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Development of Lignin Supramolecular Hydrogels with Mechanically Responsive and Self-Healing Properties

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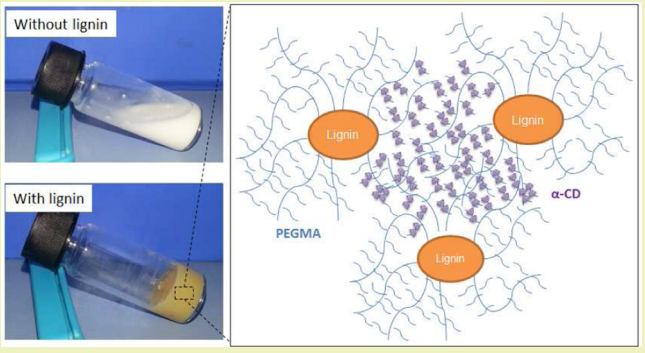
Supporting Information

ABSTRACT: The development of functional polymers from renewable lignin is attractive due to the depletion of fossil fuel and increasing environmental usage. A series of poly(ethylene glycol) methyl ether methacrylate (PEGMA)-grafted lignin hyperbranched copolymers were prepared by atom transfer radical polymerization (ATRP). The chemical structures, molecular characteristic and thermal properties of these copolymers were evaluated and such copolymers were prepared in a range of molecular weights from 38.7 to 65.0 kDa by adjusting the PEGMA-to-lignin weight ratio. As a result from their hyperbranch architecture, their aqueous solutions were found to form supramolecular hydrogels with a very low critical gelation concentration of 1 wt % copolymers, in the presence of α-cyclodextrin (α-CD). The rheological properties of the supramolecular assemblies were investigated and these hydrogel systems showed tunable mechanical response and excellent self-healing capability. Combined with good biocompatibility, these new types of green supramolecular hydrogels based on lignin−PEGMA/cyclodextrin inclusion are potentially useful as a smart biomaterial for biomedical application.



KEYWORDS: *Biomass, Cyclodextrin, PEGMA, Self-assembly, Rheology*

INTRODUCTION

Lignin is the second most abundant renewable biopolymer on the planet, surpassed only by cellulose. It is massively produced as a byproduct from papermaking and emerging cellulosic ethanol industries.[1] Annually, more than 50 million tons of lignins are produced but only less than 2% are used in value-added applications, including concrete additives, stabilizing agents as well as dispersants and surfactants, whereas the rest is used as low grade burning fuel. Because of the large consumption of fossil fuels and their negative influences on environment, lignin has been selected as a potential substitute for fossil fuel-based products. Extreme efforts have been put to develop lignin-based value added materials because of its abundant availability and renewable resources.[2] Moreover, lignin also provides various advantages, such as adequate reactive groups that can easily be functionalized, high carbon content, low density, biodegradability, antioxidant, antimicrobial and stabilizing properties, thereby making it a potential candidate to be used in diverse industrial applications.[3]

Lignin is a randomly cross-linked network biopolymer arising from enzymatic dehydrogenative polymerization of hydroxylated and methoxylated phenylpropane unit. Recently, there has been increasing interest in utilizing lignin's hydrophobic polyol structure to develop novel lignin-based functional materials.[4] Prior attempts to synthesize such polymers include using free radical polymerization, condensation polymerization and even chemo-enzymatic approach.[5−11] Atom transfer radical polymerization (ATRP) is an advanced "living" radical polymerization method to synthesize polymers with predesigned compositions, topologies and functionalities.[12−15] ATRP has proved to be a useful method to develop lignin-based functional materials. Different polymers, including poly(*N*-isopropylacrylamide), polystyrene and poly(methyl methacrylate), have been successfully grafted to lignin.[16−19] This polymerization approach offers several advantages, including the possibility to modulate molecular weight of grafted chains and the number of grafts per macroinitiator.

Supramolecular hydrogels are physical networks self-assembled by biocompatible gelators with macromolecules via noncovalent interactions, including hydrogen bonding, hydro-

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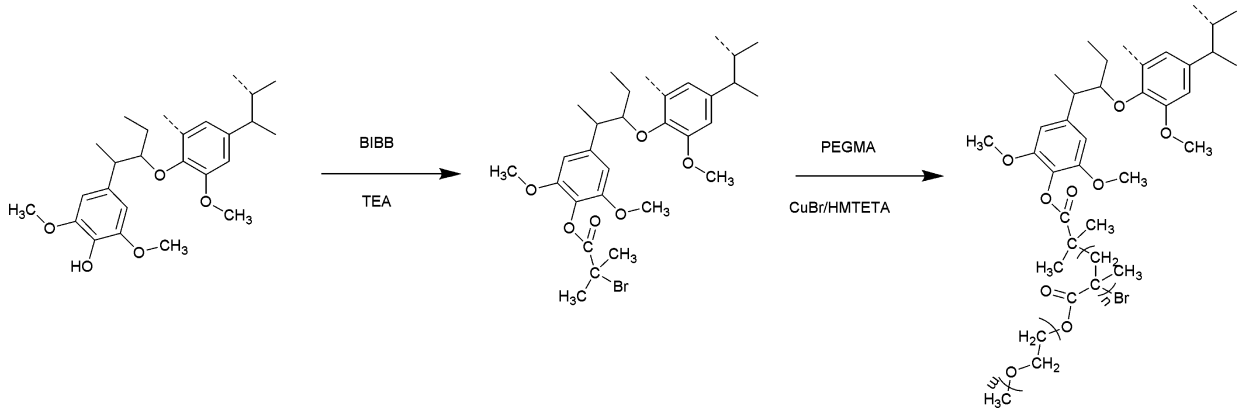
Scheme 1. Synthetic Route for Lignin−PEGMA Copolymers



phobic interactions, host−guest recognition and crystallization.[20−30] The supramolecular hydrogels based on the inclusion complexation of α-cyclodextrin (α-CD) with poly(ethylene glycol) (PEG)-grafted polymers are interesting due to their advanced properties and wide potential applications.[31−33] Although extensive studies have been carried out on PEG/α-CD hydrogels,[34−45] there is no research on the formation of hydrogels from α-CD and PEGMA-grafted lignin. The unique structure and properties of lignin remarkably influences the gelation and self-assembly behaviors of α-CD/lignin pseudopolyrotaxane hydrogels. In this paper, we designed and synthesized a series of poly(ethylene glycol) methyl ether methacrylate (PEGMA)-grafted lignin hyperbranched copolymers via ATRP (Scheme 1), which were able to form supramolecular hydrogels with α-CD. The thermal properties and molecular weights of the lignin−PEGMA copolymers were characterized and also the supramolecular hydrogels fabricated based on these copolymers and α-CD were prepared and investigated by rheological studies.

MATERIALS AND METHODS

Materials. All chemicals were purchased from Sigma-Aldrich Chemicals and used as received except where noted. Kraft lignin (obtained from kraft pulping, Product No.: 370959, M_n = 5000 g/mol, M_w = 28 000 g/mol) was dried at 105 °C overnight before use. Poly(ethylene glycol) methyl ether methacrylate (PEGMA, average M_n = 1100 g/mol) was purified by dissolving into anhydrous tetrahydrofuran (THF) and passing through a column with inhibitor remover before use. The remaining THF was then removed by a rotary evaporator and vacuum-drying.

Synthesis of Lignin ATRP Macroinitiators (Lignin−Br). Lignin (Alkali, 3.0 g, 0.6 mmol, contains −OH 22.3 mmol) was weighed in a reaction flask. Subsequently, anhydrous *N*,*N*-dimethylacetamide (DMA, 30 mL) was injected into the flask to dissolve the lignin under rapid stirring. Then triethylamine (TEA, 53.5 mmol, 7.46 mL) was added into lignin solution. After that, 10 mL of anhydrous DMA containing 2-bromoisobutyryl bromide (BIBB, 44.6 mmol, 5.51 mL) was added dropwise into the lignin solution under rapid stirring over 1 h in an ice−water bath. The reaction mixture was stirred for 1 day at room temperature and under nitrogen atmosphere. After that, the reaction mixture was centrifuged and the supernatant was precipitated with 500 mL of ether. The tan gel-like precipitate was redissolved into THF (50 mL) and the solution was then precipitated with 600 mL of ether. The brown powder of lignin macroinitiator was collected and dried under vacuum at 40 °C. The number of initiator sites on lignin was determined by ^{1}H NMR (Figure 1B) in DMSO: δ (ppm) 1.4−2.2 (−CH_3 of initiation group), 3.5−4.3 (−CH_3O−), 6.0−8.0 (aromatic protons of lignin).

Synthesis of Lignin−PEGMA Graft Copolymers. In a typical example, lignin−Br (130 mg, 0.3 mmol Br), PEGMA (1 g), 1,1,4,7,10,10-hexamethyltriethylenetetramine (HMTETA, 83 mg, 0.36 mmol) and 10 mL of degassed acetone were added into a dry flask. The mixture was stirred at room temperature and purged with dry nitrogen for 20 min. After that, CuBr (43 mg, 0.3 mmol) was added and the mixture was purged with dry nitrogen for another 10 min at room temperature. The mixture was continued to stir for overnight at room temperature. The experiment was then stopped by opening the flask and exposing the catalyst to air. The final tan mixture was diluted with THF and passed through a short neutral Al_2O_3 column with THF as eluent to remove copper catalyst. The resulting eluate solution was concentrated to 10 mL and precipitated with 1000 mL hexane. The brown product, Lig-PEG1 was collected by centrifugation and dried under vacuum at 40 °C. A series of lignin−PEGMA graft copolymers with different compositions of PEGMA were prepared under similar condition, as shown in Table 1.

Characterization of Lignin−PEGMA Graft Copolymers. Lignin, lignin−Br and lignin−PEGMA were characterized by ^{1}H NMR (NMR Bruker 400 MHz, USA). Deuterated chloroform ($CDCl_3$) and deuterated dimethyl sulfoxide (DMSO-d_6) were used as a solvent to dissolve synthesized materials.

Molecular weight and polydispersity index of polymer samples were analyzed by gel permeation chromatography (GPC, a Shimadzu SCL-10A and LC-8A system equipped with two Phenogel 5 μm 50 and 1000 Å columns in series and a Shimadzu RID-10A refractive index detector). THF was used as eluent at a flow rate of 0.30 mL/min at 40 °C. Monodispersed poly(ethylene glycol) standards were used to obtain a calibration curve.

Thermogravimetric analysis (TGA) was carried out on a thermogravimetric analyzer (Q500, TA Instruments, USA). Samples were heated at 20 °C/min from room temperature to 700 °C in a dynamic nitrogen atmosphere (flow rate = 60 mL/min).

Differential scanning calorimetry (DSC) thermal analysis was performed on a DSC (Q100, TA Instruments, USA) equipped with an autocool accessory and calibrated using indium. The following protocol was used for each sample: heating from room temperature to +180 °C at 20 °C/min, holding at +180 °C from 5 min, cooling from +180 to −20 °C at 20 °C/min, and finally reheating from −20 to +180 °C at 20 °C/min. Data were collected during the second heating run.

Preparation of Hydrogels. A weighed amount of lignin−PEGMA copolymers was added to PBS under sonication. After the copolymer was dissolved, α-CD solution (in PBS) was added into the lignin−PEGMA solution and the mixed solutions were left to stand at room temperature (25 °C) or body temperature (37 °C) without any sonication. The detailed composition of each hydrogel is given in Table 3. Gel formation was confirmed by using the inverted-tube test.

Rheological Analysis. Viscoelastic properties of lignin/ α-CD hydrogels were evaluated at 37 °C in a Discovery Hybrid Rheometer 3 (TA Instrument, USA) fitted with 20 mm parallel-plate geometry. The test methods employed were oscillatory amplitude sweeps at a

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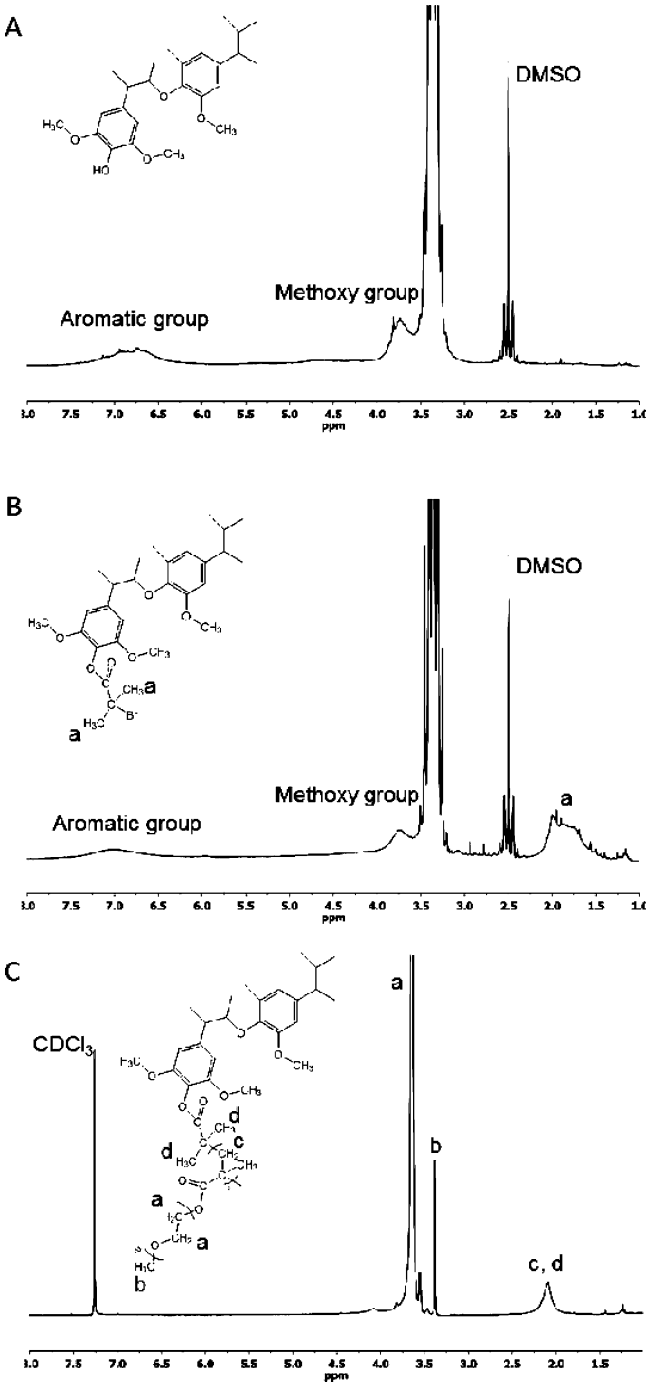

Figure 1. ^{1}H of NMR spectra of (A) lignin, (B) lignin−Br and (C) lignin−PEGMA.

constant frequency of 1 Hz. The storage (G') and the loss (G'') moduli were recorded while the strain increased from 0.01% to 100%. A frequency sweep test was also conducted on each sample to determine their viscoelastic behaviors at a constant oscillation stain of 0.1% and over a frequency range of 0.1 to 100 Hz.

The self-heal ability of lignin/ α-CD hydrogels was investigated by assembling the hydrogels at 37 °C and 1 Hz under a small strain of 0.1% for 300 s (Step A). After step A, a large strain of 10% was applied for 150 s under same temperature and frequency (Step B). After that, steps A and B were repeated alternatively 4 times.

Cell Viability Assay. MTT assays were performed to assess the metabolic activity of human dermal fibroblasts. Human dermal fibroblasts were seeded in 96-well plates (Costar, USA) at a density of 1×10^4 cells/mL. After 24 h of incubation, the medium was replaced by the Lig-PEG1, Lig-PEG2, Lig-PEG4, α-CD and P(PEGMA) (M_n = 18 000 g/mol) aqueous solutions at concentrations of 10, 5 and 1 μM, respectively. The cells were then incubated for 24, 48 and 72 h, respectively. After the designated time intervals, the wells were washed twice with 1 × PBS buffer, and 100 μL of freshly prepared MTT (0.5 mg/mL) solution in culture medium was added to each well. The MTT medium solution was carefully removed after 3 h incubation in the incubator. DMSO (100 μL) was then added into each well, and the plate was gently shaken for 10 min at room temperature to dissolve all precipitates formed. The absorbance of MTT at 570 nm was monitored by the microplate reader (Genios Tecan, Switzerland). Cell viability was expressed by the ratio of absorbance of the cells incubated with polymer solution to that of the cells incubated with culture medium only.

RESULTS AND DISCUSSION

Synthesis of lignin−PEGMA Copolymers. The PEGMA−graft lignin copolymers were synthesized through ATRP reaction, as shown in Scheme 1. Representative NMR data for unmodified lignin and lignin−Br are shown in Figure 1A,B, respectively. The natural polyhydroxyl aromatic lignin containing hydroxyl groups was readily modified by BIBB through esterification reaction. Compared to the ^{1}H NMR spectra of unmodified lignin (Figure 1A), Figure 1B confirms the formation of 2-bromoisobutyryl ester on lignin, as there were characteristic chemical shifts at 1.4−2.2 ppm corresponding to the methyl protons of the initiating sites derived from both phenolic and aliphatic alcohols. FTIR analysis of lignin−Br also showed the incorporation of the bromoisobutyryl ester moiety, as evident from the C═O and C—O stretching vibrations at 1750 and 1260 cm^{-1}, respectively. (Supporting Information Figure S1) The concentration of initiator sites (bromoisobutyrate groups) on unit weight of lignin was calculated by adding the internal standard styrene in ^{1}H NMR solution. The synthesized lignin macroinitiator had 2.3 mmol of initiator sites per gram of material. The lignin−Br was soluble in chloroform, THF and acetone, also indicating the successful modification of lignin.

PEGMA was grafted onto the lignin macroinitiator to form lignin−PEGMA copolymers via ATRP reaction. Figure 1C shows the ^{1}H NMR spectra of the lignin−PEGMA copolymer

Table 1. Molecular Characteristics of Lignin−PEGMA Copolymers

	feed ratio					
polymers	lignin−Br (g)	PEGMA (g)	M_n (g/mol)[a]	M_w (g/mol)[a]	polydispersity[a]	mass % of lignin[b]
lignin			5000	28,000	5.60	
Lig-PEG1	0.13	1	38,700	48,000	1.24	12.9
Lig-PEG2	0.13	2	49,100	59,200	1.20	10.2
Lig-PEG4	0.13	4	65,000	71,600	1.23	7.7

[a]Determined by GPC. [b]Determined by GPC based on the molecule weight of lignin (5000 g/mol).

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(6.1% lignin). Because of lignin being present in a very small mass fraction, the lignin peaks for the grafted material are difficult to see. However, characteristic peaks of PEGMA were shown at 3.3 and 3.7 ppm corresponding to methyl and methylene protons from PEGMA. The FTIR and ^{13}C NMR data also showed the characteristic peaks of PEGMA in the spectra (Supporting Information Figures S1 and S2), indicating the successful grafting of PEGMA onto lignin. The molecular weights of the copolymers are summarized in Table 1. The unmodified lignin and PEGMA monomer had molecular weights of 5 and 1.1 kDa, respectively. The copolymers showed low polydispersities (about 1.2), and their molecular weights varied according to the feed ratio of lignin:PEGMA. The M_n of copolymers increased from 38.7 kDa for Lig-PEG1 to 65.0 kDa for Lig-PEG4. On the basis of the molecular weight of lignin, the contents of lignin in the copolymers were calculated and mass % of lignin were ranged from 7.7 for Lig-PEG4 to 12.9% for Lig-PEG1. Therefore, both the NMR and GPC results demonstrated the successful synthesis of the lignin−PEGMA copolymers.

Thermal Characterization of Lignin−PEGMA Copolymers. The thermal properties of lignin and lignin−PEGMA copolymers were characterized by DSC and TGA (Table 2).

Table 2. Thermal Properties of Lignin and Lignin−PEGMA Copolymers[a]

polymers	T_m (°C)	enthalpy (J/g)	T_d (°C)	T_p (°C)	weight % remained at 500 °C	mass % of lignin[b]
lignin	N.A.	N.A.	260	349	58.0	
Lig-PEG1	33	88	352	415	8.2	14.1
Lig-PEG2	35	94	360	416	4.8	8.2
Lig-PEG4	35	103	355	412	3.4	5.8

[a] T_m is melt temperature determinated by DSC. T_d, thermal decomposition temperature, is defined as the temperature at which the mass of the sample is 5% less than its mass measured at 50 °C. T_p is the derivative peak temperature. [b] Determined by TGA based on weight % remained at 500 °C.

Unmodified lignin did not have a melting temperature (T_m) or enthalpy, whereas the lignin−PEGMA copolymers exhibited their T_m at ∼34 °C resulting from the melting of the PEGMA chains (Supporting Information Figure S3). The melting peaks became larger and clearer with the increasing PEGMA contents, and also their enthalpies increased from 88 g/mol for Lig-PEG1 to 103 g/mol for Lig-PEG4 with the increasing contents of PEGMA.

The thermal stability of lignin and lignin−PEGMA copolymers was investigated by TGA under N_2 atmosphere (Table 2 and Figure 2). The unmodified lignin thermally decomposed slowly and showed 5% of the weight loss (thermal decomposition temperature, T_d) at 260 °C. Lignin's aromatic chemical structure gave a very high char yield (around 40 wt % at 500 °C). Compared to lignin, the lignin−PEGMA copolymers showed higher T_d values (above 350 °C for 5% of the weight loss), and their derivative peak temperatures (T_p) increased to about 415 °C. Lig-PEG1, Lig-PEG2 and Lig-PEG4 remained 8.2, 4.8 and 3.4% of their original weights. As PEG segments were completely degraded at 500 °C, the residuals of the lignin−PEGMA copolymers were the remaining lignin. After calculation, the mass % of lignin in the copolymers ranged

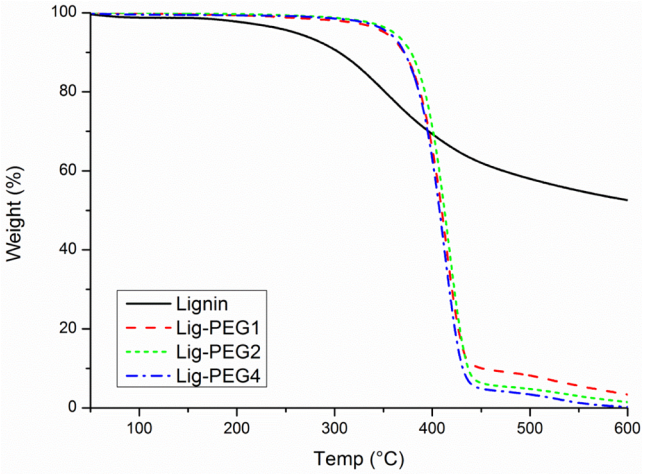

Figure 2. TGA curves of lignin and lignin−PEGMA copolymers.

from 5.8% for Lig-PEG4 to 14.1% for Lig-PEG1, in agreement with the results calculated by GPC. Overall, the grafting of PEGMA onto lignin increased lignin's thermal stability, but the length of PEGMA chain did not significantly influence the thermal stabilities of the copolymers.

Hydrogel Formation between Lignin−PEGMA and α-CD. P(PEGMA) (M_n = 18 000 g/mol) synthesized by ATRP was used as control. 10% of P(PEGMA) did not render gels even mixed with very high concentration (10%) of α-CD solution, probably due to the low molecular weight of the linear PEG chains (Figure 3A). It was reported that short PEG chains were tended to be wholly included in the cavities of α-CD to form hydrophobic polypseudorotaxane, which were easily precipitated from water.[38] On the other hand, the lignin systems became turbid dispersions or brown gels as soon as the

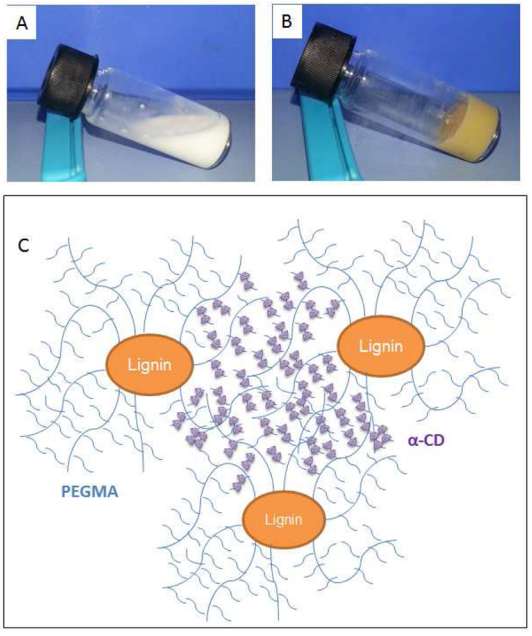

Figure 3. Photographs of (A) inclusion complex suspension of PEG 10/10 (10% P(PEGMA) + 10% α-CD) and (B) the LP1 2/10 hydrogel (2% Lig-PEG1 + 10% α-CD) and (C) schematic illustration of proposed structure of lignin-based supramolecular hydrogel by inclusion complexation between PEGMA-grafted lignin and α-CD.

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two solutions were mixed. As shown in Table 3, the systems containing 0.5% (and below) of copolymers or 5% (and below)

Table 3. Composition and Appearance of the Lignin/α-CD Formulations[a]

formulation code	polymer used	gel composition: copolymer (w/v %)	gel composition: α-CD (w/v %)	appearance of lignin/α-CD: 25 °C	appearance of lignin/α-CD: 37 °C
PEG 10/10	P(PEGMA)	10	10	sol	sol
LP1 0.5/10	Lig-PEG1	0.5	10	sol	sol
LP1 1/5	Lig-PEG1	1	5	sol	sol
LP1 1/6	Lig-PEG1	1	6	gel	gel
LP1 1/10	Lig-PEG1	1	10	gel	gel
LP1 2/6	Lig-PEG1	2	6	gel	gel
LP1 2/8	Lig-PEG1	2	8	gel	gel
LP1 2/10	Lig-PEG1	2	10	gel	gel
LP1 2/12	Lig-PEG1	2	12	gel	gel
LP1 2/14	Lig-PEG1	2	14	gel	gel
LP1 3/10	Lig-PEG1	3	10	gel	gel
LP1 4/10	Lig-PEG1	4	10	gel	gel
LP2 0.5/10	Lig-PEG2	0.5	10	sol	sol
LP2 1/5	Lig-PEG2	1	5	sol	sol
LP2 1/6	Lig-PEG2	1	6	gel	gel
LP2 1/10	Lig-PEG2	1	10	gel	gel
LP2 2/6	Lig-PEG2	2	6	gel	gel
LP2 2/8	Lig-PEG2	2	8	gel	gel
LP2 2/10	Lig-PEG2	2	10	gel	gel
LP2 2/12	Lig-PEG2	2	12	gel	gel
LP2 2/14	Lig-PEG2	2	14	gel	gel
LP2 3/10	Lig-PEG2	3	10	gel	gel
LP2 4/10	Lig-PEG2	4	10	gel	gel
LP4 0.5/10	Lig-PEG4	0.5	10	sol	sol
LP4 1/5	Lig-PEG4	1	5	sol	sol
LP4 1/6	Lig-PEG4	1	6	gel	gel
LP4 1/10	Lig-PEG4	1	10	gel	gel
LP4 2/6	Lig-PEG4	2	6	gel	gel
LP4 2/8	Lig-PEG4	2	8	gel	gel
LP4 2/10	Lig-PEG4	2	10	gel	gel
LP4 2/12	Lig-PEG4	2	12	gel	gel
LP4 2/14	Lig-PEG4	2	14	gel	gel
LP4 3/10	Lig-PEG4	3	10	gel	gel
LP4 4/10	Lig-PEG4	4	10	gel	gel

[a]Lines in the table are used to guide the reader and make it easier to compare the concentration effect and the gelation of the system.

of α-CD in PBS did not form hydrogels in both room temperature (25 °C) and body temperature (37 °C). The increase in the polymer concentration led to the gel formation within 2 min and it was found that the mixture of 1% (and above) of the copolymer solution with 6% (and above) of α-CD solution rendered hydrogels (Figure 3B). It was reported that linear PEG took a long time (several hours) to form hydrogels, and the gelation time highly depended on the molecular weight of PEG chains, polymer structure and concentration.[36] Here, our hyperbranched lignin-based copolymers were able to remarkably reduce the gelation time even at a very low polymer concentration. Only 1% of such copolymer was enough for gel formation, whereas the P(PEGMA)/α-CD only formed flowable inclusion complex emulsion in even 10% polymer concentration. Compared to a linear PEG structure, the unique complex three-dimensional network structure of lignin−PEGMA copolymers, presenting hydrophobic lignin core together with the inclusion complexes of a-CD with PEG branches, facilitated the formation of polypseudorotaxanes and accelerated self-assembly of the supramolecular hydrogels (Figure 3C). Unlike other linear PEG/α-CD hydrogels, the polypseudorotaxane formation and the association of the threaded α-CD in our lignin hydrogels almost occurred synchronously, therefore leading to the rapid self-assembly of a three-dimensional network.

Rheological Studies of Lignin/α-CD Hydrogels. To investigate the effects of the amounts of α-CD, lignin−PEGMA copolymer and their molecular weights on the viscoelastic properties of the supramolecular hydrogels, amplitude sweep measurements were carried out by a rheometer at 37 °C. Figure 4 shows the viscoelastic behavior (storage and loss moduli, complex viscosity) of lignin/α-CD hydrogels as a function of oscillation stain. Results indicated that the lignin hydrogels were mechanically responsive systems and their rheological properties varied according to the change of stress or stain. At a low oscillation stain (<0.1%), storage modulus (G') and loss modulus (G'') were constant, indicating that the gel structures were intact and undisturbed. This region is known as the linear-viscoelastic (LVE) region ($G' > G''$ or tan $\delta = G''/G' < 1$), in which the materials are highly structured and have solid-like behavior. As oscillation stain increased, G' and complex viscosity started to decrease, whereas tan δ began to increase (Supporting Information Figure S4). The materials became progressively more fluid-like and eventually G'' exceeded G' whereas tan δ values were beyond 1 with the increasing oscillation stain. The intersection point of G' and G'' ($G' = G''$ or tan $\delta = 1$) represented a transition of the hydrogel from solid-like (Gel) to liquid-like (Sol) behavior. The high Tan δ ($G' < G''$) suggested that a high stress or stain could destroy the cross-linked polypseudorotaxanes and crystalline structure in the hydrogels, resulting in largely unassociated inclusion complexes in the aqueous system.

The concentration of α-CD always affects the rheological behavior of the hydrogel systems as the polymer influences the formation of hydrophobic polypseudorotaxanes through supramolecular cross-linking. Figure 4A,B shows the rheological results of the hydrogels containing different amounts of α-CD (with the same amount of Lig-PEG1, 2%). LP1 2/6 containing only 6% of α-CD showed the lowest G', G'' and complex viscosity, and these values increased with the increase of α-CD amount up to a concentration of 10%. It was reported previously that a higher amount of α-CD favored the formation of PEG/α-CD-based supramolecular hydrogels with high mechanical properties because of the enhanced inclusion complexion.[46] Further increase in α-CD concentration did not lead to greater moduli and viscosity, but a decrease of these values was observed when the amounts of α-CD were higher than 10%. This indicated the negative effects of the excess α-CD on the cross-linking of polypseudorotaxanes.

Figure 4C,D shows the rheological properties of the hydrogels with different amounts of Lig-PEG1 (containing the same amount of α-CD, 10%). The concentration of PEG also played a significant role in the formation of supramolecular hydrogels. Inadequate PEG chains are not able to form a stable hydrogel, but an excess amount of PEG (a low α-CD:EG ratio) would result in an unstable network structure and even phase separation. In our study, 0.5% of lignin−PEGMA copolymer was not able to form hydrogel, and 1% (and above) of each copolymer could render gel after mixing with α-CD. As shown

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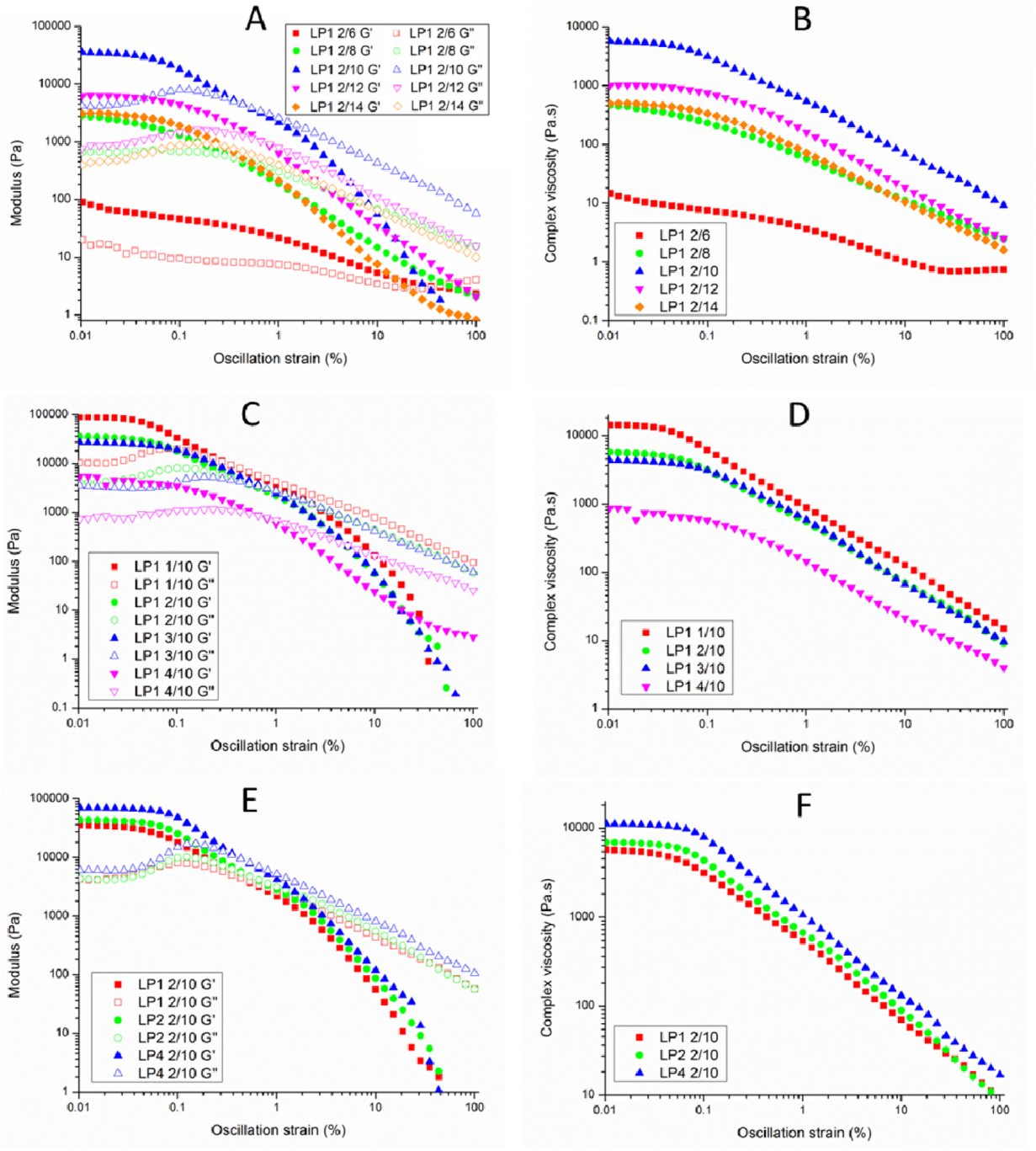

Figure 4. Dynamic rheological behaviors, (A, C, E) storage (solid symbols) and loss (open symbols) moduli, (B, D, F) complex viscosity, of lignin/α-CD hydrogels under amplitude sweep (oscillation strain from 0.01% to 100%). Panels A and B present the rheological properties of Lig-PEG1 hydrogels with different concentrations of α-CD (6% to 14%), panels C and D present the rheological properties of the hydrogels with different concentrations of Lig-PEG1 (1% to 4%) and panels E and F present the hydrogel systems made with 2% different copolymers and 10% α-CD.

in Figure 4C,D, LP1 1/10 with 1% copolymer showed the highest G', G'' and complex viscosity, and these values exhibited a decreasing trend with the increase of Lig-PEG1 amount. The explanation might be that the excess amount of copolymers hindered the stacking of the α-CD nanotubes as well as the formation of polypseudorotaxanes. Similar results were also observed in Lig-PEG2 and Lig-PEG4 hydrogels (Supporting Information Figures S5 and S6).

The chain lengths of the PEGMA segments also affected the viscoelastic properties of the hydrogels. Figure 4E,F shows the rheological properties of the hydrogels made of different copolymers (with the same polymer concentration). The Lig-PEG4 hydrogels exhibited the highest G', G'' and complex viscosity, whereas the Lig-PEG1 hydrogels displayed the lowest viscoelastic properties. The hydrogels in other concentrations showed the similar trend (Supporting Information Figure S7). It was reported that a higher ratio of PEGMA in polymers increased the cross-linking density and enhanced the moduli and viscosity of hydrogels.[47] Our results indicated that the viscoelastic properties of the mechanically responsive lignin hydrogels were easily tunable by adjusting the copolymer

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concentrations, α-CD concentrations, or even the molecular weights of the PEGMA segments.

Furthermore, the rheological properties of the hydrogels were characterized by using a frequency sweep at a strain of 0.1%. Figure 5 depicts the G' and G'' of the lignin hydrogels with different polymer concentrations and different types of the copolymers. As the strain was only 0.1%, G' of all the hydrogels were dominant over the entire frequency range. It indicated that these hydrogels exhibited a substantial elastic response, possessed a permanent network and displayed strength and rigidity. We also noticed that those stronger hydrogels with higher mechanical properties (G' and G'') tended to perform as an elastic solid and showed frequency-independent G', while the weaker systems (such as LP1 2/6, LP1 2/8 and LP1 4/10) showed a changed G' especially at high frequency. As a frequency-dependent G' is related to a fluid-like material or unstable emulsion, those weak hydrogels tended to lose their cross-linked networks under high frequencies. The complex viscosities of all the hydrogels decreased gradually and linearly with the increasing oscillation frequency, confirming that the polymers were dispersed uniformly in the medium and formed stable network systems (Supporting Information Figure S8).

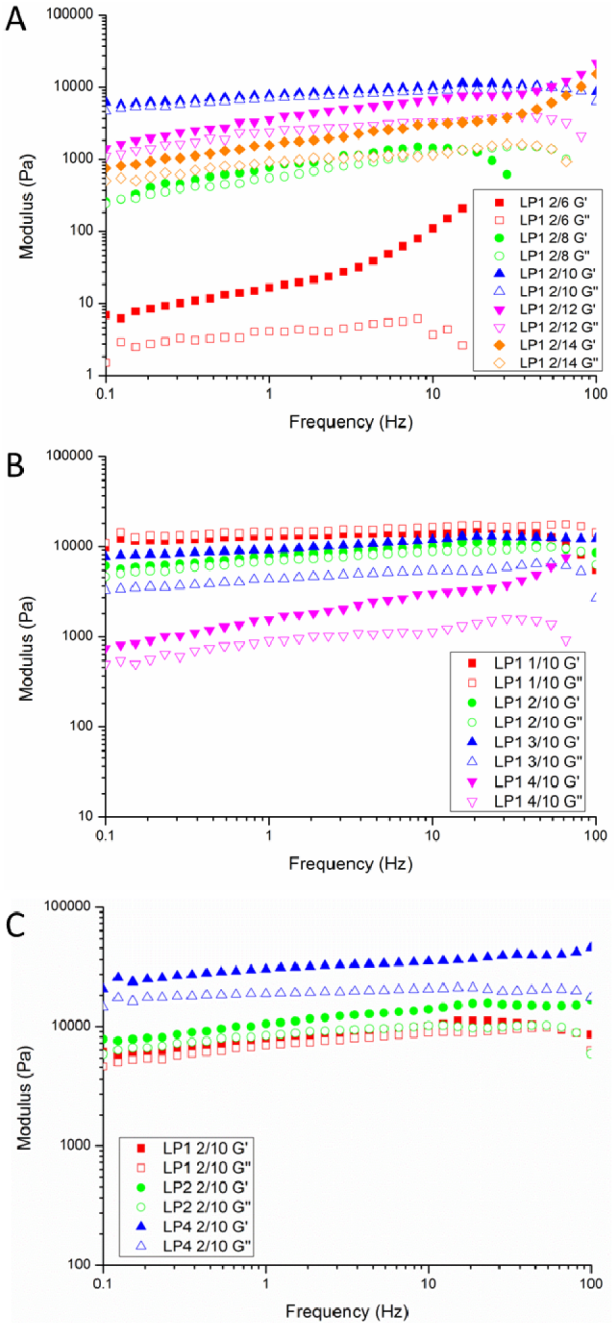

Figure 5. Dynamic rheological behaviors, storage (solid symbols) and loss (open symbols) moduli; of the lignin/α-CD hydrogels under frequency sweep (oscillation frequency from 0.1 to 100 Hz). (A) Lig-PEG1 hydrogels with different concentration of α-CD (6% to 14%), (B) the hydrogels with different concentration of Lig-PEG1 (1% to 4%) and (C) the comparison of the hydrogel systems made of different copolymers.

Self-Healing of Lignin/α-CD Hydrogels. A preliminary investigation of the potential of these lignin/α-CD hydrogels to self-heal postfailure was performed by a rheological test. The hydrogels were first set for 300 s at 37 °C under a small oscillation strain of 0.1% and a frequency of 1 Hz, then followed by the application of a large oscillation strain of 10% for 150 s to fail, and the procedures were repeated 3 more times. Figure 6 shows the typical self-healing curves of LP1 2/10, LP2 2/10 and LP4 2/10 hydrogels. All three systems formed gel under 0.01% of oscillation strain ($G' > G''$) at beginning and G' were constant against time. When the oscillation strain was shifted to 10%, the hydrogel networks were disturbed and the systems failed to form flowable liquid phase with G' below G''. After the gels were destroyed for 150 s, the low strain of 0.01% was reapplied. When the strain was reduced below the critical strain, the flowable sols rapidly recovered to form gel within 5 s. G' became dominant again and returned to their original values as prefailure. These self-healing behaviors were repeatable for at least four cycles, and the systems were always able to recover and form stable hydrogel structures. Among these three hydrogels, LP4 2/10 exhibited the best self-healing capability as its G' always recovered to the same values as before, even after the fourth disturbance. On the other hand, LP1 2/10 and LP2 2/10 showed relatively weaker recoverability as their G' decreased gradually compared to the original values after three cycles. Our

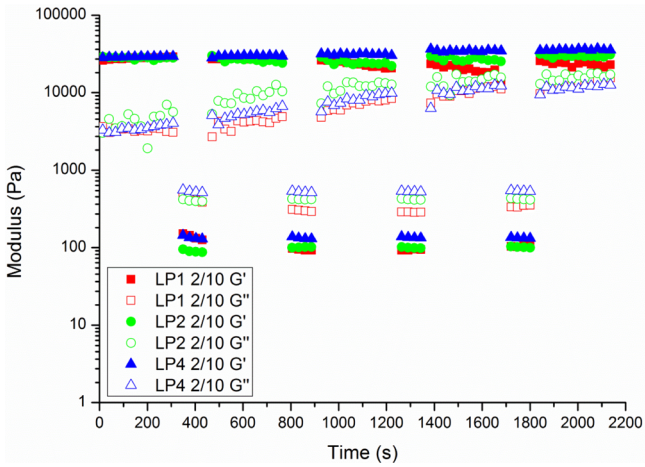

Figure 6. Self-healing of LP1 2/10, LP2 2/10 and LP4 2/10 at 37 °C under a constant frequency of 1 Hz and repeat-shifted strains of 0.01% and 10%. The solid symbols represent storage modulus, and open symbols represent loss modulus. All the three hydrogels turned into sol under 10% strain and recovered to the solid state under 0.01% strain.

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results suggested that longer chains of PEGMA would improve the self-healing capability of the hydrogels.

Recently, many different self-healing polymeric materials have been designed and reported by utilizing various noncovalent interactions and dynamic covalent bonds as a "binder".[48,49] The self-healing properties of our lignin supramolecular hydrogels are based on the reversible host−guest inclusion complexation.[21,50,51] Under high strain, the host−guest inclusion disassembles and the hydrogel was broken. When the force was removed, hydrogen bonding between the exteriors of the bound cyclodextrins formed again and led to the reformation of crystalline domains and polymer-chain cross-linking.[52−54] It is a big advantage that the self-healing process of the lignin supramolecular hydrogels occurs autonomously without any external treatment. Similarly, Harada et al. developed self-healing hydrogels composed of a host (β-CD)-gest (adamantane) polymer and the recover efficiency of the hydrogel reached almost 100% after 24 h of healing.[55] Cooper-White et al. also reported the self-healing properties of pluronic and α-CD hydrogels and they found that it took over 120 min for the destroyed hydrogel to achieve the similar G' as before the failure.[37] In comparison, our lignin hydrogel systems only took few seconds to recover to the value before, as the lignin copolymers with multiple PEGMA side chains enhanced the host−guest interaction and accelerated the cross-linking of the supramolecular networks.

Cytotoxicity of Lignin−PEGMA Copolymers. Cytotoxicity is an essential evaluation of biocompatibility, and here we evaluated the *in vitro* cell viability of lignin−PEGMA copolymers and α-CD in different molar concentrations by MTT assay using human dermal fibroblasts. All the lignin−PEGMA copolymers exhibited excellent cell viability (>95%) even at 10 μM (Supporting Information Figure S9). With the similar cell viability to that of P(PEGMA), it is suggested that lignin in the copolymers had no cytotoxicity to the fibroblasts. It is also found that even after 72 h of culture, the cells still exhibited high metabolic activity within the polymers solutions. As both the lignin−PEGMA copolymers and α-CD showed no cytotoxicity to the human cells, we conclude that our lignin-based supramolecular hydrogels are biocompatible and are able to be used as biomaterials for multiple biomedical or healthcare applications.

CONCLUSION

Potentially biodegradable and biocompatible lignin−PEGMA copolymers, with various PEGMA molecular weights, have been successfully synthesized using ATRP. Their chemical structure and molecular characteristic were examined with GPC, NMR, which proved the hyperbranched copolymer structure. Thermal stability of lignin−PEGMA was found to be better than raw lignin. Such copolymers hold a great potential as an alternative for petroleum-based polymers. The formation of supramolecular hydrogels of the aqueous copolymers with α-CD was studied by rheology. It was found that lignin−PEGMA copolymers formed supramolecular hydrogels in α-CD solution at both room and body temperature, with the copolymers concentration as low as 1 wt %. In addition, these hydrogel systems showed mechanically responsive rheological properties and excellent self-healing capabilities. With a lignin biodegradable core, the hyperbranched supramolecular hydrogels are potentially biodegradable. The PEGMA branches are easier to recycle or excrete from body after the breakdown of lignin core. Together with their good biocompatibility, the potential applications lignin-based hydrogels would be developed in biomedical and personal care fields.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssuschemeng.5b00405.

DSC of lignin and lignin−PEGMA, dynamic rheological properties of lignin/α-CD hydrogels under amplitude sweep, complex viscosities of lignin/α-CD hydrogels under frequency sweep, cell viability of lignin−PEGMA copolymers (PDF)

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Notes

The authors declare no competing financial interest.

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