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ORIGINAL PAPER

Dual-responsive release behavior of pH-sensitive PVA/PAAc hydrogels containing temperature-sensitive PVA/PNIPAAm microcapsules

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Abstract Both temperature and pH responsive drug delivery system was prepared by combining temperature-sensitive poly(vinyl alcohol) (PVA)/poly(*N*-isopropylacrylamide) (PNIPAAm) microcapsules and pH-sensitive PVA/poly(acrylic acid) (PAAc) hydrogels. The release of drug from the composite hydrogels increased as the pH increased due to the repulsion among the carboxylate anions in the PVA/PAAc hydrogels. The release of drug from the composite hydrogels also increased as the temperature decreased due to the higher hydrophilicity generated below the lower critical solution temperature of PNIPAAm. The compression moduli of composite hydrogels increased with increasing the content of PVA/PNIPAAm microcapsules. The biocompatibility of composite hydrogels was confirmed by the cytotoxicity test.

Keywords Temperature-sensitive · PH-sensitive · Microcapsule · Hydrogel

Introduction

Stimuli-responsive hydrogels have obtained a reputation as "intelligent materials" due to the ability to change their volume in response to pH [1–4], temperature [5, 6], electrical stimuli [7], and glucose [8, 9]. These materials have been used in the medical device industries, such as surgical sutures, soft tissues, artificial organs, catheters, and electrode sensors. The release of water-soluble drugs from the temperature-sensitive poly(*N*-isopropylacrylamide) (PNIPAAm) hydrogels was used to the biomedical applications [10, 11]. PNIPAAm hydrogel has a lower critical solution temperature (LCST) of 32 °C. PNIPAAm hydrogels shrank upon

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heating above LCST and swelled upon cooling below LCST reversibly [12–14]. These temperature-sensitive characteristics made the PNIPAAm hydrogels useful in various biomedical applications, such as the controlled drug delivery systems (DDS). The pH-sensitive poly(acrylic acid) (PAAc) can change the volume easily depending on the pH. As the pH decreased below the pKa of PAAc or increased above the pKa of PAAc, the PAAc would shrink or swell, respectively, due to the variation in the content of carboxylate anion.

From the viewpoint of recent biomedical applications, it would be favorable if the hydrogel could response to two different types of stimuli simultaneously, either mutually or independently, with particular emphasis on pH and temperature stimuli [15–17]. Even though many researchers have prepared the smart polymers by controlling the monomer structure [18] and copolymer structure [19], some limitations have to be solved for the practical applications. The direct chemical modifications of a thermo-responsive network were limited by the molecular design, its weak mechanical property [20–22], and low swelling ratio by other polymer chains present in a swollen state. The prolonged drug release having both pH- and temperature-sensitivity along with the improved mechanical strength would be preferable for a new DDS. In this study, the dual-responsive release of a model drug was investigated for the pH-sensitive PVA/PAAc hydrogels containing the temperature-sensitive PVA/PNIPAAm microcapsules.

Experimental

Materials

Poly(vinyl alcohol) (PVA, Mw = 31,000–50,000), acrylic acid (AAc), *N*-isopropylacrylamide (NIPAAm), glutaraldehyde (GA), ethylene glycol dimethacrylate (EGDMA), span 80, *n*-hexane, and potassium persulfate (KPS) were obtained from Sigma Chemical Co. Vitamin B12 (VB12) was purchased from Samchun Chemical and was used as a model drug. AAc was purified by vacuum distillation. NIPAAm was further purified by recrystallization in benzene/n-hexane. Others were used as received without further purification.

Preparation of PVA/PNIPAAm microcapsules

The hydrogel microcapsules having shell of hydrophilic PVA and temperature-sensitive PNIPAAm were synthesized by a three-step interfacial emulsion polymerization technique. PNIPAAm was synthesized by radical polymerization of NIPAAm with KPS as an initiator at 60 °C for 4 h under the nitrogen atmosphere. In the first step, a mixed aqueous solution of PVA (10 wt%, 10 mL) and PNIPAAm (5 wt%, 10 mL) was slowly added to the mixture of span 80 (6 mL) and *n*-hexane (100 mL) with stirring at 1000 rpm for 30 min at room temperature to form water-in-oil emulsion. The aqueous solution of GA (25 wt%) was slowly added to the mixture of span 80 (6 mL) and *n*-hexane (100 mL) with stirring at 1000 rpm for 30 min at room temperature to form another water-in-oil emulsion.

These two emulsions were mixed with stirring at 1000 rpm for 10 min at room temperature. After HCl (0.1 mL) as a catalyst was added to the mixed emulsion, the crosslinking of PVA was completed by further stirring at 1000 rpm for 30 min at room temperature. The PVA/PNIPAAm semi-IPN microcapsules were purified by multiple washing with distilled water and petroleum ether.

Drug loading in PVA/PNIPAAm microcapsules

The PVA/PNIPAAm microcapsules were dried in vacuum overnight until their weights remained unchanged. The vacuum dried PVA/PNIPAAm microcapsules (5 g) were immersed in the 0.5 wt% VB12 aqueous solution (100 mL) at 25 °C for at least 2 days to reach the equilibrated state. During this swelling period, the drug was loaded into the hydrogel networks. Drug loaded PVA/PNIPAAm microcapsules were separated from the VB12 solution followed by drying in vacuum oven for the future experiments. After separating the drug loaded microcapsules, the content of VB12 remained in the aqueous solution was measured in order to calculate the amount of VB12 loaded in the hydrogel.

Preparation of PVA/PAAc hydrogels containing PVA/PNIPAAm microcapsules

PVA/PAAc hydrogels were synthesized by the free radical copolymerization. The polymerization of AAc was carried out in de-ionized water by using EGDMA as a crosslinker and KPS as an initiator, respectively. The feed compositions of PVA, AAc, PVA/PNIPAAm microcapsule, crosslinker, and initiator are shown in Table 1. The reaction mixture was stirred for 30 min at room temperature under the nitrogen atmosphere. After the reaction mixture was stirred for 2 h at 70 °C, the PVA/PAAc hydrogels containing PVA/PNIPAAm microcapsules (MCH) were prepared. The MCHs were washed with distilled water at room temperature and replaced with fresh distilled water every few hours. All the MCHs were cut into disc-like pieces having approximately 10 mm in diameter and 10 mm in thickness for the following studies. MCH disks were dried in vacuum oven.

Morphology

The optical images of MCHs were recorded by a multi media imaging microscope (Sometech, ASTSV5-XYS). MCHs were put in a pH 10 buffer at a temperature

Table 1 Feed compositions of PVA/PAAc hydrogels containing PVA/PNIPAAm microcapsules (MCH)

	MCH0	MCH10	MCH15	MCH20
PVA (g)	15	15	15	15
AAc (g)	4	4	4	4
GA (g)	3	3	3	3
EGDMA (g)	1	1	1	1
KPS (g)	0.2	0.2	0.2	0.2
PNIPAAm/PVA microcapsules (g)	–	1.0	1.5	2.0

range from 4 to 40 °C for 72 h to reach the equilibrium swollen state. The swollen samples were quickly frozen in liquid nitrogen and followed by freeze drying. The morphologies of both PVA/PNIPAAm microcapsules and MCHs were examined by scanning electron microscope (SEM, Jeol, JSM-7000F). As a pretreatment, the samples were vacuumed up to 10^{-3} Pa and sputtered using Pt.

Mechanical properties

The mechanical properties of MCHs were measured by an Instron tester (Instron, model 5583) at 25 °C and 50% relative humidity with a compression load cell, having a full-scale range of 1.0 kN. All the samples were immersed in water at room temperature for 2 days to reach the equilibrium swollen state. All the MCHs were cut into disc-like pieces having $\sim$10 mm in diameter and 10 mm in thickness (5 ± 0.2 g) for the following studies. The sample was then placed on the top plate of a compression load cell and compressed by a cylindrical metal rod probe (diameter 10 mm) at a constant crosshead speed of 2 mm/min until the fragmentation of the sample occurred. Initial compression modulus was calculated from the initial slope of the stress–strain curve. The mechanical measurement was carried out twelve times to obtain the average compression modulus and the error range.

Swelling behavior

Three different kinds of pH buffers (2, 7, and 10) were used as swelling media and the swelling behavior was determined by gravimetric method. The dried samples were immersed into the buffer solutions for a certain period of time until the swelling equilibrium was reached and then these hydrogels were taken out for wiping with tissue paper to remove excess of buffer solution on the surface. They were weighed immediately. The equilibrium swelling (%) of the hydrogels was calculated as follows:

$$\text{Swelling } (\%) = (W_s - W_d)/W_d \times 100,$$

where W_d and W_s are the weights of dry and swollen samples, respectively. The swelling behavior was also evaluated in three different temperatures (4, 25 and 40 °C) to investigate the temperature effect on swelling behavior of hydrogel.

Release behavior

The standard calibration curve for the VB12 concentration was obtained by measuring the absorbance at 361 nm with the UV spectrophotometer (Shimadzu, UV-1201). Releasing of VB12 from MCHs was conducted in pH 2, 7, and 10 buffers at various temperatures (4, 25, and 40 °C). During the drug releasing experiment, 2 mL aliquots of the releasing media were taken out with reconstitution of 2 mL fresh buffers at every predetermined time interval and the concentration of the VB12 released from MCHs was determined by measuring the absorbance at 361 nm using the standard calibration curve. All the experiments were carried out in triplicate.

Indirect cytotoxicity evaluation

The indirect cytotoxicity evaluation of samples was carried out by using Cell Counting Kit-8 (CCK-8), which was purchased from Dojindo Laboratory, Japan. Experimental design was conducted according to the ISO 10993-1, 5, and 12 standard test methods [23]. Briefly, the samples were taken to 0.1 g, and the extraction media were prepared by immersing the samples in a 96-well microtiter plate at 5×10^3 cells/well and incubated for 24 h. Mouse fibroblasts (L 929, KCLB No 10001) were seeded in a 96-well plate at a density of 5×10^3 cells/well and incubated at 37 °C under a 5% CO_2 humidified atmosphere in DMEM (Dulbecco's Modified Eagle's Medium) containing 10% FBS (Fetal Bovine Serum) and penicillin (5000 U/mL)/streptomycin (50 μg/mL). The culture medium was removed after 24 h and then the prepared extraction media were added to the 96-well. The cells were incubated for 24 h and the number of viable cells was quantified with the CCK-8. The absorbance of CCK-8 was measured at 450 nm using an automated ELISA (Enzyme linked immunosorbent assay) microplate reader (Bio-Tek Instruments, USA). The analysis was carried out in triplicate.

Results and discussion

Morphology of PVA/PNIPAAm microcapsules

Scanning electron microscope images of PVA/PNIPAAm microcapsules are presented in Fig. 1. Microcapsules had quite smooth surfaces and thin shells. The size of microcapsules was in the range of 10–50 μm. The optical microscope images of various MCHs are presented in Fig. 2. The PVA/PNIPAAm microcapsules were uniformly distributed in the PVA/PAAc hydrogel matrix, even though some microcapsules were aggregated as the content of microcapsules increased. To observe PVA/PNIPAAm microcapsules in PVA/PAAc hydrogel more clearly, SEM images were taken. SEM images of various MCHs are shown in Fig. 3. PVA/PNIPAAm microcapsules were found intact in the PVA/PAAc hydrogel matrix with weak interactions between microcapsules and hydrogel matrix.

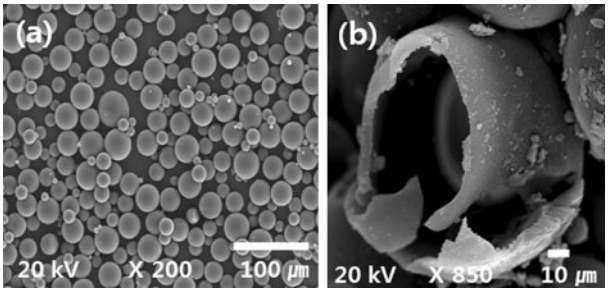

Fig. 1 SEM images of **a** PVA/PNIPAAm microcapsules and **b** mechanically fractured PVA/PNIPAAm microcapsules

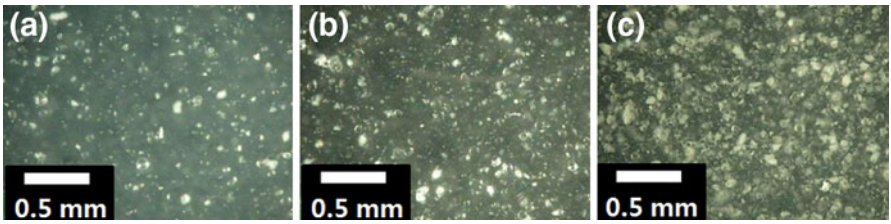

Fig. 2 Optical microscope images of various MCHs: **a** MCH10, **b** MCH15, and **c** MCH20

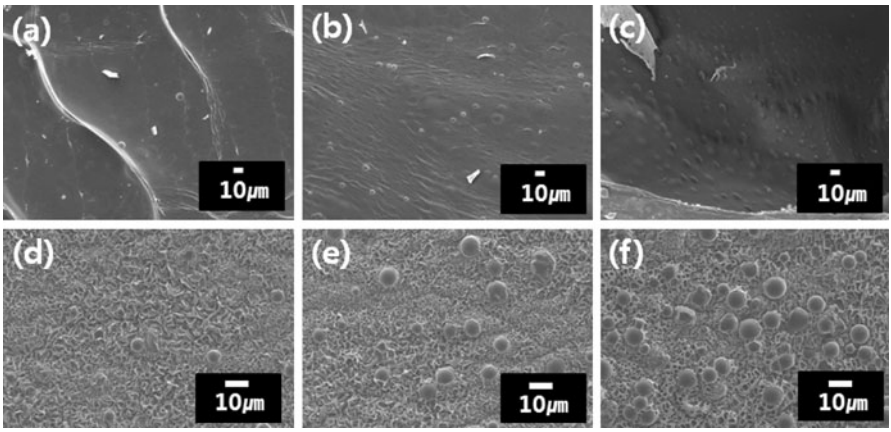

Fig. 3 SEM image of various MCHs: **a** surface of MCH10, **b** surface of MCH15, **c** surface of MCH20, **d** fractured section of MCH10, **e** fractured section of MCH15, and **f** fractured section of MCH20

PVA/PNIPAAm microcapsules were also found on the surface and in the fractured section of PVA/PAAc hydrogel matrix and were distributed uniformly.

Mechanical properties of MCHs

The swelling behavior of hydrogels is closely related to their structures and the degree of swelling also influences the mechanical properties of hydrogels. Therefore, it is essentially important to examine the relationships between the degree of swelling and mechanical properties of hydrogels. The mechanical properties of MCHs were evaluated by the compression modulus as shown in Fig. 4. The mechanical strength of MCHs increased greatly by incorporating PVA/PNIPAAm microcapsules into the PVA/PAAc hydrogel matrix due to the higher compression modulus of PVA/PNIPAAm microcapsules. The mechanical strength of MCHs increased gradually as the content of PVA/PNIPAAm microcapsules increased.

Swelling behavior of MCHs

The temperature-sensitive swelling behavior of MCHs is presented in Fig. 5. The swelling ratio of MCHs decreased quickly and showed the similar behavior

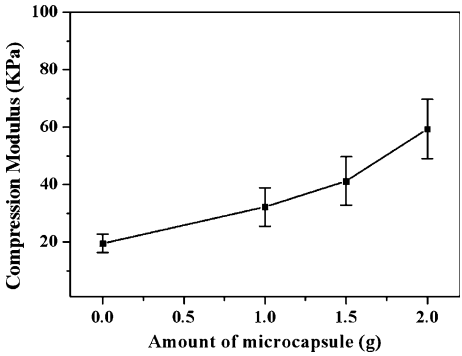

Fig. 4 Compression modulus of various MCHs depending on the content of PVA/PNIPAAm microcapsules in PVA/PAAc hydrogel matrix (23 g)

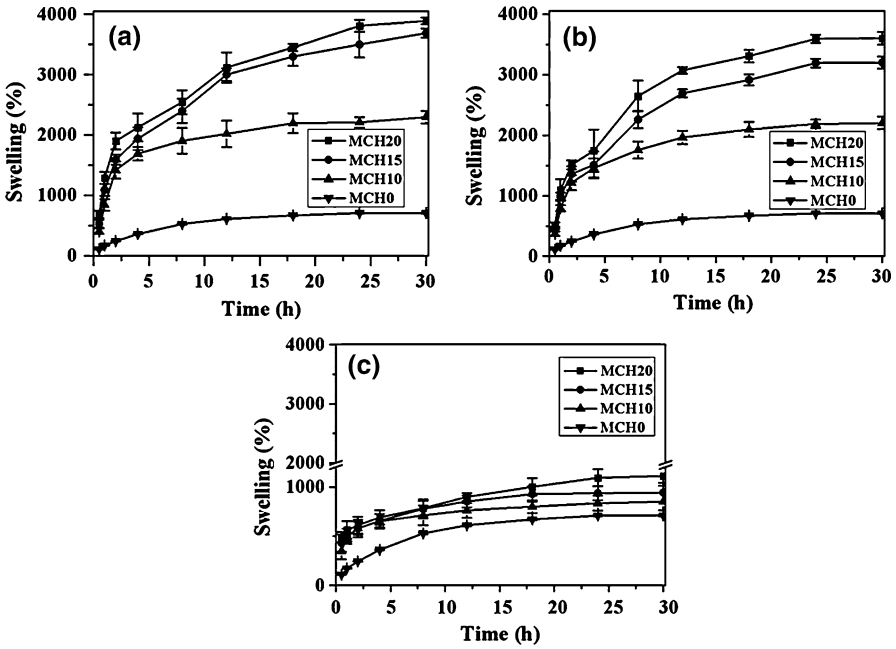

Fig. 5 Swelling ratios of various MCHs in pH 10 buffer at **a** 4 °C, **b** 25 °C, and **c** 40 °C

regardless of the content of microcapsules as the temperature increased over the lower critical solution temperature (LCST) of PNIPAAm (32 °C). However, the swelling ratio of MCHs increased as the content of temperature-sensitive PVA/PNIPAAm microcapsules increased in the temperature ranges below the LCST.

Temperature-sensitive variation in the size of PVA/PNIPAAm microcapsules is clearly shown in Fig. 6. The size of microcapsules decreased drastically as the temperature increased above the LCST consequently resulting in the apparent deswelling of MCH.

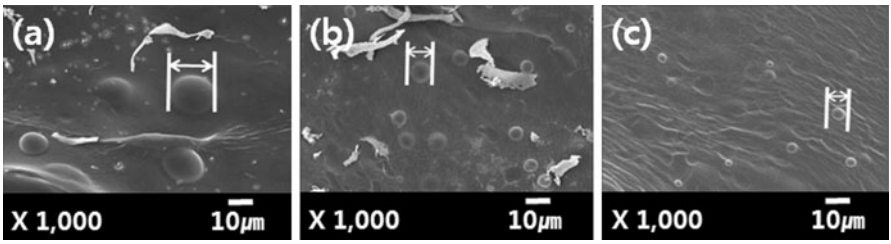

Fig. 6 Variation in the size of PVA/PNIPAAm microcapsules of MCH20 swollen in pH 10 buffer at **a** 4 °C, **b** 25 °C, and **c** 40 °C

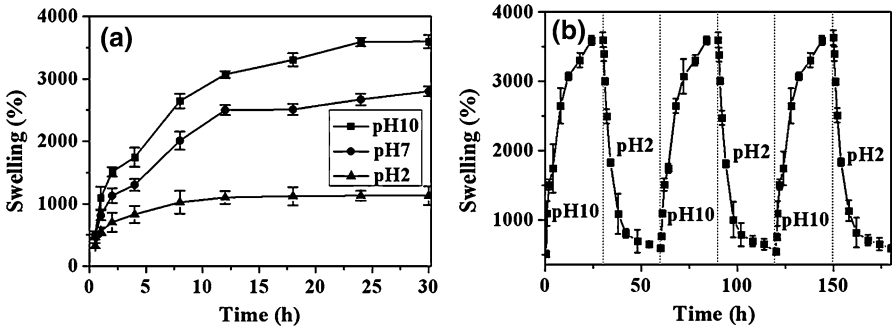

Fig. 7 Swelling ratios of MCH20 **a** in various different buffers and **b** under cyclic swelling and deswelling in pH 10 and pH 2 buffers, respectively, at 25 °C

The pH-sensitive swelling behavior of MCH20 is presented in Fig. 7. The swelling of MCH20 increased greatly as the pH of buffer increased. pH-sensitive PVA/PAAc hydrogels have pendant carboxylic acid groups which can be ionized into carboxylate anion above its pKa of 4.7. The amount of carboxylate anion increased as the pH increased. The carboxylate anions cause more electrostatic repulsion and hydrophilicity to the polymer chains. In the lower pH range, the polycarboxylic groups were nonionized and the higher H^+ concentration also decreased the osmotic pressure of the hydrogel. The electrostatic repulsion between the nonionic polycarboxylic groups within the network became dominant because of the decreased ionic strength. Therefore, PVA/PAAc hydrogels show pH-sensitive swelling behavior.

Scanning electron microscope images of the cross section of the fractured MCH20 are presented in Fig. 8. The void size in the hydrogel matrix increased gradually as the pH of buffer increased due to the higher swelling ratio in the basic condition of buffer.

Dual-responsive release behavior of MCHs

The standard calibration curve for the VB12 concentration is shown in Fig. 9 and has a linear relationship with a correlation coefficient (r) of 0.9999. This linear

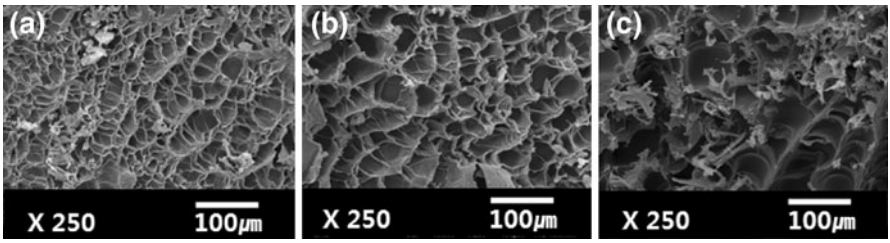

Fig. 8 Variation in the fracture morphology of MCH20 swollen at 25 °C in **a** pH 2, **b** pH 7, and **c** pH 10 buffers

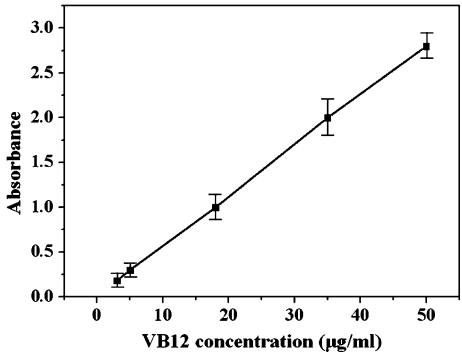

Fig. 9 The standard calibration curve of the absorbance as a function of VB12 concentration at 361 nm on the UV spectrophotometer

relationship is described as the following equation: $A = (55.927c - 11.02) \times 10^{-3}$, where A is the absorbance, and c is the concentration (μg/mL) of the VB12 drug.

The standard calibration curve was used for the determination of the released amount of drug from MCHs.

The dual-responsive release profile of MCH20 was evaluated in detail based on both pH and temperature variations as shown in Fig. 10. For the pH-sensitive characteristics of MCH20, there was no noticeable release of drug at pH 2. This result matched with the swelling results of MCH20 shown in Fig. 7. The release of drug was accelerated as the swelling of PVA/PAAc hydrogel matrix increased at higher pH. For the temperature sensitivity of MCH20, the drug release increased significantly as the temperature decreased below the LCST due to the higher swelling of PVA/PNIPAAm microcapsules.

Indirect cytotoxicity evaluation

The cytotoxicity test was carried out to investigate the biocompatibility of MCHs as shown in Fig. 11. All the MCHs showed the high viability over 85%. All the materials used for the preparation of MCHs did not show any noticeable cytotoxicity because the higher viabilities were maintained for all the MCHs.

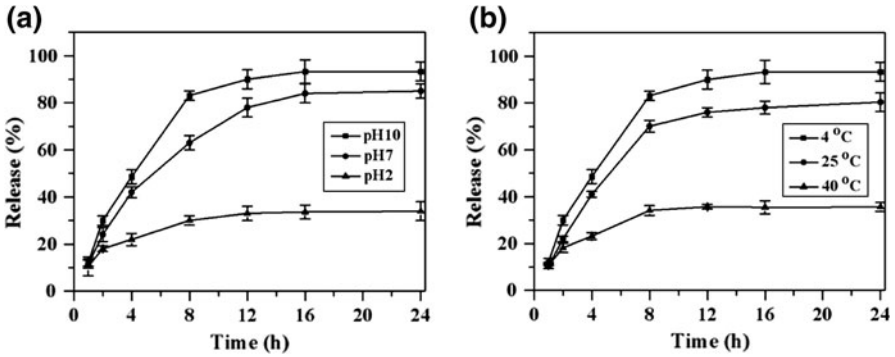

Fig. 10 Drug release behavior of MCH20 **a** at 4 °C in various pHs and **b** at various temperatures in pH 10

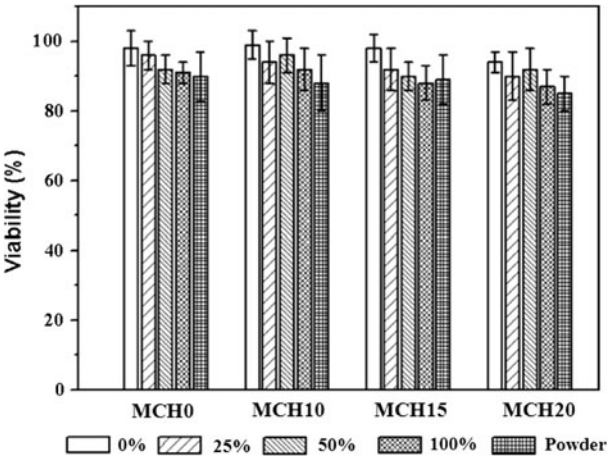

Fig. 11 Cytotoxicity evaluation of various MCHs

Therefore, MCHs could be applied to the dual-responsive drug delivery system without causing cytotoxic problems.

Conclusions

The pH-sensitive PVA/PAAc hydrogels and the temperature-sensitive PVA/PNIPAAm microcapsules were combined to prepare the dual-responsive drug delivery system. The release of drug from MCHs increased with increasing the pH because of the higher swelling caused by the repulsions among the carboxylate anions in the PVA/PAAc hydrogel matrix. On the other hand, the release of drug from MCHs increased with decreasing the temperature because of the higher swelling below the LCST of PNIPAAm. The mechanical properties of MCHs increased with increasing the content of PVA/PNIPAAm microcapsules. MCHs had the high enough viability over 85% in the cytotoxicity test to be suitable as biocompatible materials.

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