, PVCL has a continuous coil-to-globule phase transition behavior with the LCST ranging from 32–50°C, which depends on PVCL molecular weight and concentration.^{34,35} This unique feature affords controlling temperature sensitivity of the polymer by varying its molecular weight.

Layer-by-Layer (LbL) assembly is a simple and facile method to fabricate nanothin films and capsules with well-defined composition and specifically designed properties.^{36,37,38,39} The method relies on the sequential assembly of synthetic polyelectrolytes via hydrogen bonding, electrostatic or covalent intermolecular interactions.^{40,41,42} Typically, temperature-sensitive LbL films have been made by incorporating PNIPAM and its co-polymers. Thermoresponsive films of PNIPAM-*co*-acrylic acid microgels assembled with poly(allylamine hydrochloride) were explored by Lyon and co-workers.⁴³ Electrostatically-bound multilayers of PNIPAM-based copolymers have been also reported by Schlenoff's group.⁴⁴ Hydrogen-bonded temperature-responsive multilayers of PNIPAM and poly(acrylic acid) were developed by Caruso and co-workers.⁴⁵ Zhu and Sukhishvili produced hydrogen-bonded multilayers of PNIPAM block-copolymer micelles with temperature-induced de-swelling/swelling behavior.¹¹ In thermosensitive capsules of electrostatically-bound multilayers, the capsule wall underwent transitions from hydrophilic to hydrophobic states upon temperature variations leading to capsule shrinkage or swelling.^{46,47,48}

However, one of the challenges in utilizing polyelectrolyte multilayer systems is that the LbL films can dissolve or re-arrange under environmental changes which results in an irreversible response. Although some ionically-bound multilayers exhibited a reversible response, the stability of the films is still limited to a certain pH-range.^{43,49} In this regard, introducing covalent cross-links at the LbL assembly or post-assembly steps is an efficient way to stabilize multilayer films.^{42,50,51,52,53,54} These multilayer hydrogels undergo reversible swelling/shrinking provided by the functional groups not involved in covalent cross-links. This way, thermosensitive capsules of alkyne- and azide-functionalized PNIPAM were generated using a “click-chemistry” LbL approach.⁵⁵ The effect of deposition temperature has been found to affect morphology and thickness of those capsules. In other studies, a PNIPAM copolymer and poly(acrylic acid) were alternatively assembled through covalent bonding between the components during the assembly step.^{56,57} The assembly was driven by chemical linkages between amino and carboxylic groups of a PNIPAM copolymer and poly(acrylic acid) or poly(vinylamine), respectively.

Another interesting aspect of LbL hydrogels is that the hydrogel swelling/shrinking depends not only on the cross-link density but also on polymer conformations. Indeed, the conformational effects were found to be a major factor for the difference in de-swelling ratios for LbL hydrogels prepared through the covalent assembly of poly(acrylic acid-*co*-NIPAM) with poly(vinylamine hydrochloride).⁵⁸ In that study, the copolymers with higher carboxylic acid content had a stronger ability to swell in water, attributed to the fact that they were assembled under extended conformations and could easily transform into coiled conformations after swelling.

In contrast to the vast majority of PMIPAM-containing multilayers, PVCL-based LbL materials have been less thoroughly investigated.^{59,60,61,62} Initially, PVCL was included in multilayer films to enhance interlayer hydrogen-bonded interactions to improve pH-stability

of the LbL films. In one example, Erel-Unal et al. reported on PVCL capability to stabilize interchain binding in hydrogen-bonded PVCL/poly(methacrylic acid) (PMAA) and PVCL/poly(L-aspartic acid) films.⁵⁹ The stabilization of intermolecular bonding was attributed to the relatively hydrophobic nature of PVCL due to the presence of extra methyl groups in the caprolactam ring. The effect of deposition temperature on the stability of PVCL/PMAA films towards pH variations has been investigated by Zhuk et al.⁶⁰ Recently, our group has shown that PVCL could be successfully assembled with silk fibroin, but not with the more hydrophilic poly(N-vinylpyrrolidone) (PVPON).⁶²

Despite past experiments with PVCL incorporated into LbL films, temperature responsive properties of the multilayers have been rarely explored. One of us investigated temperature-induced changes in dye permeability through hydrogen-bonded multilayer membranes of PVCL/poly(methacrylic acid) (PMAA).⁶³ However, the system permeability was not reversible due to irreversible morphological changes within the (PVCL/PMAA) membranes upon temperature response. An important point is that the presence of the second component in the LbL film can significantly weaken the temperature sensitivity expected from the functional component. Indeed, several studies illustrated that the thermoresponse of PNIPAM-containing films was suppressed or less pronounced when ionic components participated in those assemblies.^{45,64,65}

Previous studies on thermo-responsive polyelectrolyte multilayer capsules were focused solely on the spherical systems. Köhler and coworkers found that poly(allylamine hydrochloride)/poly(styrene sulfonate) (PAH/PSS) and poly(diallyldimethyl ammonium chloride)/PSS multilayer capsules exhibited response to elevated temperature despite the absence of a thermosensitive component.^{47,48} The response was irreversible and was expressed through either capsule shrinkage or swelling depending on the dominance of either hydrophobic or electrostatic forces. Glinel et al. studied PSS-block-PNIPAM and poly(dimethyldiallyl ammonium chloride)-block-PNIPAM capsules made by electrostatic LbL.⁴⁶ The 5.6- μm capsules shrunk by 15% when exposed to 60°C. A higher shrinkage of ~60% was observed for the sub-micron PNIPAM capsules when temperature was raised to 35°C.⁶⁶ Those capsules were produced by cross-linking of PNIPAM polymer brushes grown on silica particles using ATRP.

In this work, we demonstrate the first example of temperature responsive anisotropically-shaped (cubical) hydrogel microcapsules that reversibly decrease in size when the temperature is raised to 50°C while retaining their cubical shape. The nanothin capsule wall of the cubical hydrogel capsules is comprised of a single-component non-ionic PVCL multilayer hydrogel which exhibits a distinctive and highly reversible thermoresponsive behavior. Changes in the volumes of nanothin PVCL hydrogel films and hollow PVCL hydrogel capsules of spherical and cubical shapes in response to temperature variations are investigated and compared. The single-component PVCL hydrogels are formed by the selective cross-linking of PVCL in multilayer films of PVCL-NH₂ copolymers assembled with PMAA via hydrogen bonding. The temperature response is controlled by the varied hydrogel cross-link density provided with the amino groups in the PVCL-NH₂ copolymers. *In situ* ATR-FTIR spectroscopy and *in situ* ellipsometry are utilized to elucidate the composition and temperature-induced shrinking/swelling of the hydrogel films. Scanning electron microscopy and confocal laser scanning microscopy are exploited to investigate surface morphology and temperature-induced behaviors of cubical PVCL capsules. Our study introduces new fundamental aspects of thermoresponsive nanostructured hydrogels and provides a new platform for designing novel stimuli-sensitive films and capsules for controlled delivery and sensing.

Experimental

Materials

N-(tert-butoxycarbonyl-aminopropyl)methacrylamide (*t*BOC), and monodisperse silica microspheres of 4.0 μm in dry form were purchased from Polysciences, Inc. 2,2'-Azobis(2-methylpropionitrile) (AIBN) (Aldrich) was recrystallized from methanol before use. *N*-vinylcaprolactam (VCL) (Aldrich) was distilled under low pressure before copolymerization. Poly(methacrylic acid) (Sigma), (PMAA, average M_w 4000–6000 g mol^{-1} , 40 wt.%) was diluted to 0.05 wt%. Glutaraldehyde, 25% aqueous solution (GA, Alfa Aesar) was used as 5% aqueous solution in an appropriate buffer. 1,4-Dioxane, diethyl ether, hexane, methanol, ammonium hydrogen carbonate, manganese (II) sulfate monohydrate, 2-propanol were purchased from Fisher Scientific and used as received. Ultrapure de-ionized water with resistivity of 0.055 $\mu\text{S/cm}$ (18.2 $\Omega\text{ cm}$) was used in all experiments (Siemens). To control pH, buffer solutions of NaH_2PO_4 and Na_2HPO_4 (Sigma) of ACS grade were used. Phosphate buffer solutions (0.01 M) were used in pH-dependent experiments. The HCl and NaOH aqueous solutions were used to adjust pH values. Alexa Fluor® 488 hydrazide sodium salt fluorescent dye was purchased from Invitrogen.

Synthesis of poly(N-vinylcaprolactam-co-(aminopropyl)methacryl amide) copolymers, PVCL-NH₂-m

Amino-containing poly(N-vinylcaprolactam) copolymers, PVCL-NH₂-m, (m represents molar percentage of amino group-containing polymer units) were synthesized using gradual feeding copolymerization of VCL and *t*BOC as previously reported.⁶⁷ For PVCL-NH₂-7 synthesis, 2.00 g of VCL and 0.14 g of *t*BOC were dissolved in 10 mL and in 5 mL of dioxane, respectively (to yield PVCL-*co-t*BOC copolymer with 7% of *t*BOC units). Solutions were bubbled with nitrogen for 15 min to remove oxygen. After the VCL solution was heated to 73°C, 0.01 g of AIBN was added to the stirred solution. *t*BOC solution was then added dropwise for 3.5 h from a fitted funnel. The reaction was terminated by pouring the mixture into a 10-fold excess volume of hexane. The precipitated PVCL-*co-t*BOC copolymer was dissolved in tetrahydrofuran, precipitated in hexane two times, and dried in vacuum. *t*BOC groups were hydrolyzed in 1 M methanol solution of HCl for 3 days, followed by neutralizing acid with 1 M NaOH. The copolymer was dialyzed against deionized water using a Spectra/Por Float-A-Lyzer with a MWCO of 10,000 Da, and lyophilized. Composition of PVCL-*co-t*BOC copolymers prior and after hydrolysis was determined using NMR.⁶⁷ ¹H NMR spectra of the copolymers (10 mg mL⁻¹ in D₂O) were recorded on a Bruker 400 MHz NMR spectrometer (see Supporting Information). The molecular weight of PVCL-NH₂-m copolymers was determined with GPC (Waters) using linear polystyrene standards (an average M_w of PVCL-NH₂-7 was 18,500 g mol^{-1} ; an average M_w of PVCL-NH₂-14 was 23,600 g mol^{-1}).

Turbidimetric analysis

To determine the scattering intensity of the copolymers as a function of temperature, 5 mL PVCL-NH₂ copolymer aqueous solution (1 mg mL⁻¹) was mixed with GA solution (5%, 0.5 mL) and stirred overnight. During measurements using a fluorescence spectrophotometer (Varian, Cary Eclipse), the temperature was ramped from 25 to 50°C with the temperature increase rate of 0.2°C min⁻¹ and the scattering intensity of the sample was measured at a wavelength of 700 nm. The measurements were carried out in 0.01 M phosphate buffer at pH=7.5. The increase in optical density with increasing temperature resulted from the copolymers phase transition. The LCST of thermoresponsive polymers was calculated as the inflection point of the optical density curve, in which density is a function of temperature; OriginPro 8 software was used.

Hydrogel fabrication on flat templates

Prior to PVCL-NH₂/PMAA film deposition, Si wafers (University Wafers) were cleaned as described elsewhere.⁶¹ Briefly, the wafers were cut into 5 cm × 2 cm substrates and cleaned by immersion in piranha solution (H₂SO₄:H₂O₂ (1:3, v:v)) for 1 hour (Caution: Piranha solution is highly corrosive and reacts violently with organic matter!) Prior to use, substrates were thoroughly rinsed with DI water, dried under a stream of filtered nitrogen and used immediately thereafter. Hydrogen-bonded films of (PMAA/PVCL-NH₂)_n, where 'n' denotes a number of deposited polymer bilayers, were built up using spin-assisted (SA) or dipping LbL method. To enhance the surface adhesion of subsequently grown PMAA/PVCL-NH₂ multilayers to the SiO₂ surface of silicon wafers, a PEI/PSS/PEI precursor was first spin-coated to the substrates from 0.5 mg mL⁻¹ polymer solutions (0.01 M, pH=5) starting from PEI. The average thickness of the precursor film was 4.5±0.5 nm. Alternatively, a layer of poly(glycidyl methacrylate) (PGMA) was covalently bound to silica surface first to enhance the surface adhesion of the following PVCL-NH₂/PMAA multilayers. PGMA was synthesized from glycidyl methacrylate by radical polymerization with AIBN in 2-butanone as described elsewhere.⁶⁸ The PGMA solution (0.5 mg mL⁻¹) in 2-butanone was deposited on a wafer through the SA-LbL and then the wafer was heated at 110°C for 1 hour. Unattached PGMA was removed by 30 s sonication in acetone. The wafer was heated at 70°C in 1 mg mL⁻¹ PVCL-NH₂ copolymer solution in 2-propanol for 4 hours to covalently attach the copolymer to PGMA priming layer. Unattached PVCL-NH₂ was removed by rinsing in ethanol and then water. For dipped films, PMAA was then adsorbed stepwise from 0.5 mg mL⁻¹ buffer solutions at pH=3 (10 min deposition time). For SA-LbL, the deposition was carried out at 3000 rpm for 60 s per layer. After depositing a desired number of polymer bilayers, the films were chemically cross-linked with GA. For that, wafers with PVCL-NH₂/PMAA films were immersed into 5% GA buffer solution at pH 6.5 for overnight followed by their exposure to pH=8 for 5 hours to release PMAA.

In situ Ellipsometry

Film thickness measurements were performed using a M2000U spectroscopic ellipsometer (Woollam). For dry-film measurements, surface-tethered films were dried under a stream of nitrogen. Measurements were performed between 400 nm and 1000 nm at 65°, 70°, and 75° angles of incidence. For data interpretation, the ellipsometric angles, ψ and Δ , were fitted using a multilayer model composed of Si, SiO₂, and the multilayer film to obtain the thickness of films. The thickness of SiO₂ layers was determined using known optical constants. Thickness of SiO₂ was measured for each wafer. The thickness of the multilayer film was obtained by fitting data with the Cauchy approximation with the refractive index as $n(\lambda) = A_n + B_n/\lambda^2 + C_n/\lambda^4$, with $A_n=1.5$, $B_n=0.01$, and $C_n=0.0$. pH-Dependent measurements of the films were performed *in situ* using a 5 mL heated liquid flow-through cell (Woollam). The cell was filled with 0.01 M phosphate buffer solution at various pH values and measurements were taken after 30 min of equilibration. The thickness of the multilayer film at each pH and temperature was obtained by fitting data with the Cauchy approximation with permitted fitting of A_n , B_n , and C_n . The mean squared error for data fitting was less than 50. For temperature experiments, the equilibration time was set to 30 min.

Fourier Transform Infrared Spectroscopy (FTIR)

For characterization of synthesized polymers, infrared spectra were collected using a Bruker Vertex 70 FTIR spectrometer. Spectra were collected from the polymers evaporated from polymer solutions in methanol with a concentration of 0.5 mg mL⁻¹. The solutions were mixed and then transferred onto potassium bromide pellets followed by methanol evaporation. For monitoring *in situ* growth and chemical cross-linking of (PVCL-NH₂/PMAA) films, *in situ* ATR-FTIR measurements were performed. A Bruker FTIR spectrometer (Vertex 70) was equipped with a narrow-band mercury cadmium telluride

detector. The internal compartment of the FTIR spectrometer containing the liquid cell was purged with dry nitrogen. The ATR surface was rectangular trapezoidal multiple reflection Si crystal of dimension 50 mm × 10 mm × 2 mm (Harrick Scientific) whose beam entrance and exit surfaces were cut at 45°. Interferograms were collected at 4 cm⁻¹ resolution, and the number of averaged scans was 120. Each interferogram was corrected on the corresponding background, measured for the same ATR cell with the same D₂O buffer solution. The bare ATR crystal was used as a background. To eliminate overlap of the IR bands in the 1700–1500 cm⁻¹ region with the strong water band, D₂O with 99.9% isotope content was utilized. Use of multiple-reflection ATR along with a custom-made flow-through liquid cell (Harrick Scientific) allowed us *in situ* deposition and compositional monitoring of the ultrathin films (several nanometers in thickness). Briefly, a PEI/PSS/PEI film was adsorbed first from 0.5 mg mL⁻¹ (0.01 M buffer) in D₂O solutions onto the surface of the oxidized Si crystal at pH=5 with deposition time of 10 min, and after that the polymer solution was replaced by the pure buffer solution in D₂O. Then, PMAA was adsorbed in a similar way from 0.5 mg mL⁻¹ buffer solutions in D₂O at pH=3 for 10 min followed by a rinse at the same pH value and then by PVCL-NH₂ deposition. The assembly was repeated until a desired multilayer was built. The absorption peaks were baseline-corrected and analyzed with Galactic Grams/32 software using curve-fitting of the absorption peaks as described elsewhere.⁶⁹ In the fitting procedure, the wavenumbers, widths, and Gaussian band profiles were fixed, but peak intensities were varied for different spectra.

Synthesis of manganese carbonate particles

Monodisperse cubic manganese carbonate particles were synthesized as reported previously. Briefly, for cubic manganese carbonate particles with an average size of 3 μm, fresh nanoseed solution was first prepared by mixing 20 mg of ammonium bicarbonate and 1 mg of manganese (II) sulfate in 100 mL of DI water. Afterwards, 75 mL of the fresh nanoseed solution was added to 500 mL of 6 mM aqueous manganese (II) sulfate solution with 0.5% of 2-propanol. Then, 500 mL of 0.06 mM ammonium bicarbonate solution with 0.5% of 2-propanol were poured into the target solution. Immediately, the mixed solution was heated to 80°C and kept at this temperature for 40 minutes. The precipitate was collected by filtering through 0.45 μm pore size aluminum oxide filters (Whatman), followed by rinsing with DI water, and drying at ambient conditions.

Fabrication of capsules

For all deposition steps, 0.5 mg mL⁻¹ polymer solutions were used to reach saturated adsorption within 15 min deposition time. Hydrogen-bonded multilayers of (PMAA/PVCL-NH₂)_n, where 'n' denotes a number of polymer bilayers, were deposited on PEI-coated MnCO₃ particles. PEI precursor was allowed to adsorb from 0.5 mg mL⁻¹ aqueous solution at pH=6. Assembly of the hydrogen-bonded layers was then performed at pH=3.5, starting from PMAA. Each deposition cycle was followed by rinsing three times with a buffer solution at pH=3.5 to remove excess polymer, followed by centrifugation of suspensions at 5000 rpm for 2 min to remove supernatant. Deposition and rinsing steps were performed in a shaker (Fisher Scientific). After a desired number of layers were deposited, chemical cross-linking of PVCL-NH₂ layers was performed as described for the films on Si wafers. For that, the core-shell particles were exposed to GA solution at pH=6.5 overnight. After that, coated microparticles were exposed to pH=8 for 4 hours, followed by three rinsing steps at pH=4. The coated carbonate cores were transferred into a Lab-Tek chambered coverglass (Electron Microscopy Sciences) for analysis using confocal microscopy and then dissolved using 1 M HCl solution to yield (PVCL)_n hollow hydrogel capsules. In the case of the multilayer films produced on silica microparticles, silica cores were dissolved in 8% aqueous solution of hydrofluoric acid and dialyzed in aqueous solution at pH=4 using Float-A-lyzer tubes (Spectrum Labs) with MWCO of 20,000 Da for 2 days.

Confocal scanning laser electron microscopy (CLSM)

Confocal images of capsules were obtained with Zeiss LSM 710 confocal microscope equipped with a 63x oil immersion objective. Capsules were visualized with Alexa Fluor 488 hydrazide sodium salt solution used for capsule staining. The excitation wavelength was 488 nm. To observe capsule shape and size, a drop of either hollow capsule or core-shell dispersions was added to a Lab-Tek coverglass chamber. Coated particles or hollow capsules were allowed to settle in the chamber before analysis. For temperature-dependent capsule size measurements, capsules were incubated at 50°C for 30 min and then quickly transferred to a chamber with a solution preheated to 50°C and imaged.

Results and Discussion

Chemical structures of the polymers used in hydrogel film fabrication are shown in Figure 1. To enable a fabrication of thermoresponsive multilayer PVCL hydrogels with controlled properties, PVCL copolymers bearing a known amount of amino-containing cross-link centers were utilized. For that, poly(N-vinylcaprolactam-*co*-(aminopropyl)methacrylamide) (PVCL-NH₂-*k*) copolymers, with 'k' representing molar percentage of amino-group-containing polymer units, were synthesized using gradual feeding copolymerization of N-vinylcaprolactam (VCL) and N-(*tert*-butoxycarbonyl-aminopropyl)methacrylamide (tBOC) as described earlier.⁶⁷ After copolymerization, *tert*-butoxycarbonyl protective groups were hydrolyzed in methanol (to yield PVCL-NH₂-7 and PVCL-NH₂-14) and HCl (36%) under room temperature. Synthesis and characterization of the copolymers was carried out using FTIR and ¹H NMR as described in the Experimental and Supporting Information.

To produce PVCL hydrogel films, PVCL-NH₂ copolymers were first assembled with PMAA into hydrogen-bonded LbL films (Scheme 1a). The hydrogen-bonded films were then converted to non-ionic hydrogels by introducing covalent links between PVCL-NH₂ layers using glutaraldehyde (GA) followed by PMAA removal (Scheme 1b). GA is a difunctional molecule known to react with amino groups in synthetic polymers or proteins in aqueous solutions.^{70,71,72,73}

In our study we investigated the effects of assembly conditions, thickness, and the cross-link density on temperature-triggered dynamic behavior of the produced hydrogel films (Scheme 1c, d). Indeed, the known amount of amino groups involved in cross-linking allows a fine control over the cross-link density, and, consequently, the shrinking or swelling behavior of the resultant hydrogel. In the case of hydrogel capsules, the effect of the core shape on change in capsule dimensions was also examined.

Temperature responsive properties of PVCL-NH₂ copolymers in solutions

Prior to exploring temperature responsive behavior of PVCL hydrogels, PVCL-NH₂ copolymers were tested in aqueous solutions at pH=7.5 under varied temperatures. The LCST values (also denoted as cloud points) were found from turbidimetry measurements performed for PVCL-NH₂-7 (average M_w 18,500 g mol⁻¹) and PVCL-NH₂-14 (average M_w 23,600 g mol⁻¹) before and after cross-linking. The LCST of the copolymers were then compared to the LCST of a PVCL homopolymer of comparable molecular weight (average M_w 31,500 g mol⁻¹) which was found to be 38°C. It was reported previously that the LCST of PVCL is dependent on molecular weight of the polymer ranging from 33°C to 46°C for PVCL of 150,000 g mol⁻¹ and 18,000 g mol⁻¹, respectively.⁷⁴

Figure 2 shows that the optical density of PVCL-NH₂-7 drastically increased when the solution temperature reached 47°C. For copolymer cross-linking, GA was added to the aqueous copolymer solutions to allow it to react with amino groups overnight. As expected, the cloud point of PVCL-NH₂-7 significantly shifted to a lower temperature of 38°C after

cross-linking (Fig. 2a). In drastic difference to PVCL-NH₂-7, the LCST of PVCL-NH₂-14 was much higher, with its cloud point above 60°C (Fig. 2b). However, the value dramatically decreased to ~40°C after cross-linking (Fig. 2b). LCST depression due to cross-linking is consistent with the behavior of PVCL-NH₂-7 solutions (Fig. 2a, b). Transitions observed before and after copolymer cross-linking were completely reversible.

These data indicate that the cloud points of PVCL-based systems strongly depend on the amount of free amino groups. Introducing amino groups in PVCL chains results in an increase in the LCST from 38°C to 47°C for the PVCL-NH₂-7 copolymer. The LCST is further shifted above 60°C for PVCL-NH₂-14 copolymer which contains a higher ratio of amino-containing units. The results correlate well with those reported for PNIPAM copolymers where the presence of amino groups shifted the LCST to higher temperatures due to the increase in hydrophilicity of the copolymers.⁷⁵ Apparently, cross-linking amino groups with GA lowers the LCST values due to the consumption of the charged hydrophilic amino groups. Importantly, the cloud points of both copolymers after cross-linking are very close to the LCST of the PVCL homopolymer of comparable molecular weight; this indicates that all amino groups of the copolymers were consumed in the reactions.

Fabrication of multilayer (PVCL)_n hydrogel films

To produce PVCL hydrogel films, PVCL-NH₂ copolymers were assembled with PMAA via hydrogen-bonded interactions on silicon wafers at pH=3 followed by GA-assisted cross-linking. To investigate the effect of the cross-linking degree on hydrogel response, copolymers with a varied amount of cross-linkable groups, PVCL-NH₂-7 and PVCL-NH₂-14, were used.

Figure 3 shows that the 'dipped' (PVCL-NH₂-7/PMAA)_n films (obtained by alternating exposure to the polymer solutions) grew linearly with the bilayer thickness of 4.4 nm as measured with ellipsometry. When the spin-assisted method was used instead of dipping for LbL deposition, (PVCL-NH₂/PMAA)_n films were thinner, i.e., 2.5 nm per bilayer. The result is consistent with the previous studies on spin-assisted hydrogen-bonded multilayers and was explained by the enhanced interlayer interactions provided by spin-assisted procedure.^{76,77} The (PVCL-NH₂-7/PMAA) film growth results also suggest that 7% of positively charged amino groups do not hinder hydrogen bonding between carbonyl units of PVCL and carboxylic acid groups of PMAA. There could be additional electrostatic binding between PVCL-NH₂ copolymer and carboxylic groups in PMAA/PVCL-NH₂, where protonated amino groups of the copolymers could also ionically pair with locally ionized carboxylic groups. It was reported previously that the combination of hydrogen bonding and electrostatic interactions could increase pH-stability of the hydrogen-bonded tannic acid/PVPON films from pH=8.5 to pH=11.7 when poly(vinylpyrrolidone-*co*-aminoethyl(2-hydroxypropyl)amine methacrylate) was used instead of PVPON homopolymer.⁵⁹ However, in our case, the (PVCL-NH₂-7/PMAA) film was not stable at basic pH values and could be dissolved at pH > 6.5 unless cross-linked.

Figure 4 compares pH-stability of a 20-bilayer (PVCL-NH₂-7/PMAA) film before and after cross-linking. The (PVCL-NH₂-7/PMAA)₂₀ film was assembled by spin-assisted deposition on silicon wafers at pH=3 and exposed to the elevated pH values. The thickness of the film decreased drastically upon its exposure to pH>6.5. A similar result was observed when a (PVCL/PMAA)₂₀ film assembled with PVCL homopolymer was exposed to the elevated pH (Fig. 4). This film behavior is typical for the hydrogen-bonded PMAA-containing films where pH-induced ionization of PMAA (pK_a ~6–6.5) leads to disruption of hydrogen bonds.^{67,78} To cross-link PVCL within the films, the (PVCL-NH₂-7/PMAA)₂₀ film was immersed in GA solution followed by the film exposure to pH=8 buffer in order to release unbound PMAA from the film. As a result, total film thickness decreased by 41%±4%. The

resultant film remained stable in the pH range from 2 to 8 with no thickness loss (Fig.4). Thickness of a 'dipped' (PVCL-NH₂-7/PMAA)₂₀ film also decreased by 46%±3% upon cross-linking. Similar results on pH-dependent film behavior before and after cross-linking were obtained for (PVCL-NH₂-14/PMAA)₂₀ films.

To confirm the composition of the hydrogel films before and after cross-linking, *in situ* ATR-FTIR was applied. Figure 5 demonstrates ATR-FTIR spectra of a 10-layer (PVCL-NH₂-7/PMAA)₅ film grown at pH=3 before and after GA-assisted cross-linking, as well as after the hydrogel exposure to pH=8 followed by pH=3. The FTIR spectrum of the as-deposited film shows two major peaks centered at 1700 cm⁻¹ and 1633 cm⁻¹, which correspond to carbonyl stretching vibrations of protonated carboxylic groups (COOH) and carbonyl groups of the caprolactam ring, respectively^{59,67} (Fig. 5a). There is also a small peak centered at 1550 cm⁻¹ associated with asymmetric stretch vibrations of carboxylate (COO⁻), indicating a small fraction of ionized PMAA (~5%). After cross-linking, a new broad band centered at 1710 cm⁻¹ appeared in the spectrum which is associated with C=O vibrations from GA (Fig. 5b). The spectrum also shows that the absorption band associated with the stretching vibration of the carbonyl group in the caprolactam ring (1633 cm⁻¹) was present in the film after the cross-linking, while the band at 1700 cm⁻¹ associated with COOH groups disappeared, indicating the release of PMAA layers at pH=8 (Fig.5b). ATR-FTIR spectra of the cross-linked PVCL hydrogel exposed to pH=8 and pH=3 are shown in Figure 6, as spectrum 1 and 2, respectively. As seen, there is some PMAA left in the hydrogel which manifests itself as absorbance peaks from ionized COO⁻ at 1550 cm⁻¹ (pH=8, spectrum 1) and from protonated COOH at 1700 cm⁻¹ (pH=3, spectrum 2). This PMAA is, most probably, ionically paired to the PEI priming layer through local ionization of PMAA and thus is close to the substrate. The amount of the PMAA was estimated through the integration of the corresponding peak areas and compared to the integrative intensities of initially deposited layers of PMAA, as FTIR allows taking an individual spectrum of each layer. The amount of PMAA left corresponded to less than one PMAA layer. The effect of the trapped PMAA layer on the temperature-responsive properties of (PVCL) multilayer hydrogels is discussed below.

Thermoresponsive properties of multilayer (PVCL) hydrogel films—The temperature-induced phase transitions of PVCL hydrogels were investigated using *in situ* ellipsometry. For that, thickness of the PVCL multilayer hydrogels attached to the surface using PEI-based priming layer (see Experimental) was measured in a temperature-controlled liquid cell. Hydrogel responses to temperature changes in the temperature range from 25°C to 50°C were monitored.

We found that the hydrogel obtained from PVCL-NH₂-7 undergoes approximately two-fold shrinking in response to heating. The shrinkage profile of (PVCL)₂₀ hydrogel is shown in Figure 7. As seen, the wet thickness of the hydrogel film decreased drastically from 73 nm to 35 nm when the temperature was raised from 25°C to 50°C, with the shrinkage ratio of 2.1 (the ratio of the wet hydrogel thicknesses at 25°C to that at 50°C). The corresponding refractive indices of the film increased from 1.40 at 25°C to 1.45 at 50°C, which indicates the internal collapse of the PVCL hydrogel film. This is in contrast with the as-deposited non-cross-linked two-component (PVCL-NH₂-7/PMAA)₂₀ film, which was not responsive to temperature and showed no thickness changes in the studied temperature range (Fig. 7). This result is in good agreement with previous reports on hydrogen-bonded films made of temperature-sensitive polymers. Indeed, the extensive hydrogen-bonding was shown to suppress the LCST behavior of PNIPAM upon complexation with tannic acid.^{64,79} In our case, the hydrogen bonds between PVCL copolymer and PMAA displaced water from the polymeric LbL structure and inhibited the coil-to-globule transition of PVCL chains. In

drastic difference, the LCST behavior of PVCL in the single-component hydrogel is restored due to the fact that PMAA was released from PVCL hydrogel after cross-linking.

The result is similar to the temperature-induced behavior of a single-component hydrogel derived from PVCL-NH₂-14. The temperature response of the hydrogel was, however, significantly suppressed as compared to that derived from PVCL-NH₂-7. Indeed, the PVCL hydrogel produced from PVCL-NH₂-14 experienced only 1.3-fold shrinking when the temperature increased from 25°C to 50°C (inset in Figure 7).

The hydration ratios were different for the two types of hydrogels. The hydration ratio was computed as $(t_w - t_d)/t_d$, where t_w and t_d respectively correspond to the wet hydrogel thickness measured at $T=25^\circ\text{C}$ and to the dry thickness of the hydrogel. The hydrogel derived from PVCL-NH₂-14 shows as twice less the hydration than does the hydrogel produced from PVCL-NH₂-7 (Fig. 7b). The hydration ratios are found to be 1.8 and 0.8, for the hydrogels made of PVCL-NH₂-7 and PVCL-NH₂-14, respectively.

To explore the effect of the multilayer hydrogel thickness on its responsive behavior, PVCL-NH₂-7-derived hydrogels comprising of 20, 30, and 40 layers were studied. Figure 8a demonstrates a linear dependence of the hydrogel thickness on the number of PVCL layers. The result indicates that the hydrogel thickness can be precisely controlled by varying the amount of PVCL layers. Moreover, the hydrogels of various thicknesses showed similar hydration ratios (Fig. 8b). The temperature-induced hydrogel shrinkage (calculated from equilibrated thicknesses) was also independent of number of layers and was found to be 1.9 ± 0.1 (Fig. 8c). Similar hydration and shrinkage ratios of the hydrogel indicate the consistent cross-link density, despite the various hydrogel thicknesses (Fig. 8b,c).

Importantly, the temperature shrinking/swelling cycles of PVCL hydrogels were highly reversible. The data on the (PVCL)₄₀ hydrogel displays that the wet thickness temperature transitions are reversible with the ratio amplitude of 1.9 (Fig. 8d). Slightly lower shrinkage ratios of ~ 1.7 were reported by Sukhishvili and co-authors for two-component thermosensitive films of PNIPAM-based block-copolymers assembled within LbL films via electrostatic or hydrogen bonding interactions.^{11,49} In contrast to those studies, the covalently cross-linked PVCL hydrogels allow for films with improved pH stability. Moreover, the single-component PVCL films afford a larger free volume and more pronounced temperature response in a wide pH range as opposed to double-component films. As was mentioned above, the second component in the film may suppress film response by restricting chain conformational changes due to various non-specific interactions, which results in less pronounced properties.^{56,64} Moreover, the ionic component can provide excess charge which can result in more hydrophilic local environment which increases the local LCST. On the other hand, the full charge compensation between two oppositely charged film components may produce the hydrophobic environment within the assembly which may lead to a lowered LCST. In fact, an acrylic acid was found to hinder thermosensitivity of PNIPAM-*co*-acrylic acid copolymer microgel thin films upon ionization of acrylic acid.⁴³

To elucidate the effect of the small amount of PMAA trapped in surface-attached PVCL hydrogel multilayers primed with PEI-based film on the temperature-dependent shrinkage of the hydrogel, poly(glycidylmethacrylate) (PGMA) priming layer was used to prime silicon wafers instead of a PEI/PSS/PEI precursor film.⁶⁸ After PGMA anchoring to the surface, a PVCL-NH₂-7 layer was covalently anchored to the priming PGMA layer (see Experimental). The thickness of the resultant (PVCL)₂₀ decreased from 90 nm to 48 nm upon the temperature increase from 25°C to 50°C (Fig 8a). The (PVCL)₂₀ hydrogel experienced a 1.86-fold shrinkage which is similar to that of 1.89 for the hydrogel built up

on PEI/PSS/PEI priming layer (Fig. 7). Therefore, PMAA ionically paired to PEI had a negligible effect on the surface-attached PVCL hydrogel temperature response. Importantly, the (PVCL)₂₀ multilayer hydrogels were stable towards hydrolysis at pH=3 or pH=7.5 and did not show any thickness change after their exposure to both 25°C and 50°C under those conditions for at least two days (Fig. 9). As known, aldehydes are expected to form Schiff bases upon nucleophilic attack by amino groups of aminopropyl residues in the copolymer. However, Schiff bases are unstable under acidic conditions and tend to break down to the aldehyde and amine. In our work, the linkages formed by the reaction of GA with amino groups at pH=6.5 has shown exceptional stability at low and neutral pH values and high temperatures (Fig. 9). Also, no absorbance peaks corresponding to C=C or C=N bonds can be observed for cross-linked (PVCL) hydrogels at 1540 cm⁻¹ and 1648 cm⁻¹, respectively, and a new absorbance band appeared at 1100 cm⁻¹ which was assigned to -C-O-C- bond (Fig. S5). Following this, a simple Schiff base with both ends of monomeric GA has been ruled out as a mechanism for GA cross-linking of the copolymers. The cross-linking reaction most probably occurred through either the monomeric cyclic hemiacetal form of GA or its multimeric form known to react under acidic or neutral conditions (Fig. S6) as previously reported for hydrolytically stable enzymes and proteins immobilized by GA under acidic or neutral conditions.⁸⁰

To investigate how the temperature-induced changes in hydrogel thicknesses correlate with the amount of cross-linkable groups in the copolymer used for the hydrogel construction, the cross-link densities were calculated using two approaches. In the first approach, the theoretical number of possible cross-links per chain (the cross-link density) was estimated based on the number of amino-bearing units in the copolymer and its molecular weight. Thus, given 7% of the amino-bearing units in PVCL-NH₂-7 copolymer and M_w of 18,500 g mol⁻¹, the calculations suggested that there were 15 PVCL monomer units between two cross-links (assuming saturated conversion of all amino groups), or, on average, 9 cross-links per polymer chain.⁸¹ For the hydrogel with 14% of amino-bearing units and the copolymer M_w of 23,600 g mol⁻¹, we calculated that there were 7 PVCL monomer units between two cross-links, or 23 cross-links per polymer chain. Thus, the increase in the amount of cross-linkable groups from 7% to 14% resulted in the decreased number of PVCL units between two cross-links from 15 to 7. The corresponding cross-link density increased from 9 to 23 for the hydrogels derived from PVCL-NH₂-7 and PVCL-NH₂-14, respectively.

In the second approach, the cross-link density was found from experimental liquid ellipsometry data using the Flory equation for swelling of non-ionic gels.^{82,83} To obtain the density of the cross-links in hydrogels, the equilibrated wet hydrogel thickness values at T=25°C were considered. According to the Flory equation, the equilibrium expansion of a polymer gel is

$$0 = \ln(1 - \phi_2) + \phi_2 + \chi \phi_2^2 + \left(\frac{v_1}{\bar{v}}\right) \left(\frac{1}{M_c} - \frac{2}{M}\right) \left(\phi_2^{1/3} - \frac{\phi_2}{2}\right) \quad (\text{Eq. 1})$$

In which ϕ_2 is the volume fraction of the polymer in the swollen gel, v_1 is the molar volume of the solvent, $\bar{v}$ is the specific volume of the polymer, χ is the Flory-Huggins interaction parameter characterizing polymer-solvent interactions, M_c is the average molecular weight between cross-links, and M is the molecular weight of the polymer before cross-linking.⁸⁴ It has been shown⁸² that for surface-attached hydrogel films constrained to swell in only one direction, Eq. 1 can be re-written as follows

$$0 = \ln(1 - \phi_2) + \phi_2 + \chi \phi_2^2 + \left(\frac{v_1}{v} \right) \left(\frac{1}{M_c} - \frac{2}{M} \right) \left(\phi_2^{1-2/d} - \frac{\phi_2}{2} \right) \quad (\text{Eq. 2})$$

In which $d=1$ for the surface-anchored hydrogel swelling in one direction and ϕ_2 set as the ratio of dry to swollen thicknesses. In the case of (PVCL)₂₀, derived from PVCL-NH₂-7, the ratio $\phi_2=0.36$ considering that dry and equilibrated swollen films were 32.4 nm and 90 nm thick, respectively (Fig. 8). We used 0.953 cm³ g⁻¹ for the specific volume of the polymer, 18.1 cm³ mol⁻¹, for the molar volume of the solvent, 0.522 for the Flory-Huggins interaction parameter of PVCL in water,²³ and 18,500 g mol⁻¹ for the molecular weight before cross-linking. The calculations from swelling of the PVCL hydrogels of the studied thicknesses showed that there were, on average, 9±1 cross-links per polymer chain. The result showed excellent agreement with the theoretical estimation of the cross-link density calculated from the ratio of the amino-bearing units in the copolymer. Thus, taking into account that the maximum possible cross-link density resulted in 9 cross-links per polymer chain, the cross-linking reaction was complete and involved all available amino groups in the copolymer.

The hydration ratio correlated well with the number of PVCL units between two cross-links. As calculated above, the number of PVCL units between two cross-links was found to be 15 and 7 for the hydrogels derived from PVCL-NH₂-7 and PVCL-NH₂-14, respectively. The two-fold decrease in the number of PVCL units between the cross-links resulted in two-fold decrease in the hydration ratio from 1.8 to 0.8. Moreover, the temperature-induced hydrogel shrinkage ratio was significantly affected by the hydrogel cross-link density. The shrinkage ratio of ~2 was observed for the PVCL-NH₂-7 hydrogel with the cross-link density of 9 cross-links per chain. However, the increase in the cross-link density to 23 cross-links per chain in PVCL-NH₂-14 hydrogels resulted in the significantly suppressed shrinkage ratio of 1.3. The data presented here are in good agreement with the previously reported dynamic behavior of micro-sized and bulk gels controlled by the cross-link density.^{85,23,53} For nanothin multilayer hydrogels, the degree of chain expansion and the corresponding change in the hydrogel free volume was also found to be dependent on the cross-link density. For instance, pH-induced swelling of PMAA-containing hydrogel films and capsules was suppressed at higher cross-linking degrees.^{67,82}

Multilayer (PVCL) hydrogel capsules—To explore the capabilities of the hydrogel to form self-supporting 3D membranes in solutions, hollow capsules comprised of the ultrathin multilayer PVCL hydrogel walls were fabricated. To investigate the effect of capsule geometry on their thermoresponsive properties, cubical and spherical capsules were produced. For that, monodisperse cubical particles of manganese carbonate and spherical silica were used as sacrificial templates using previously developed procedures.⁸⁶

To obtain PVCL hydrogel capsules, the (PVCL-NH₂-7/PMAA)₇ multilayer was constructed via hydrogen-bonded LbL at pH=3.5 (0.01 M) on the sacrificial cores. To convert the coating into a hydrogel, (PVCL-NH₂-7/PMAA)₇-coated particles were exposed to GA solution at pH=6.5 as was described for the PVCL hydrogel films (see Experimental).

FTIR spectroscopy of the freeze-dried (PVCL-NH₂-7) copolymer and non-cross-linked (PVCL-NH₂-7/PMAA)₇ hydrogen-bonded capsules initially templated on silica cores revealed that the 1629 cm⁻¹ band associated with the carbonyl groups in the caprolactam ring in the free homopolymer (Fig.10a, spectrum 1) is shifted to a lower frequency band of 1610 cm⁻¹ upon complexation with PMAA (Fig.10a, spectrum 2). This result agrees with the previously reported shift of the carbonyl vibrations to lower frequencies in poly(N-

vinylpyrrolidone) upon hydrogen bonding.⁸⁷ Comparison of FTIR data on freeze-dried (PVCL-NH₂-7/PMAA)₇ hydrogen-bonded and (PVCL)₇ hydrogel capsules in Figure 10b demonstrates that all PMAA was released from the hydrogel capsules after their exposure to the high pH value. As seen, the absorption band centered at 1718 cm⁻¹ and corresponding to COOH groups of PMAA (Fig. 10b, spectrum 2) in the non-cross-linked hydrogen-bonded capsules disappeared in the spectrum of (PVCL)₇ hydrogel capsules (Fig. 10b, spectrum 1). It is worth noting that the absorption band of C=O in the caprolactam ring for (PVCL)₇ centered at 1610 cm⁻¹ in the non-cross-linked hydrogen-bonded capsules shifted back to ~1627 cm⁻¹ (Fig. 10b, spectrum 1), similar to that in the FTIR spectrum of PVCL-NH₂ copolymer, which confirms complete PMAA release from the hydrogel capsules.

Optical microscopy images in Figures 11(a,b) demonstrate complete dissolution of the cubic MnCO₃ cores with the (PVCL)₇ hydrogel capsules of the cubical geometry left behind. The single wall thickness of the produced cubical capsules was 13 nm, which corresponds to ellipsometry measurements for the dry hydrogel film fabricated under the same conditions. Figure 11c shows SEM image of hollow cubical (PVCL)₁₅ capsules dried from pH=3 after core dissolution and dialysis. As seen, the PVCL cubical hydrogel capsules partially collapsed after drying on silicon wafers. Despite this, the edges and corners of the hollow hydrogel cubes were still well-preserved and could be observed in the image. This result correlates well with our recent work on PMAA-based hydrogel capsules capable of retaining cubical shapes upon core dissolution.^{86,88}

The temperature-responsive behavior of the cubical (PVCL)₇ capsules was observed using CLSM microscopy (Fig. 12). The capsules were imaged first at 25°C (Fig. 11a,b) and then after heating to 50°C (Fig. 11c,d). As revealed by CLSM, the initial cubic geometry of cubical capsules persisted through exposure to higher temperature, even after capsule size decreased (Fig. 12e). Analysis of capsule dimensions before and after the temperature treatment revealed the cubical capsules decreased in size by 21%±1%. The fact that the capsules retained their cubical shape at the elevated temperature indicates a uniform decrease in the capsule size upon heating. The capsule size variations at 25°C and 50°C were completely reversible. As seen in Figure 12e, the cubical (PVCL) hydrogel capsules preserved their shape after two cycles of heating from 25°C to 50°C.

To address the possible effect of capsule shape on their thermoresponsive properties, spherical PEI(PVCL)₇ hydrogel capsules were investigated for comparison to cubical. The spherical capsules of 4.0±0.1 μm (size similar to that of the cubical capsules) were made by depositing hydrogen-bonded multilayers on surfaces of spherical silica particles. The capsules experienced temperature-induced shrinkage by 23%±1%, which is similar to that of the cubical capsules showing 21%±1% shrinkage. (Fig. 12f). Thus, our results suggest that shape does not impose any significant effect on the temperature response of (PVCL)₇ capsules. Studies of temperature-dependent permeability of the PVCL multilayer hydrogel capsules are underway.

Conclusions

We report on a novel type of single-component PVCL multilayer hydrogel films and capsules with a distinct and highly reversible thermoresponsive behavior. Importantly, the cubical (PVCL)₇ hydrogel capsules retained their cubical shape when temperature was changed from 25°C to 50°C while showing a size decrease of 21%±1%. Spherical (PVCL)₇ hydrogel capsules demonstrated the similar shrinkage of 23%±1%. The temperature-triggered size changes of both types of capsules were completely reversible. The hydrogels were derived from hydrogen-bonded multilayers of PMAA and PVCL-NH₂ by chemical cross-linking of PVCL-NH₂ copolymer layers and subsequent release of PMAA at basic pH

values. We found that the degree of hydrogel temperature-triggered shrinkage was controlled by the cross-link density ranging from 9 to 23 cross-links per chain for the hydrogels derived from PVCL-NH₂-7 and PVCL-NH₂-14 copolymers, respectively. The hydrogel films made from PVCL-NH₂-7 exhibited shrinkage of 1.9±0.1. Increase in the cross-link density in PVCL-NH₂-14 hydrogels significantly suppressed the shrinkage ratio down to 1.3±0.1. No temperature-responsive behavior was observed for non-cross-linked two-component films due to the presence of PMAA. Our work opens new prospects for developing biocompatible hydrogel-based nanothin coatings and shaped containers for temperature-regulated drug delivery, cellular uptake, sensing, and transport behavior in microfluidic devices.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Chemical structures of PVCL-NH₂ copolymer (a), PMAA (b), GA (c), PGMA (d), and PEI (e).

Scheme 1.

Layer-by-layer multilayers of $(\text{PVCL-NH}_2/\text{PMAA})_n$ (a) converted to $(\text{PVCL})_n$ multilayer hydrogel film (b) using GA cross-linking of PVCL-NH₂ copolymer layers within the LbL film. Swollen $(\text{PVCL})_n$ multilayer hydrogel (c) reversibly shrinks upon a temperature decrease (d).

Figure 2. Turbidimetry of PVCL-NH₂-7 (a) and PVCL-NH₂-14 (b) copolymer solutions before and after cross-linking with GA. Scattering experiments were carried out using a spectrofluorometer at 700 nm with aqueous copolymer solutions at pH=7.5.

Figure 3. Layer-by-layer growth of (PVCL-NH₂-7/PMAA) multilayers using dipping (circles) and spin-assisted LbL assembly (squares). The films were grown on PEI-based priming layer.

Figure 4. pH-Dependent thickness of (PVCL/PMAA) (squares), non-cross-linked (PVCL-NH₂-7/PMAA)₂₀ (circles), and cross-linked (PVCL)₂₀ (triangles) films. The films were fabricated using spin-assisted LbL deposition of polymers using the PEI-based anchoring coating. The *in situ* ellipsometry on the surface-attached films was carried out using 0.01 M phosphate buffers with appropriate pH values.

Figure 5.

(a) An FTIR spectrum of a (PVCL-NH₂-7/PMAA)₅ multilayer film grown at pH=3 and individual spectra of PVCL-NH₂-7 and PMAA monolayers as monitored by *in situ* ATR-FTIR. (b) FTIR spectra of a (PVCL-NH₂-7/PMAA)₅ film before cross-linking at pH=3 (a) and after cross-linking at pH=8 as monitored by *in situ* ATR-FTIR. The films were fabricated using the PEI-based priming film. The absorption bands associated with protonated carboxylic groups (–COOH), ionized carboxylic groups (–COO[–]), and carbonyl vibration band of PVCL are centered at 1700 cm^{–1}, 1550 cm^{–1}, and 1610 cm^{–1}, respectively.

Figure 6. FTIR spectra of a (PVCL-NH₂-7/PMAA)₅ film after cross-linking at pH=8 (spectrum 1) and after cross-linking at pH=3 (spectrum 2) as monitored by *in situ* ATR-FTIR. The absorption bands associated with protonated carboxylic groups (–COOH), ionized carboxylic groups (–COO[–]), and carbonyl vibration band of PVCL are centered at 1700 cm^{–1}, 1550 cm^{–1}, and 1610 cm^{–1}, respectively.

Figure 7.

Temperature-dependent thicknesses of non-cross-linked (PVCL-NH₂-7/PMAA)₂₀ (squares), and cross-linked (PVCL)₂₀ films (triangles). The *in situ* ellipsometry on surface-attached films was carried in 0.01 M phosphate buffer at pH=3 for the hydrogen-bonded (PVCL-NH₂-7/PMAA)₂₀ films and at pH=7.5 for (PVCL)₂₀ films in a temperature controlled liquid cell. The films were assembled on silicon wafers primed with the PEI-based film. The inset shows the hydration ratios of the corresponding (PVCL)₂₀ hydrogels fabricated from (PVCL-NH₂-7/PMAA)₂₀ or (PVCL-NH₂-14/PMAA)₂₀ hydrogen-bonded multilayers.

Figure 8.

(a) Dependence of (PVCL) hydrogel thickness on a number of PVCL layers. Inset in (a) shows temperature-induced shrinkage of PVCL hydrogel films of 20, 30, 40 layers. (b) Reversible thickness changes of (PVCL)₄₀ hydrogel film in response to temperature alterations from 25°C to 50°C in 0.01 M phosphate buffer solution at pH=7.5. (c) Hydration ratios of (PVCL) multilayer hydrogel films. (d) Temperature induced shrinkage of (PVCL) multilayer hydrogel films. In all graphs data on (PVCL) hydrogels produced from PVCL-NH₂-7 copolymer are presented. The hydrogels were built up on a PGMA priming layer using spin-assisted LbL.

Figure 9. Stability of (PVCL)₂₀ hydrogel films attached to the surfaces of silicon wafers through PGMA covalent anchoring. PVCL-NH₂-7 was used for the multilayer hydrogel formation. Dry films were exposed to pH=3 or pH=7.5 (0.01 M phosphate buffer solutions) at 25°C and 50°C for 48 hours followed by the ellipsometry measurements of their dry thickness.

Figure 10.

(a) FTIR spectra of PVCL-NH₂-7 copolymer (spectrum 1) and hydrogen-bonded (PVCL-NH₂/PMAA)₇ freeze-dried capsules (spectrum 2). (b) FTIR spectra of (PVCL)₇ hydrogel (spectrum 1) and freeze-dried (PVCL-NH₂/PMAA)₇ hydrogen-bonded capsules (spectrum 2). The capsules were made using silica microspheres without PEI priming layer. The spectra were taken from the samples pelleted in potassium bromide.

Figure 11.

Optical microscopy images of PEI(PVCL)₇-coated manganese carbonate cubical particles before (a) and after (b) carbonate core dissolution. Core dissolution was performed in a Lab-Tek microscopy chambered coverglass. The scale bars are 5 μm. (c) SEM image of PEI(PVCL)₁₅ hydrogel cubical hollow capsules dried on Si wafer from aqueous solution at pH=3.

Figure 12.

Hollow cubical (PVCL)₇ multilayer hydrogel capsules at T=25°C (a, b) and T=50°C (c, d) in 0.01 M phosphate buffer solutions at pH=7.5. The capsules are shown in CLSM mode (a, c) with their respective images in transmitted light mode (b, d). The scale bar is 3 μm . (e) (PVCL)₇ cubical capsules imaged after 25°C-50°C-25°C-50°C temperature cycles, the scale bar is 2.7 μm . (f) The shrinkage ratios (the ratio of the capsule size at 25°C to that at 50°C, d_{25}/d_{50}) of (PVCL)₇ multilayer hydrogel hollow spherical and cubical capsules.