Hybrid Hydrogels

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Thermally Responsive Hydrogel Blends: A General Drug Carrier Model for Controlled Drug Release**

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Abstract: *Thermally responsive hydrogels have drawn significant research attention recently because of their simple use as drug carrier at human body temperature. Here we design a hybrid hydrogel that incorporates a hydrophilic polymer, polyethyleneimine (PEI), into the thermally responsive hydrogel poly(N-isopropylacrylamide) (PNIPAm), as a general drug carrier model for controlled drug release. In this work, on one hand, PEI modifies the structure and the size of the pores in the PNIPAm hydrogel. On the other hand, PEI plays an important role in tuning the water content in the hydrogel and controls the water release rate of the hydrogel below the lower critical solution temperature (LCST), resulting in a tunable release rate of the drugs at human body temperature (37°C). Different release rates are shown as different amounts of PEI are incorporated. PEI controls the release rate, dependent on the charge characteristics of the drugs. The hydrogel blends described in this work extend the concept of a general drug carrier for loading both positively and negatively charged drugs, as well as the controlled release effect.*

Hydrogels are synthesized from hydrophilic monomers with a network promoted by functional crosslinkers.[1] They have attracted increasing attention because of their outstanding properties including biocompatibility and high permeability for water-soluble nutrients and drugs, leading to wide-ranging applications such as tissue-engineering scaffolds,[2] in vivo drug carriers[3] or in vitro[4] delivery systems. Among them, the stimuli-responsive hydrogel exhibits a conformation transition between swollen and de-swollen states when experiencing external environmental changes such as temperature,[5] pH,[6] electric field[7] or even light changes.[8]

Among many kinds of hydrogels with thermoresponsive behavior, poly(*N*-isopropylacrylamide) (PNIPAm) gel is one of the most studied gels, which has an obvious coil–globule transition at 32 °C (the lower critical solution temperature or LCST), being hydrophilic below this temperature or hydrophobic above it.[9] This temperature corresponds to the part in the phase diagram where the entropic gain of the system overcomes the enthalpic contribution associated with hydrogen bonds.[9] The unique property endows PNIPAm-based materials with a wide array of applications including chemosensors,[10] DNA separation medium,[11] fluorescent thermometer[12] or even catalyst for hydrolysis.[13] Moreover, they also take a more active role as drug carriers, which are designed to retain loaded drugs in collapsed state and release drugs in swollen state because of the volume transition under environmental stimuli.[14] However, there are two significant limitations of the PNIPAm hydrogel when used as a drug delivery device:[15] One is the poor mechanical property under its highly swollen state; the other is the lack of sustained release capability. In most cases, the drug molecules are wrapped in the swollen gels by simple physical interaction forces, resulting in uncontrolled high drug release rates because of the relatively weak intermolecular interactions. Moreover, for applications in medicine, the materials should show their responsive properties under biological conditions. Till now, some modifications of PNIPAm have been presented in order to overcome the deficiencies as well as "regulate" the LCST, such as co-polymerization with other monomers,[16] which may lead to a significantly lowered thermal sensitivity of the PNIPAm-based hydrogel after the introduction of a non-thermosensitivity moiety.[15] Another route to control the phase transition behavior is to physically incorporate hydrophilic or hydrophobic constituents into the gel network. It has been believed that the LCST of the gels can be elevated by increasing the intrasystem hydrogen bonds after introducing appropriate hydrophilic ingredients.[17] This method is attractive and inexpensive and has many advantages over the traditional co-polymerization approach in obtaining new structural materials, including facile device fabrication and manipulation, and ready control of drug loading.[18]

Here, we designed a hybrid gel based on PNIPAm hydrogel with branched polyethyleneimine (PEI) incorporated, to enable tunable release of a drug wrapped in the hydrogel network. The LCST for the PNIPAm gel is increased by the incorporation of PEI, a water-soluble cationic polymer widely used in gene delivery because of its ease to complex with DNA.[19] Additionally, chemical integration of PEI transforms the neutral PNIPAm hydrogel to ionized PNIPAm, realizing the feasibility of tuning its swelling ratio,[20] leading to advantageous features of ionic gels over their neutral counterparts in many potential applications, especially in controlled release devices.[21] This work demonstrates the effective control of the drug releasing speed by tuning the ionization degree of the hydrogels. The resultant release rate depends on different interactions between the

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loaded drug and the PEI, including electrostatic repulsion or attraction.

In our study, methylene blue (MB) and folic acid (FA) were used as drug models to investigate the controlled release effect of the hydrogel blends. In brief, a certain amount of drugs and PEI were dispersed together with *N*-isopropylacrylamide (NIPAm) as the monomer and *N*,*N*′-methylenebisacrylamide (MBAAm) as the crosslinker, followed by nitrogen bubbling and adding ammonium persulfate (APS) as the initiator for the free-radical polymerization of PNIPAm. The drug could also be loaded after polymerization and dialysis of the PNIPAm/PEI hydrogel by a "drying and reswelling" procedure for practical application. Four different PNIPAm/PEI hydrogel blends were prepared from mixture solutions with compositions shown in Table S1 in the Supporting Information. The four samples are referred to as PEIAm-0, PEIAm-18, PEIAm-88, and PEIAm-160 with increasing PEI content, and the numbers indicate the mass ratio of the PEI raw solution and PNIPAm monomer. It was reported that polyelectrolyte can strongly reduce the free-radical polymerization rate.[22] PNIPAm hydrogel cannot form if too much PEI is employed. Meanwhile, the amount of drug loading can be varied over a wide range as long as the drug molecule itself does not react with PEI or PNIPAm. The highest loading amount in our studies is 0.36 mg of drug per milligram of hydrogel which is already higher than the concentrations of drugs commonly used.

Generally, it is believed that the drug release rate is closely related to the degree of water absorption, as well as the moieties that have interactions with drugs if introduced in the hydrogels.[23] In our hybrid hydrogel system, PEI plays an important role in enhancing the hydrogen-bond intensity in the hydrogel matrix, which can be regarded as water-retaining effect and is investigated by employing MB as drug model (Scheme 1 a). In addition, as an amine-rich polymer, it is straightforward to introduce different interactions between PEI and drugs. Previously researchers have used cyclodextrins that have covalent bonding with PEI to realize the controlled release of drugs.[23] To demonstrate this interaction effect (different from the water-retaining effect) in our hybrid hydrogel system, negatively charged folic acid (FA) is selected as the drug model to investigate its controlled release property because it enables electrostatic attraction with PEI (Scheme 1 b).[24]

Figure 1 shows the Fourier transform infrared (FT-IR) spectra of the dried hydrogel blends. Characteristic peaks marked in Figure 1 give the evidence of co-existence of PNIPAm and PEI in PEIAm-18, PEIAm-88, and PEIAm-160. The peaks at around 780 cm^{-1} are more intensive for PEIAm-88 and PEIAm-160 because they are associated with the deforming vibration and rocking vibration of PEI amines.[25] The intensities of the peaks at about 1305 and 2823 cm^{-1} increase in PEIAm-88 and PEIAm-160, which may be assigned to stronger wagging and twisting motions and symmetric vibrations of the CH_2 group in PEI, respectively.[26] Moreover, compared to the other two spectra, PEIAm-88 and PEIAm-160 samples also show a significant increase of the C-H stretching band at about 2937 cm^{-1} as it is more common in PEI.[27] There is a slight blue shift for the N-H stretching vibration around 3300 cm^{-1} because of the formation of hydrogen bonds between the PNIPAm hydrogel and PEI.[28]

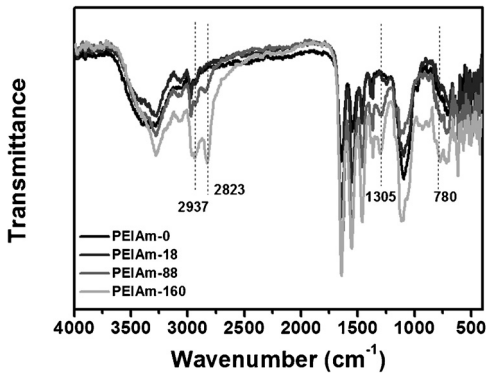
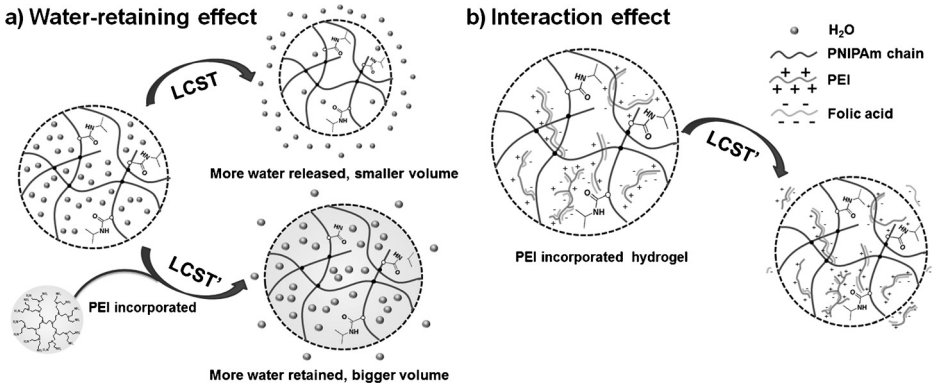


Figure 1. FT-IR spectra of PEIAm-0, PEIAm-18, PEIAm-88, and PEIAm-160.

The swollen samples of PEIAm-0, PEIAm-18, PEIAm-88, and PEIAm-160 are processed by freeze-drying and characterized by scanning electron microscopy (SEM; Figure 2). The structure of the hydrogel blends gradually shifts from a loose network with randomly oriented sheet-like structure to more compact structures with pores occupied by PEI, as the content of PEI increases.[29] However, PEIAm-18 has a markedly increased pore size compared to the other three samples, probably because of the repulsion of the ionized pendant amine groups after a small amount of PEI is introduced.[29,30] As the size of the pores is often considered to influence the diffusion-controlled speed of the drug release, PEIAm-18 of significantly larger pores is inclined to accelerate the release of drug molecules that have no interactions with PEI.



Scheme 1. Schematic of two effects influencing the drug release rate after incorporation of PEI: a) the water-retaining effect and b) the interaction effect.

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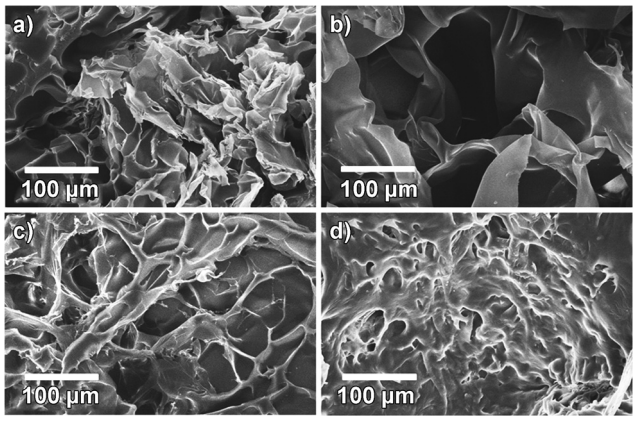

Figure 2. Microstructural characterization by SEM imaging for a) PEIAm-0, b) PEIAm-18, c) PEIAm-88, and d) PEIAm-160.

among the amine groups in PEI as mentioned before. Thus we make the hypothesis that slight incorporation of PEI can accelerate the releasing speed of drugs that have no interaction with PEI since no confining force is applied but only natural diffusion dominates the release speed. According to the previous study,[29] incorporating hydrophilic components can reduce the loss of the water content in a hydrogel. Therefore, when the amount of PEI is increased, the water-retaining effect prominently slows down the release speed, meanwhile alleviating the burst release effect (Figure 3a). Interestingly, the release of FA is different from that of MB (Figure 3b). The highest speed appears in PEIAm-0, suggesting the diffusion of FA is dominated by chemical interaction rather than physical diffusion of MB. This finding provides a unique perspective for future approaches to controlling the release speed of drugs that are attracted by PEI. For both cases of MB and FA, the sample PEIAm-88 shows the desired controlled release, with the substantially weakened burst release relative to PEIAm-0 and PEIAm-18, and the release times are 25 and 35 h for MB and FA, respectively.

The oscillatory tests were also carried out at variable frequencies and fixed stress to analyze the dynamic mechanics of various hybrid gel samples, providing storage modulus (G′) and loss modulus (G″), of which the former (G′) represents the ability to store deformation energy that can be recovered after removing the load cycle,[31] and the latter (G″) stands for viscous properties.[32] Although the storage modulus decreases after PEI incorporation and further attenuates with increasing amount of PEI (Figure S1a), nevertheless, it is larger than the loss modulus at all frequencies, as expected for soft solids, suggesting that the present hydrogels display a predominantly elastic-like behavior (Figure S1a and b).[32–33] In addition, the ratio G′/G″, which is regarded as the stiffness of the hydrogels,[33,34] reaches its highest value for PEIAm-88, indicating that it is more rigid,[35] and more suitable for sustained drug release (Figure S1c). During the strain sweep test, the four samples all show a liner region from 0.1 to 10% strain at a fixed frequency of 2% which is within the liner region, confirming the validity of our test (Figure S2). The G′ value decreases as the amount of PEI increases, indicating that the hybrid gel becomes weaker, which is consistent with the results of oscillatory tests (Figure S1a).

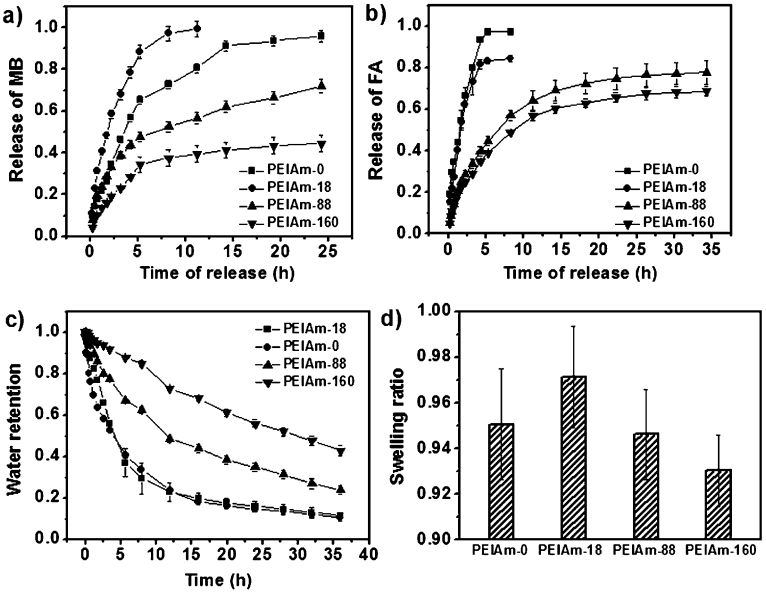

Figure 3. Release curves in 37°C water of a) MB and b) FA. c) Shrinking kinetics and d) swelling ratios of the four hydrogel blends.

The in vitro release of MB and FA was carefully investigated and the results are shown in Figure 3a and b, respectively. The drug carriers PEIAm-0, PEIAm-18, PEIAm-88, and PEIAm-160 are immersed into 37°C water as imitation of the body environment. The cumulative release amount was calculated at specific time. For both drug models, there is a general trend that the release rate decreases with increasing amount of PEI, showing its role for the controlled release effect. The release speed of MB from PEIAm-18 is a little higher than that of PEIAm-0 (Figure 3a), although both show initial burst release within the first 5 h. This is likely due to the ease in diffusion through the larger pore in the network of PEIAm-18 (Figure 2b) caused by repulsion

In this work, the MCF-7 (Michigan Cancer Foundation-7, a breast cancer cell line) cells were used to evaluate the cytocompatibility of the four samples. The cytotoxicity was examined through testing the cell viability upon exposure to our sample by using the standard cell counting Kit-8 (CCK-8) assay. No apparent reduction in cell viability was found after incubation of the cells with the hydrogel blends even at its concentration of 100 $\mu g\,mL^{-1}$ (Figure S3), suggesting good biocompatibility of our hybrid gel system. In drug release devices, it is usually required that the drug carrier have the ability to maintain its original shape and integrity, as well as smooth surface during the entire release process.[36] Typically, the PEIAm-88, which has the smoother surface and is more

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transparent compared to PEIAm-0 (Figure S4), shows another advantage of our design. The hydrogel blends maintain their plasticity, thus any shape and size of the hydrogel can be formed by altering the template, which can deliver molecules with desired release kinetics over long periods when properly designed. In addition, the total amount of drug released is around 80%, which is higher than or comparable with the previous controlled release systems.[37] For the investigation of shrinking behaviors of the hydrogels, similar results to release of MB were obtained (Figure 3c). Compared with PEIAm-0, PEIAm-18 releases water even more quickly (Figure S5) with a similar degree of water retention, which may be attributed to the larger pores shown in Figure 2b. In the swelling ratio test, PEIAm-18 reaches the highest level among the four samples, indicating that the larger pores can hold more water than the other three. But more pores will be occupied if more PEI is introduced, as shown in Figure 2c and d. Therefore, the swelling ratio decreases for PEIAm-88 and PEIAm-160 (Figure 3d), although more PEI can hold more water at lower temperature as shown in the deswelling ratio test (Figure S5). Meanwhile, the LCST is increased to about 37 °C from 32 °C for PEIAm-88 and PEIAm-160, facilitating the practical use at human body temperature (Figure S5).

In summary, we have developed a general drug carrier based on a hybrid hydrogel that incorporates a hydrophilic polymer PEI with many functional groups into a PNIPAm hydrogel network for tunable drug release. This gel design provides a general drug carrier model with facile tenability of the drug release rate since the composition of the hydrogel blends can be easily modulated by the amount of PEI in the hydrogel matrix. In this work, the PEIAm-88 hydrogel blend is considered as the optimized sample for release of MB and FA, with prolonged release time and decent total release amount. As demonstrated herein, the factors controlling the release speed vary with the type of the drug from simple diffusion to chemical interaction-dominated diffusion. This fundamental concept of the hybrid hydrogel as drug carrier medium can open up exciting opportunities for developing various drug carrier systems with a desired release rate.

Keywords: controlled drug release · drug carrier · hydrogel blends · polyethyleneimine (PEI) · thermoresponsive materials

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