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Tuning the Hydrophobicity of a Hydrogel by Self-assembly of Polymer Cross-linkers

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Abstract: Hydrogels incorporated with hydrophobic motifs have received considerable attention to recapitulate the cellular microenvironments, specifically for the bio-mineralization of a 3D matrix. Introduction of hydrophobic motifs into a hydrogel often results in irregular arrangement of the motifs, and further phase separation of hydrophobic domains, but limited efforts have been made to resolve this challenge in the hydrophobically-modified hydrogel. Therefore, this study presents an advanced integrative strategy to incorporate hydrophobic domains regularly in a hydrogel by self-assembling of polymer cross-linkers, building blocks of a hydrogel. Self-assemblies between polymer cross-linkers were examined as micro-domains to incorporate hydrophobic motifs in a hydrogel. The self-assembled structures in a pre-gelled solution were confirmed with the fluorescence analysis and the hydrophobicity of a hydrogel could be tuned by incorporating the motifs in a controlled manner. Overall, the results of this study would greatly serve to tuning performance of a wide array of hydrophobically-modified hydrogels in drug delivery, cell therapies and tissue engineering.

Keywords: Hydrogel; Hydrophobicity; Self-assembly; Degree of swelling

1. Introduction

Hydrogels have been extensively studied for use in various biomedical applications including drug delivery, tissue engineering and recently, Bio-MEMS(bio-microelectromechanical system) [1-3]. The successful use of the hydrogels in these applications greatly relies on their physical and chemical properties for maximizing their functionality [4-7]. For example, mineralized hydrogel systems are being increasingly studied to understand bio-mineralization processes related to the development, repair, regeneration and remodeling of bone tissue [8,9]. In here, the hydrogels incorporated with hydrophobic motifs are required to recapitulate the hydrophobic/hydrophilic microenvironments in mineralized bone tissue [10,11]. However, incorporating of hydrophobic motifs into a hydrogel often results in irregular arrangement of the motifs, and further phase separation of hydrophobic domains, but limited efforts have been made to resolve this challenge in the hydrophobically-modified hydrogel.

Therefore, we hypothesized that self-assemblies of polymer cross-linkers in a pre-gelled solution would allow us to incorporate hydrophobic motifs regularly as micro-domains in a hydrogel. This hypothesis was examined using a model system for a hydrophobically-modified hydrogel formed from the cross-linking of poly(ethylene glycol) diacrylate (PEGDA). The self-assembled structures of PEGDAs in a pre-gelled solution were confirmed with the fluorescence analysis (Fig. 1(b)). Then, poly(propylene glycol) methacrylate (PPGMA) with varying of mass fraction was used as model hydrophobic motif (Fig. 1(c)). The effects of the hydrophobic domains incorporated into a hydrogel were studied by measuring swelling ratio and contact angle of a hydrogel. The underlying mechanism by which micro-domains provided by self-assembling of

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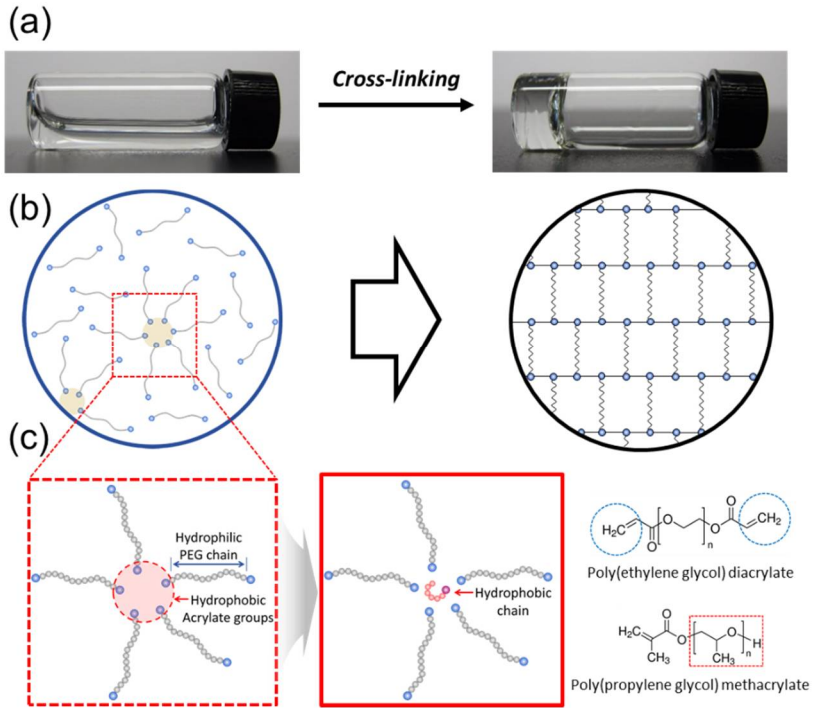
polymer cross-linkers tuned the hydrophobicity in a hydrogel was examined by evaluating average pore size of a hydrogel and characterizing by fluorescence analysis. Overall, this study demonstrates a novel strategy to create a hydrogel incorporated with hydrophobic chains in a controlled manner by self-assembling of polymer cross-linkers.

Figure 1. Schematic description of hydrogel forming form polymer cross linkers in a pre-gelled solution (a). The internal structures of self-assemblies associated with polymer cross-linkers in a



pre-gelled solution (b) and incorporating of hydrophobic chains using the self-assemblies (c).

2. Materials and Methods

2.1. Fluorescent analysis of self-assembling of polymer cross-linkers in a solution

The self-assemblies of polymer cross-linkers in a pre-gelled solution were investigated using a pyrene probe. [12,13]. The pyrene (Sigma) was dissolved in acetone to prepare a stock solution with concentration of 6.0×10^{-4} M. Polymer cross-linker pre-gelled solutions were prepared in DI (deionized) water (2 mL) by poly(ethylene glycol) diacrylate of M_n 575 g/mol (PEGDA-575, Sigma) and M_w 3400 g/mol (PEGDA-3400, Sigma) respectively. In parallel, acrylate group-free poly(ethylene glycol) of M_n 3350 g/mol (PEGdiol, Sigma) were dissolved in DI water as a control. Then, the pyrene solution was dropped into the polymer solutions with varying of polymer concentrations. The mixture of the polymer solution and pyrene was sonicated for 10 min to ensure dispersion of pyrene in the polymer solution. The mixture was further incubated at room temperature for at least 12 hour in the dark, so the pyrene was preferentially associated with hydrophobic domains of polymers. The mixture loaded in a quartz cuvette was excited at wavelength of 330nm and resulting emission spectrum was obtained using photo luminescence (QM40, Photon Technology International). The band-width was adjusted to 2.0 nm for both excitation and emission.

2.2. Hydrogel preparation

Pre-gelled solutions were prepared by mixing 10 wt% PEGDA-575 which has hydrophilic property and poly(propylene glycol) methacrylate (PPGMA, M_n of 375 g/mol, Sigma), acting hydrophobic chain, by increasing of mass fraction under fixed total polymer concentration, 10 wt%. The PEGDA was dissolved in DI water at 40 wt% stock solution, and PPGMA was dissolved in dimethyl sulfoxide (DMSO, Sigma Aldrich) at 20 wt% stock solution. The photo-initiator,

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2-hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone (Irgacure 2959, Sigma) was dissolved DMSO at 10 wt% stock solution and added to the 1mL pre-gelled solution to form 0.2 wt% as the final concentration. First, the 1mL of pre-gelled solution mixed by vortex mixer. Second the mixed pre-gelled solution was cast between glass plates with spacer of 1mm of thickness. Then, the cast pre-gelled solution was exposed by UV lamp (365 nm, VL-4.LC, VILBER LOURMAT) for gelation about 10 min, after then, gel was punched with 8mm diameter. Gel disks were immersed DI water to remove unreacted hydrophilic residuals and then, subsequently immersed DMSO to residual hydrophobic polymers in the cross-linked gel disk. Finally, gel disk was immerged to exchange DMSO to DI water and further incubated in DI water for 24 h before characterizations described below.

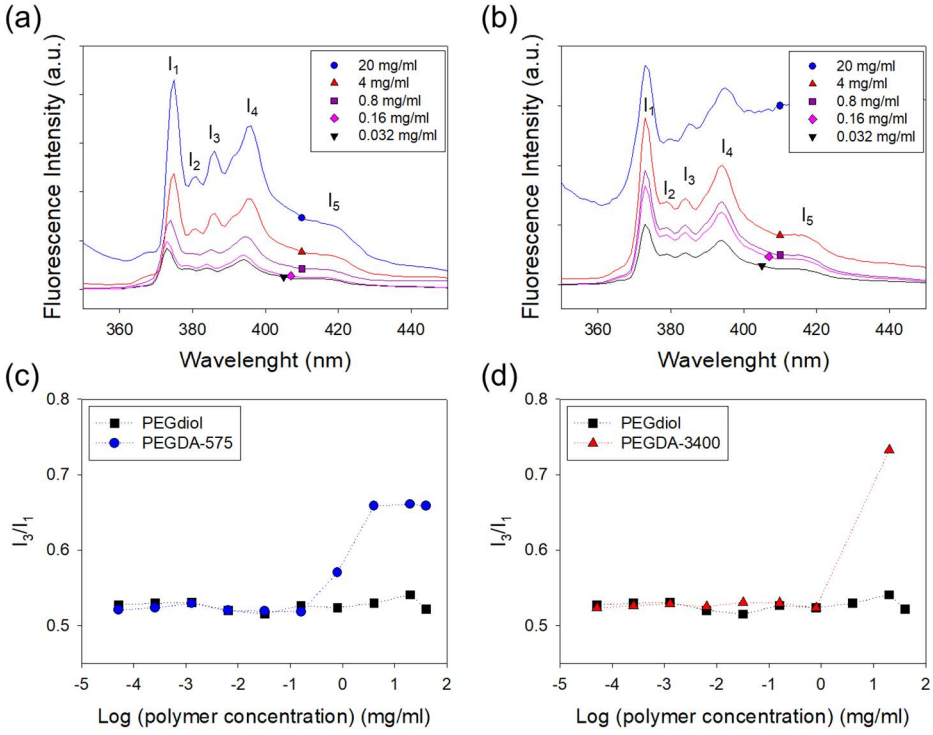

Figure 2. Fluorescence emission spectra of pyrene loaded in the (a) PEGDA-575 solution, (b) PEGDA-3400 solution were captured at various polymer concentrations. The ratio of third-to-first vibrational fine structure (I_3/I_1) in PEGDA increased while that of PEGdiol kept constant. The increase of I_3/I_1 ratio of pyrene in the presence of polymer indicated that polymers formed aggregation. In (c) and (d), ● represents the solution of PEGDA-575, ▲ the solution of PEGDA-3400, and ■ the solution of PEGdiol.

2.3. Hydrogel characterization

The swelling ratio of the hydrogel at equilibrium was determined by measuring the weight of the hydrated gel and that of the dried gel. The degree of swelling (Q), defined as the reciprocal of the volume fraction of a polymer in a hydrogel (v_2), was calculated from the following equation (1),

$$Q = v_2^{-1} = \rho_p \left[\frac{Q_m}{\rho_s} + \frac{1}{\rho_p} \right] \quad (1)$$

where ϱ_s is the density of water, ϱ_p is the density of polymer and Q_m is the swelling ratio, the mass ratio of swelled gel to the dried gel.

The average pore size (ξ) of hydrogel was calculated from the polymer volume fraction ($v_{2,s}$) and the unperturbed mean-square end-to-end distance of the monomer unit (r_o^2) using equation (2) and (3):

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$$\xi = (\nu_{2,s}^{-1/3})(r_0^{-2})^{1/2} \quad (2)$$

$$(r_0^{-2}) = l(2\frac{\bar{M}_c}{\bar{M}_r})^{1/2}\, C^{1/2} = l(2n)^{1/2}C^{1/2} \quad (3)$$

where l is the average value of the bond length between C–C and C–O bonds in the repeatable unit of PEG [–O–CH_2–CH_2–], which is taken as 1.46 Å ; M_c is the average molecular mass between cross-links in the network; M_r is the molecular mass of the PEG repeating unit; n is the number of repeat unit, which is taken as 7 (PEGDA M_n of 575 g/mol); C is the characteristic ratio for PEG, which is taken here as 4 [14].

The inner micro-structures of the prepared final hydrogels were analyzed with SEM (FE-Scanning electron microscope, JEOL-7001F). Values of the water contact angle (θ_w) on the surfaces of the hydrogels were measured by depositing a drop of water (4.0 μL) under atmospheric condition with DSA100 (KRÜSS). Also, bovine serum albumin (BSA, Sigma) was used as a model protein to evaluate the protein release rate from the hydrophobically-modified hydrogels. BSA amount released from the hydrogel was quantitatively measured using Pierce™ BCA protein assay kit (Thermo Scientific) according to the manufacturer's instructions. The absorbance was measured at 562 nm using ELISA (Multiskan GO, Thermo Scientific, USA). The BSA release profile obtained was fitted to the Ritger-Peppas equation [15].

$$\frac{M_t}{M_\infty} = \mathrm{k} \cdot t^n \quad (4)$$

where M_t is the cumulative amount of protein released at the time, t; M_∞ is the total amount of protein in the hydrogels; k is the kinetic rate constant; and n is the exponent related to the release mechanism.

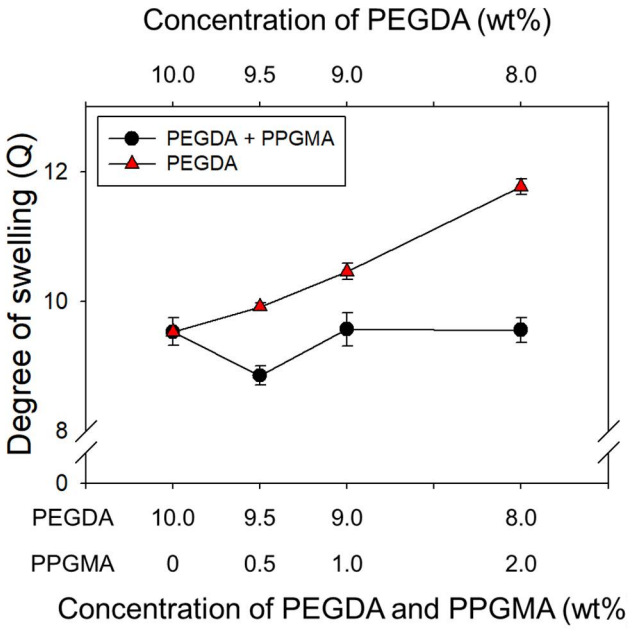

Figure 3. The degree of swelling (Q) of hydrogel was calculated 10% PEGDA-575 hydrogels with decrease mass fraction of PEGDA (▲), and with increase mass fraction of PPGMA while keeping total polymer concentration constant (●). The hydrophobic chain in the hydrogel was the major cause of decreasing degree of swelling from 10 to 9.5%.

3. Results and Discussion

3.1. Analysis of self-assembling of polymer cross-linkers in a solution

This study presents an effective method to incorporate hydrophobic domains regularly in a hydrogel by self-assembling of polymer cross-linkers. First, the self-assembled structures formed in a pre-gelled solution were confirmed with the fluorescence analysis. The hydrophobic association between the polymer cross-linkers with acrylate groups which are slightly hydrophobic was examined with a ratio of the third-to-first vibrational fine structure (I_3/I_1) in the fluorescence spectrum of pyrene prove (Fig. 2). Generally, the I_1 peak arises from the transition that can be enhanced by the distortion of the π-electron cloud [12,13]. Therefore, as the microenvironment of pyrene becomes more polar, the I_1 peak becomes more notable at the expense of other peaks (I_3). That means the ratio of I_3/I_1 represents the degree of self-assemblies between acrylate groups linked to the polymer cross-linkers. I_3/I_1 of the PEGDA polymer cross-linker solutions increased as the PEGDA concentration exceeded a critical concentration (critical aggregation concentration, CAC) which means the self-assemblies are formed at this point, as shown in Figure 2. In contrast, I_3/I_1 of the PEGdiol polymer without acrylate groups was independent of PEGdiol concentration. The I_3/I_1 of the PEGdiol solution was approximately 0.5, which is characteristic value for pyrene dispersed in water. Therefore, this fluorescent analysis demonstrated that polymer cross-linkers with acrylate groups are self-assembled in an aqueous solution because of the hydrophobic association between acrylate groups [13].

Table 1. Composition and characterization of the hydrophobically-modified hydrogel.

	Composition		Theoretical pore-size (Å)	Experimental degree of swelling	Contact angle (°)
	[2]PEGDA	[3]PPGMA			
[1]HMH-1	10.0	0.0	23.16	9.53 ± 0.21	44.30 ± 4.00
HMH-2	9.5	0.5	23.47	8.85 ± 0.15	53.40 ± 0.96
HMH-3	9.0	1.0	23.89	9.56 ± 0.26	54.84 ± 0.30
HMH-4	8.0	2.0	24.85	9.55 ± 0.19	56.87 ± 1.05

[1] Hydrophobically modified hydrogel.
[2] Poly(ethylene glycol) diacrylate (wt%).
[3] Poly(propylene glycol) mathacrylate (wt%).

3.2. Effects of the hydrophobic domains incoported into a hydrogel

The hydrophobically-modified hydrogels were prepared *via* in situ radical polymerization of self-assembled PEGDAs and PPGMAs with varying of mass fraction. As pointed out above, the concentration of polymer cross-linkers used to form hydrogel was higher than the CACs of cross-linkers as shown in Fig. 2(c). Figure 3 shows that in general, the degree of swelling (Q) of hydrogels increases as the concentration of pure PEGDAs decreases due to the increase of average pore-size (ξ) of hydrogel. For example, 2.31 nm of ξ in 10 wt% of PEGDAs increases to 2.48 nm of that in 8.0 wt% of PEGDAs as shown in Table 1, calculated using the eqn. (1). However, interestingly, in case of the hydrophobically-modified hydrogels, the Q of hydrogel was decreased with increasing PPGMA portion from 0 to 0.5 wt%, despite of the decrease of PEGDA concentration. And then, the Q was increased with increasing PPGMA from 0.5 to 2.0 wt%. These results indicated that PPGMA regulates the degree of swelling of the hydrogel at a mass fraction of less than 0.5 wt% of PPGMA as a hydrophobic repulsion. In contrast, above 0.5 wt% of the mass fraction of PPGMAs, the decreased mass fraction of PEGDAs has a predominant role to the Q of hydrogel. Note that PPGMAs are not related to regulate the ξ of hydrogel, because PPGMA molecule have a single acrylate group which is not acting as a cross-linker. However, the ξ of hydrogel from 0 to 0.5 wt% of PPGMAs was decreased despite of the decrease of PEGDAs' concentration. As addressed above, this result is attributed to hydrophobic repulsion by PPGMA.

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The inner micro-structures of hydrophobically-modified hydrogels (HMHs) were examined with freeze-dried gels by SEM. Figure 4 shows the images of hydrogels depending on the introduced amounts of PPGMAs. Structural difference between pure PEGDA hydrogel (HMH-1) and HMH-2 at 0.5 wt% of PPGMA was not significant and their 3D networked microstructures were both well formed. However, HMH-4 at 2.0 wt% of PPGMAs exhibited relatively large hydrophobic micro-domains (Fig. 4a-3 & 4b-3). It was seem to be taken place of a phase separation between hydrophilic PEGDA and hydrophobic PPGMA molecules. It means that a certain amount of PPGMA in hydrogel has functioned as hydrophobic repulsion. Hydrophobic parts, PPGMAs over a certain amount which is likely the loading capacity of the self-assemblies of PEGDAs in pre-gelled solution, caused the phase separation. Also, water contact angles (θ_w) measured at the surface of hydrogel increased with increasing of incorporating PPGMAs (Fig. 4). As a result, the hydrophobicity of a hydrogel could be tuned by incorporating the hydrophobic motifs into the self-assembled structures in a pre-gelled solution.

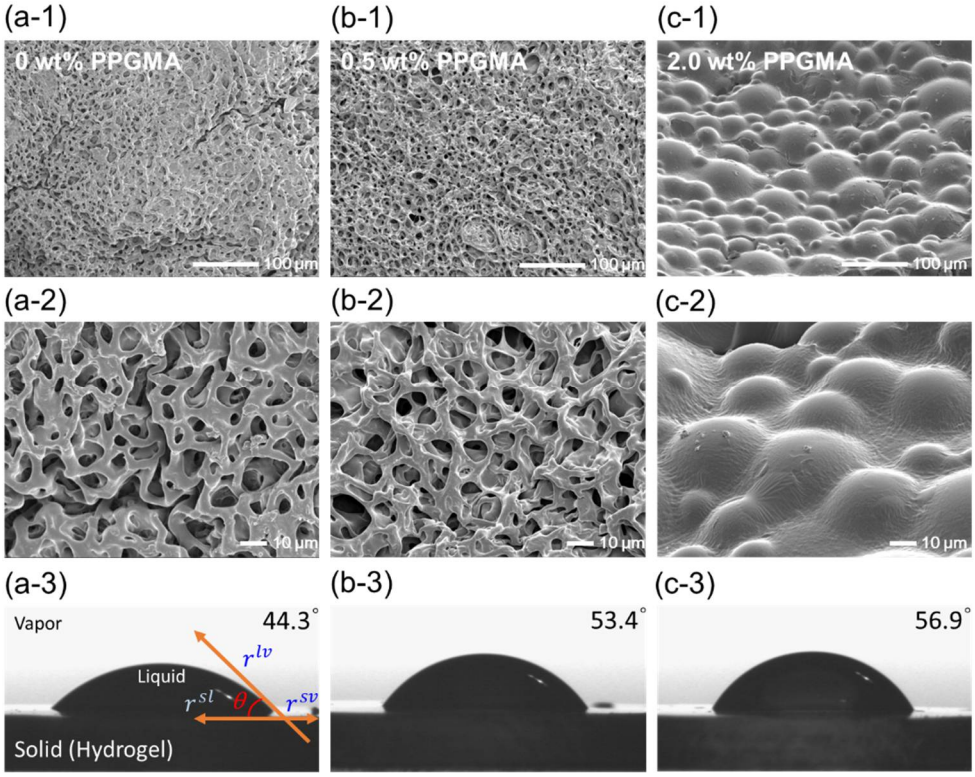

Figure 4. FE-SEM images and water contact angles of (a) pure PEGDA hydrogel (HMH-1), hydrophobically-modified hydrogel, HMH-2 (b), and HMH-4 (c) at 2.0 wt% of PPGMAs.

BSA was encapsulated into the HMHs using PEGDA-3400 to evaluate the effects of the hydrophobic motifs on protein release rate. The release rate of BSA from the HMH gel was quantified by measuring the amount of BSA released into the incubation media on a daily basis over ten days (Fig. 5). Increasing the hydrophobicity from 0 to 2 wt% of PPGDAs significantly decreased the amount of BSA initially released from the HMH gels (Fig. 5(a)). The cumulative mass fraction of BSA released from HMHs over 12 hours, M_t/M_∞, was fitted with the Ritger-Peppas equation to calculate a kinetic rate constant (k) (Fig. 5(b)). The k of the HMHs decreased by 30% as PPGMAs was increased from 0 to 2 wt%. The hydrogels incorporated with hydrophobic motifs would be useful to study for releasing and secreting of soluble and insoluble factors in the cellular microenvironment. In summary, the self-assemblies formed with polymer cross-linkers in a pre-gelled solution could be a place of incorporation of hydrophobic motifs regularly and stably in thermodynamics upon the critical range. However, above this critical point, the self-assemblies

would not provide enough room for the hydrophobic parts, so that the phase will be separated and consequently irregular hydrophobic macro-domains in size and arrangement will be generated.

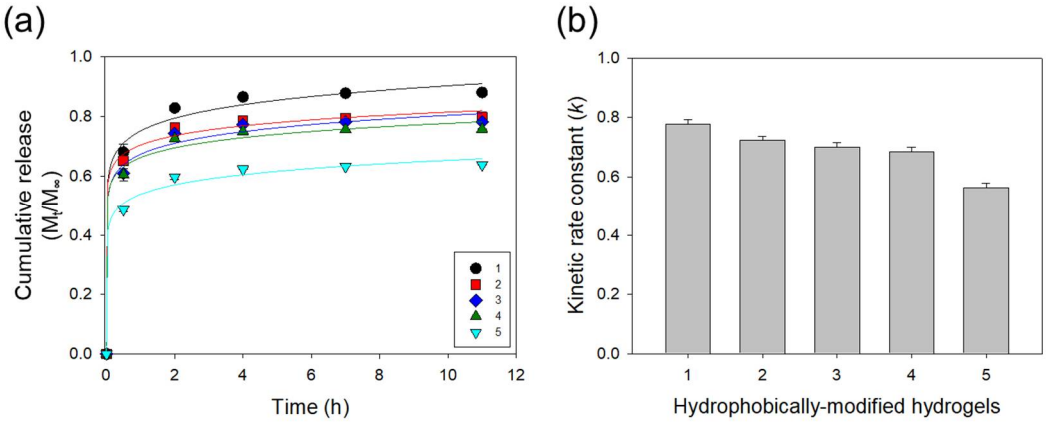

Figure 5. (a) Cumulative release profiles of BSA from the hydrogels. The solid lines represent the fitting curves used to quantify the kinetic rate constant (*k*) of release presented in (b).

5. Conclusions

Taken together, this study presents a new strategy to incorporate hydrophobic domains regularly in a hydrogel by self-assembling of polymer cross-linkers, building blocks of a hydrogel. Self-assemblies between polymer cross-linkers were examined as micro-domains to incorporate hydrophobic motifs in a hydrogel. The self-assembled structures in a pre-gelled solution were confirmed with the fluorescence analysis and the hydrophobicity of a hydrogel could be tuned by incorporating the motifs in a controlled manner. Overall, the results of this study would greatly serve to tuning performance of a wide array of hydrophobically-modified hydrogels in drug delivery, cell therapies and tissue engineering.

Author Contributions: Conceptualization, H.J. King, H.W. Ryu, J.H. Jeong; methodology, H.J. Kim, S. Cho, J.H. Jeong; formal analysis, H.J. Kim, S.J. Oh, S.G. Shin; data curation, H.J. Kim, S.J. Oh, J.H. Jeong; writing—original draft preparation, H.J. Kim, J.H. Jeong; writing—review and editing, X.X.; project administration, J.H. Jeong; funding acquisition, J.H. Jeong

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Conflicts of Interest: The authors declare no conflict of interest.

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