In-Situ Formation of Biodegradable Hydrogels by Stereocomplexation of PEG−$(PLLA)_8$ and PEG−$(PDLA)_8$ Star Block Copolymers

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Eight-arm poly(ethylene glycol)−poly(L-lactide), PEG−$(PLLA)_8$, and poly(ethylene glycol)−poly(D-lactide), PEG-$(PDLA)_8$, star block copolymers were synthesized by ring-opening polymerization of either L-lactide or D-lactide at room temperature in the presence of a single-site ethylzinc complex and 8-arm PEG ($M_n = 21.8 \times 10^3$ or 43.5×10^3) as a catalyst and initiator, respectively. High lactide conversions (>95%) and well-defined copolymers with PLLA or PDLA blocks of the desired molecular weights were obtained. Star block copolymers were water-soluble when the number of lactyl units per poly(lactide) (PLA) block did not exceed 14 and 17 for PEG21800−$(PLA)_8$ and PEG43500−$(PLA)_8$, respectively. PEG−$(PLA)_8$ stereocomplexed hydrogels were prepared by mixing aqueous solutions with equimolar amounts of PEG−$(PLLA)_8$ and PEG−$(PDLA)_8$ in a polymer concentration range of 5−25 w/v % for PEG21800−$(PLA)_8$ star block copolymers and of 6−8 w/v % for PEG43500−$(PLA)_8$ star block copolymers. The gelation is driven by stereocomplexation of the PLLA and PDLA blocks, as confirmed by wide-angle X-ray scattering experiments. The stereocomplexed hydrogels were stable in a range from 10 to 70 °C, depending on their aqueous concentration and the PLA block length. Stereocomplexed hydrogels at 10 w/v % polymer concentration showed larger hydrophilic and hydrophobic domains as compared to 10 w/v % single enantiomer solutions, as determined by cryo-TEM. Correspondingly, dynamic light scattering showed that 1 w/v % solutions containing both PEG−$(PLLA)_8$ and PEG−$(PDLA)_8$ have larger "micelles" as compared to 1 w/v % single enantiomer solutions. With increasing polymer concentration and PLLA and PDLA block length, the storage modulus of the stereocomplexed hydrogels increases and the gelation time decreases. Stereocomplexed hydrogels with high storage moduli (up to 14 kPa) could be obtained at 37 °C in PBS. These stereocomplexed hydrogels are promising for use in biomedical applications, including drug delivery and tissue engineering, because they are biodegradable and the in-situ formation allows for easy immobilization of drugs and cells.

Introduction

Hydrogels are highly attractive materials for use in biomedical applications, such as tissue engineering and drug delivery, because they possess good biocompatibility due to their high hydrophilicity. Recently, much effort has been directed to hydrogels that can be formed in situ under physiological conditions. In-situ gelation is preferred, because bioactive compounds and/or cells can be mixed homogeneously with the polymer solutions prior to gelation. Also, in-situ gelation allows preparation of complex shapes and applications using minimally invasive surgery. The most common in-situ-formed, chemically cross-linked hydrogels are based on photocross-linkable, (meth)-acrylate functionalized polymers.[1−4] Other groups have prepared hydrogels by disulfide bond formation[5] and Michael addition reactions between thiols and either acrylates or vinyl sulfones.[6] Physically cross-linked hydrogels have been prepared by self-assembly of polymers through several types of secondary interactions, such as hydrophobic and ionic interactions.[7−14] Cross-linking by physical interactions has several advantages over chemical cross-linking, because it avoids the use of photoirradiation, organic solvents, auxiliary cross-linking agents, and/or other reactive molecules that may damage cells or proteins to be incorporated. Recently, several research groups have shown that hydrogels can be prepared in situ from water-soluble poly(L-lactide) (PLLA) and poly(D-lactide) (PDLA)-based block copolymers, in which the physical cross-links are provided by stereocomplexation between the enantiomeric PLLA and PDLA blocks.[15−19] De Jong et al. have shown that stereocomplexed dextran−poly(lactide), dextran−PLA, graft copolymer hydrogels quantitatively release proteins over a period of 1 week with full preservation of the protein activity.[16] Moreover, in-vivo tests showed that these stereocomplexed hydrogels are biocompatible and effective tools for local IL-2 delivery.[20,21] Li et al.[18] have shown that proteins can be released from stereocomplexed PLA−PEG−PLA triblock copolymer hydrogels over a prolonged period of time (up to 15 days). These results show that in-situ-formed, stereocomplexed hydrogels are interesting materials for use in biomedical applications. Synthesis of the dextran−PLA graft copolymers, however, requires several steps, while the PLA−PEG−PLA triblock copolymer stereocomplexed hydrogels show low mechanical strength and slow gelation kinetics as compared to the dextran−PLA graft copolymers, due to a low cross-linking density. We found previously that PEG−$(PLLA)_8$ and PEG−$(PDLA)_8$ star block copolymers gelate faster and form stereocomplexed hydrogels with improved mechanical strength as compared to PLLA−

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PEG−PLLA and PDLA−PEG−PDLA triblock copolymers.[15] In this paper, the effects of PLA block length, PEG molecular weight, and polymer concentration on the temperature-dependent phase behavior, gelation kinetics, and mechanical properties of PEG−$(PLLA)_8$ and PEG−$(PDLA)_8$ star copolymer stereocomplexed hydrogels were studied. Furthermore, the gelation mechanism by stereocomplexation between PLLA and PDLA blocks was confirmed by wide-angle X-ray scattering (WAXS) measurements.

Materials and Methods

Materials. D-Lactide and L-lactide were obtained from Purac and recrystallized from dry toluene. Star PEGs ($M_{n,NMR} = 21.8 \times 10^3$, denoted as PEG21800, and $M_n = 43.5 \times 10^3$, denoted as PEG43500) were supplied by Nektar and were used as received. GPC data, provided by the producer, showed that both starting PEG21800 and PEG43500 have a low polydispersity of 1.11 and 1.12, respectively. The single site Zn-complex catalyst (Zn(Et)[SC_6H_4(CH(Me)NC_4H_8)-2]) was kindly provided by Professor G. van Koten of the University of Utrecht (The Netherlands). Dichloromethane (CH_2Cl_2) was dried over calcium hydride and distilled prior to use.

Synthesis. Eight-arm poly(ethylene glycol)−poly(L-lactide), PEG-$(PLLA)_8$, and poly(ethylene glycol)−poly(D-lactide), PEG−$(PDLA)_8$, star block copolymers were prepared at room temperature by ring-opening polymerization of L-lactide and D-lactide in CH_2Cl_2, respectively. The single site Zn-complex catalyst Zn(Et)[SC_6H_4(CH(Me)-NC_4H_8)-2] and 8-arm star PEG were used as catalyst and initiator, respectively.[15] Briefly, PEG21800 (0.730 g, 0.0335 mmol) and lactide (0.270 g, 1.88 mmol) were dissolved in 7.5 mL of CH_2Cl_2 ($[LA]_0 = 0.25$ M). To this solution was added a solution of single site Zn-complex catalyst (0.040 g, 0.134 mmol) in 1 mL of CH_2Cl_2, and the reaction mixture was stirred for 4 h. The polymerization was terminated by the addition of an excess of glacial acetic acid, and the polymer was precipitated in a mixture of cold diethyl ether/methanol (20/1 v/v). Conversion, 98%; yield, 89%. ^{1}H NMR ($CDCl_3$): δ 1.4 (m, CH_3CHOH end group PLA), 1.5 (m, CH_3CH), 3.6 (m, CH_2O), 4.2−4.3 (m, CH_2-CO, linking unit PEG), 4.3−4.4 (q, CHOH end group PLA), 5.1 (m, CHCO).

Characterization. ^{1}H NMR spectra ($CDCl_3$) were recorded on a Varian Inova spectrometer (Varian, Palo Alto) operating at 300 MHz. The average number of lactyl units per poly(lactide) (PLA) block was calculated on the basis of the methyl protons of lactyl units at $\delta = 1.5$ and the methylene protons of PEG at $\delta = 3.6$. Cloud point measurements were performed on a homemade light scattering setup at 670 nm using a polymer concentration of 5 w/v % in water. The samples were heated from 20 to 60 °C at a heating rate of 1 °C/min. Critical association concentrations (CACs) were determined at 20 °C with the hydrophobic dye solubilization method using 1,6-diphenyl-1,3,5-hexatriene (DPH).[22] UV/vis absorption spectra were recorded in the 300−500 nm range using a Cary 300 Bio UV−visible spectrophotometer (Varian). Dynamic light scattering experiments were performed on a Zetasizer 4000 (Malvern), and the data were analyzed by the CONTIN method. Critical gel concentrations (CGCs) were determined as described before.[22] Briefly, PEG21800−$(PLA)_8$ and PEG43500−$(PLA)_8$ star block copolymer solutions were prepared with concentration increments of 2.5 and 1 w/v %, respectively, by dissolving the polymers overnight. Subsequently, polymer solutions containing equimolar amounts of PEG−$(PLLA)_8$ and PEG−$(PDLA)_8$ star block copolymers were mixed and equilibrated overnight. The critical gel concentrations were determined by inverting the vials. When the sample showed no flow within 20 s, it was regarded as a gel. The thermostability of stereocomplexed hydrogels was studied using the vial tilting method at temperatures between 5 and 70 °C with intervals of 2 °C. At each temperature, the samples were allowed to equilibrate for 10 min. X-ray diffractions were performed with a Bruker D8 Discovery equipped with a copper source (X-ray wavelength $\lambda = 0.154$ nm) and a two-dimensional detector (Hi-Star). All measurements were conducted in reflection geometry. After the measurements, the two-dimensional X-ray scattering images were integrated into one-dimensional intensity profiles with 2θ as x-axis, where θ is the scattering angle. The data were corrected with a background profile collected from pure water. Cryo-TEM was carried out with a CM12 apparatus (Philips) at 100−120 kV. Rheology experiments were performed on a US 200 rheometer (Anton Paar), using a flat plate measuring geometry (25 mm diameter, gap 0.5 mm), a frequency of 1 Hz, and a strain of 1%, as described previously.[15] Polymer solutions containing equimolar amounts of PEG−$(PLLA)_8$ and PEG−$(PDLA)_8$ star block copolymers were mixed, homogenized, and quickly applied to the rheometer.

Table 1. Synthesis of PEG−$(PLLA)_8$ and PEG−$(PDLA)_8$ Star Block Copolymers[a]

polymer	conversion (%)	N_{LA}[b] theory[c]	N_{LA}[b] ^{1}H NMR	M_n ^{1}H NMR	PEG content (wt %)
PEG21800−$(PLLA)_8$	97	10	9	27 200	81
	98	12	12	28 400	76
	98[d]	14	14	29 500	74
PEG21800−$(PDLA)_8$	97	10	10	27 400	79
	99	12	12	28 700	76
	98[d]	14	14	29 800	73
PEG43500−$(PLLA)_8$	∼95	14	13	50 900	85
	96	18	17	53 300	82
PEG43500−$(PDLA)_8$	∼95	14	13	50 800	86
	98	18	17	53 300	82

[a] The ring-opening polymerization of lactide was performed in CH_2Cl_2 for 4 h at room temperature using 8-arm PEG and the single site Zn-complex Zn(Et)[SC_6H_4(CH(Me)NC_4H_8)-2] as initiator and catalyst, respectively ($[LA]_0 = 0.25$ M, PEG−OH:Zn catalyst = 2:1). [b] Number of lactyl units per PLA block. [c] Based on feed composition and conversion. [d] Data for these star block copolymers have been reported previously.[15]

Results and Discussion

Synthesis of Water-Soluble PEG−PLA Star Block Copolymers. To prepare materials that are well soluble in water, poly(ethylene glycol)−poly(L-lactide), PEG−$(PLLA)_8$, and poly(ethylene glycol)−poly(D-lactide), PEG−$(PDLA)_8$, star block copolymers were synthesized by a Zn-complex catalyzed ring-opening polymerization of L- and D-lactide, respectively, initiated by 8-arm star PEG (Table 1). The use of the single site Zn-catalyst allowed excellent control over the degree of polymerization of the poly(lactide) (PLA) blocks, as determined by ^{1}H NMR. ^{1}H NMR showed that all hydroxyl groups of PEG had initiated the ring-opening polymerization.[15] Star block copolymers of PEG with a molecular weight of 21.8×10^3 and PLA with average block lengths of 10, 12, 14, or 16 lactyl units were prepared. Also, PEG−$(PLLA)_8$ and PEG−$(PDLA)_8$ star block copolymers with a PEG molecular weight of 43.5×10^3 and PLA with average block lengths of 13, 17, or 20 lactyl units were synthesized. The solubility of PEG21800−$(PLA)_8$ and PEG43500−$(PLA)_8$ in distilled water at room temperature decreased rapidly upon increasing the PLA block length. When the number of lactyl units per PLA block for PEG21800−$(PLA)_8$ was higher than 14, the copolymer was not water-soluble above polymer concentrations of ∼0.1 w/v % (data not shown). PEG43500−$(PLA)_8$ star block copolymers were water-soluble up to 17 lactyl units per PLA block. At 17 lactyl units per PLA block, PEG43500−$(PLA)_8$ was only soluble at low concentrations of ∼0.1 w/v % (data not shown). Moreover, with increasing PLLA block length, the critical gel concentration (CGC) of the PEG−$(PLA)_8$ star block copolymers decreased (Table 2). For instance, PEG21800−$(PLA_{10})_8$ star block copolymers showed a CGC of 40 w/v %, while PEG21800−$(PLA_{14})_8$ star block copolymers showed a CGC of 15 w/v %.

Table 2. Critical Gel Concentrations (CGC) of PEG−$(PLLA)_8$ Single Enantiomers and of Mixed Aqueous Solutions Containing Equimolar Amounts of PEG−$(PLLA)_8$ and PEG−$(PDLA)_8$ Star Block Copolymers in Distilled Water at Room Temperature

polymer	CGC single enantiomer (w/v %)	CGC mixed enantiomers (w/v %)
PEG21800−$(PLA_{10})_8$	40	25
PEG21800−$(PLA_{12})_8$	20	10
PEG21800−$(PLA_{14})_8$[a]	15	5
PEG43500−$(PLA_{13})_8$	9	6

[a] Data for the PEG21800−$(PLA_{14})_8$ star block copolymers have been reported previously.[15]

Star block copolymers based on PEG43500 are much less water-soluble, possibly due to the high molecular weight of the PEG. Similar to the PEG21800−$(PLA)_8$ star block copolymers, the CGC of PEG43500−$(PLA)_8$ star block copolymers decreases rapidly at higher PLA block lengths and only the PEG43500−$(PLA_{13})_8$ copolymer, which is soluble up to 9 w/v %, was used for further studies (Table 2).

Critical association concentration (CAC) values were determined at 20 °C using the hydrophobic dye 1,6-diphenyl-1,3,5-hexatriene solubilization method.[22] CAC values at 20 °C for PEG21800−$(PLA_{14})_8$, PEG21800−$(PLA_{12})_8$, and PEG21800−$(PLA_{10})_8$ were found to be 0.07, 0.22, and 0.44 w/v %, respectively. The decreasing CAC value with increasing PLLA block length is due to increased hydrophobic interactions and subsequent increased aggregation tendency. PEG43500−$(PLA_{13})_8$ showed a CAC of 0.11 w/v %. For these star block copolymers, the PEG molecular weight has little influence on the association behavior. The cloud points of 5 w/v % PEG−$(PLA)_8$ solutions in water increased with decreasing PLA block length and were found to be 27, 49, and 61 °C for PEG21800−$(PLA)_8$ star block copolymers with 14, 12, and 10 lactyl units per PLA block, respectively.

Critical Gel Concentrations and Phase Behavior. The influence of the PLA block length and PEG molecular weight on the gelation behavior of aqueous solutions containing equimolar amounts of PEG−$(PLLA)_8$ and PEG−$(PDLA)_8$ star block copolymers was studied at room temperature. Aqueous solutions of the star block copolymers with similar PLA blocks lengths and PEG molecular weight were mixed, and after equilibration it was tested whether the sample had turned into a gel by the vial tilting method. Stereocomplexed hydrogels could be prepared in a polymer concentration range of 5−25 w/v % for PEG21800−$(PLA)_8$ star block copolymers and of 6−8 w/v % for PEG43500−$(PLA)_8$ star block copolymers. From these experiments, it can be seen that the CGC decreases with increasing PLA block length and gelation is possible even at very short PLA block lengths of 10 lactyl units (Table 2). It was assumed that a PEG with a higher molecular weight would increase the copolymer solubility, which would allow stereocomplexed hydrogel formation at higher polymer concentrations. However, doubling of the PEG molecular weight decreased the block copolymer solubility to a large extent (Table 2). At 17 lactyl units, the PEG43500−$(PLLA)_8$ copolymer was soluble only at low polymer concentrations of ∼0.1 w/v %. At these low concentrations, no stereocomplexed hydrogel could be formed.

The thermostability of PEG21800−$(PLA)_8$ stereocomplexed hydrogels was studied by the vial tilting method in a temperature range of 5−70 °C. Upon increasing the temperature, the gel phase was lost and the stereocomplexed hydrogels phase separated into a mobile phase consisting of a clear fluid and a viscous opaque phase (Figure 1). In a few cases, at relatively short PLA block lengths and low concentrations, a sol phase was obtained. The hydrogels containing only the single enantiomers, which can be formed at high polymer concentrations, showed much lower phase separation temperatures (data not shown). Therefore, stereocomplexation appears to be a key factor to maintain the gel structure. In general, the thermostability of the gels increases with increasing polymer concentration and PLA block length. However, PEG21800−$(PLA_{12})_8$ stereocomplexed hydrogels showed unexpected phase behavior. A steep increase in the phase separation temperature from 35 to 67 °C occurred when increasing the polymer concentration from 7.5 to 10 w/v %. At 14 w/v %, however, the phase separation temperature was found at 60 °C, and it further decreased to 43 °C at 15 w/v %. In the preparation of the PEG21800−$(PLA_{12})_8$ stereocomplexed hydrogels, the stereocomplexation efficiency may be changing with the polymer concentration. It appears that at 10 w/v % polymer concentration, the stereocomplexation for these star block copolymers is most efficient.

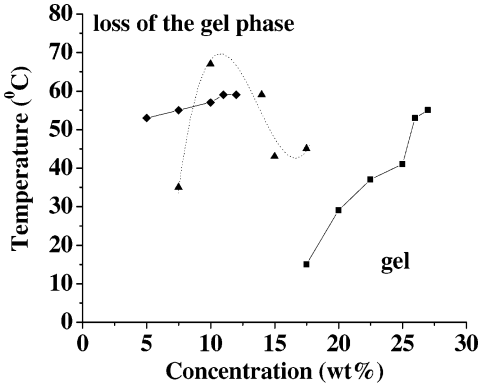

Figure 1. Thermostability of PEG−$(PLA)_8$ stereocomplexed hydrogels. PEG21800−$(PLA_{10})_8$ (■), PEG21800−$(PLA_{12})_8$ (▲), PEG21800−$(PLA_{14})_8$ (◆). Data for the PEG21800−$(PLA_{14})_8$ stereocomplexed hydrogel have been reported previously.[15]

Gelation Mechanism and Gel Morphology. To confirm the gel formation by stereocomplexation of the PLA blocks, WAXS experiments were performed on stereocomplexed hydrogels of PEG21800−$(PLA_{14})_8$, PEG21800−$(PLA_{12})_8$, and on a PEG21800−$(PDLA_{14})_8$ single enantiomer solution as a control (Figure 2). Both PEG21800−$(PLA_{14})_8$ and PEG21800−$(PLA_{12})_8$ stereocomplexed hydrogels showed diffraction peaks at $2\theta = \sim 12.2°$, 23°, 24° (Figure 2a), which are known to correspond to the PLA stereocomplex crystal.[23] The single enantiomer solution showed no diffraction peaks, because at these short block lengths PLA is amorphous. The amorphous nature of the PLA blocks was shown previously by differential calorimetry measurements (DSC).[15] The stereocomplex crystals in PEG21800−$(PLA_{12})_8$ hydrogels were still present at 30 °C, but melted when the temperature was increased to 50 °C (Figure 2b). This result agrees well with the decreasing gel strength upon increasing temperature, as was also shown by Vert et al.[24] for PLA−PEG−PLA triblock copolymer stereocomplexed hydrogels. The disappearance of the stereocomplex crystal peaks at 50 °C also agrees well with the phase separation temperature of 43 °C of the PEG21800−$(PLA_{12})_8$ 15 w/v % stereocomplexed hydrogel, as determined by the vial tilting method.

The effect of stereocomplexation on polymer aggregation in dilute solutions was also investigated. For this purpose, micellar solutions containing the single PEG21800−$(PLLA_{12})_8$ or equimolar amounts of PEG21800−$(PLLA_{12})_8$ and PEG21800−$(PDLA_{12})_8$ were prepared. Dynamic light scattering (DLS) revealed that both the *Z*-average particle size (Z-av) and the

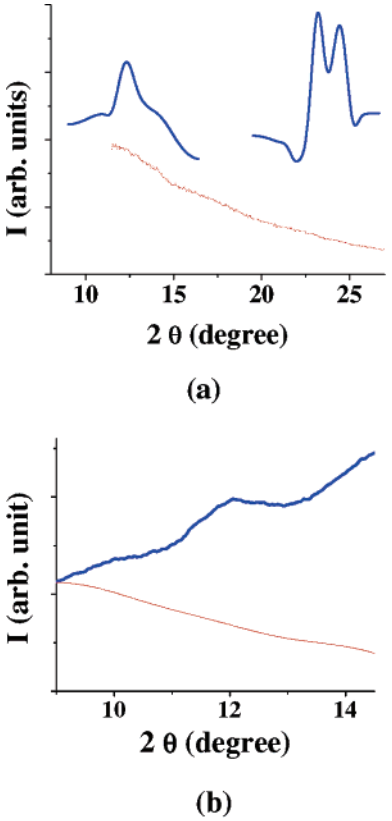

Figure 2. WAXS measurements on PEG21800−$(PLA)_8$ stereocomplexed hydrogels. (a) 10 w/v % PEG21800−$(PLA_{14})_8$ stereocomplexed hydrogel (blue line) and 10 w/v % PEG21800−$(PDLA_{14})_8$ single enantiomer solution (red line) at 20 °C; (b) 15 w/v % PEG21800−$(PLA_{12})_8$ stereocomplexed hydrogel at 30 °C (blue line) and 50 °C (red line).

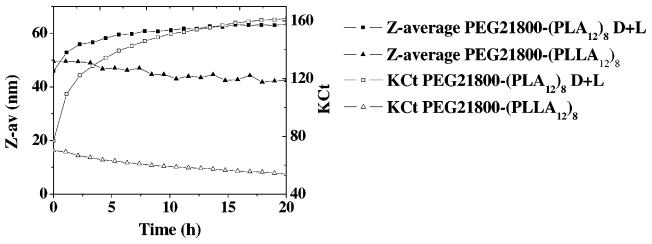

Figure 3. The *Z*-average particle size (Z-av) (nm) and the photon count rate (KCt) of 1 w/v % aqueous copolymer solutions as a function of time at 25 °C.

photon count rate (KCt) increased in time upon mixing enantiomers of opposite chirality (Figure 3), indicating that larger aggregates are formed upon stereocomplexation. In contrast, solutions containing only the single enantiomer or a D- to L-enantiomer ratio of 75/25 showed a slight decrease in the count rate and *Z*-average particle size.

Cryo-TEM was performed to show the influence of stereocomplexation on the morphology. Cryo-TEM images of 10 w/v % PEG21800−$(PDLA_{14})_8$ aqueous solutions and 10 w/v % PEG21800−$(PLA_{14})_8$ stereocomplexed hydrogels both showed dark spots and gray areas, corresponding to PEG and PLA domains, respectively (Figure 4). Both the PEG21800−$(PDLA_{14})_8$ aqueous solutions and the PEG21800−$(PLA_{14})_8$ stereocomplexed hydrogel show wormlike particles with a PEG corona and a PLA core with a core size ranging from ca. 10 to 20 nm. The cryo-TEM image of the PEG21800−$(PLA_{14})_8$ stereocomplexed hydrogel (Figure 4A) shows a somewhat coarser structure as compared to the PEG21800−$(PDLA_{14})_8$ aqueous solution (Figure 4B). In contrast to the PEG21800−

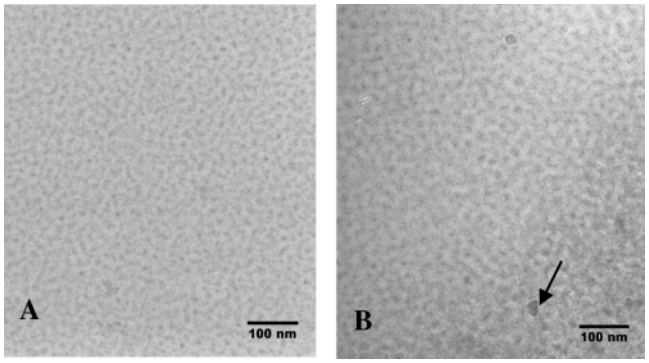

Figure 4. Cryo-TEM images of (A) a PEG21800−$(PDLA_{14})_8$ aqueous solution and (B) a PEG21800−$(PLA_{14})_8$ stereocomplexed hydrogel at a polymer concentration of 10 w/v %. Some ice crystals were present due to contamination during sample preparation, as indicated by the arrow.

$(PDLA_{14})_8$ aqueous solutions, the gray areas of the stereocomplexed hydrogel show small, darker spots, indicating more phase separation on a smaller scale upon stereocomplexation.

Rheology. The mechanical properties of the stereocomplexed hydrogels were studied by oscillatory rheology experiments on polymer solutions containing equimolar amounts of PEG−$(PLLA)_8$ and PEG−$(PDLA)_8$ star block copolymers. Gel formation kinetics was followed by monitoring the storage modulus (G') and loss modulus (G'') in time (Figures 5 and 6). Comparing PEG21800−$(PLA_{12})_8$ and PEG21800−$(PLA_{14})_8$ stereocomplexed hydrogels, it can be seen from Figure 5a that increasing the PLA block length increases the storage modulus from 0.9 to 7.0 kPa at 10 w/v % polymer concentration. The gelation time, indicated by the crossing of the storage and loss modulus,[25] increased with decreasing PLA block length. The enantiomeric mixture of PEG21800−$(PLA_{14})_8$ gelated instantly, whereas the gelation time was 40 min for a similar PEG21800−$(PLA_{12})_8$ mixture. To study the influence of PEG molecular weight, stereocomplexed hydrogels of PEG21800−$(PLA_{14})_8$ and PEG43500−$(PLA_{13})_8$ were prepared at 7.5 wt % polymer concentration at 20 °C. Figure 5b shows that the gelation time for the PEG43500−$(PLA_{13})_8$ stereocomplexed hydrogel significantly increased as compared to that of the PEG21800−$(PLA_{14})_8$ stereocomplexed hydrogel (200 min vs instant gelation). Also, after gelation the storage modulus of the PEG43500−$(PLA_{13})_8$ stereocomplexed hydrogel increases slower than the storage modulus of the PEG21800−$(PLA_{14})_8$ stereocomplexed hydrogel.

The longer gelation time and slower increase in storage modulus of the PEG43500−$(PLA_{13})_8$ stereocomplexed hydrogel show that the gel formation kinetics are much slowed as compared to the PEG21800−$(PLA_{14})_8$ stereocomplexed hydrogel, due to the high PEG molecular weight. Both stereocomplexed hydrogels have similar storage moduli of ca. 1 kPa, although the PEG43500−$(PLA_{13})_8$ stereocomplexed hydrogel contains almost twice less PLA than the PEG21800−$(PLA_{14})_8$ stereocomplexed hydrogel at the same polymer concentration. The loss modulus of the PEG43500−$(PLA_{13})_8$ stereocomplexed hydrogel is, however, much higher as compared to the PEG21800−$(PLA_{14})_8$ stereocomplexed hydrogel (220 vs 60 Pa), indicating that the PEG43500−$(PLA_{13})_8$ stereocomplexed hydrogel has a less perfect network structure and contains more viscous components.[25] Therefore, the comparable storage moduli of the PEG43500−$(PLA_{13})_8$ stereocomplexed hydrogel and the PEG21800−$(PLA_{14})_8$ stereocomplexed hydrogel is attributed to the presence of more chain entanglements at the higher PEG molecular weight, which act as physical cross-links.

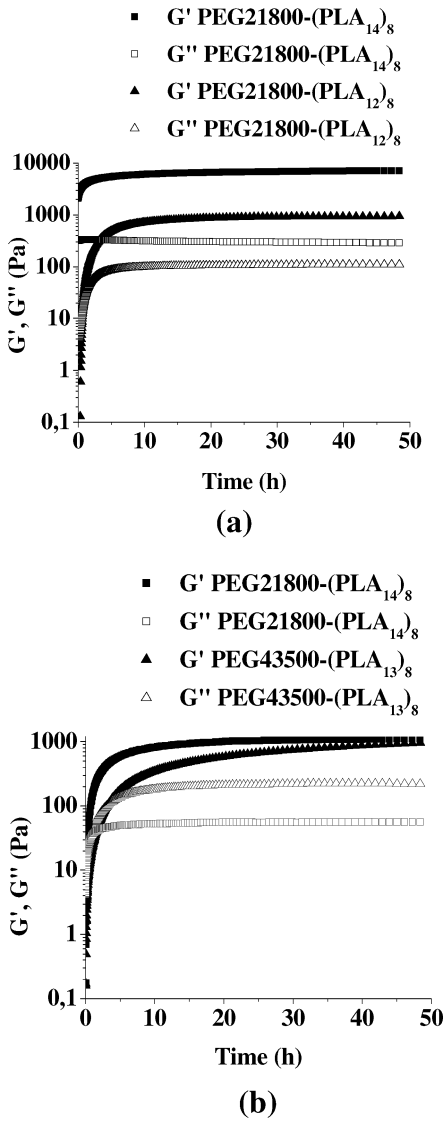

Figure 5. The storage modulus (*G′*) and loss modulus (*G″*) as a function of time after mixing solutions of PEG−(PLLA)$_8$ and PEG−(PDLA)$_8$ star block copolymers in equimolar amounts in water at 20 °C. (a) PEG21800−(PLA$_{14}$)$_8$ and PEG21800−(PLA$_{12}$)$_8$ at a polymer concentration of 10 w/v %; (b) PEG21800−(PLA$_{14}$)$_8$ and PEG43500−(PLA$_{13}$)$_8$ at a polymer concentration of 7.5 w/v %. Data for stereocomplexed hydrogels containing 10 w/v % of PEG21800−(PLA$_{14}$)$_8$ star block copolymers have been reported previously.[15]

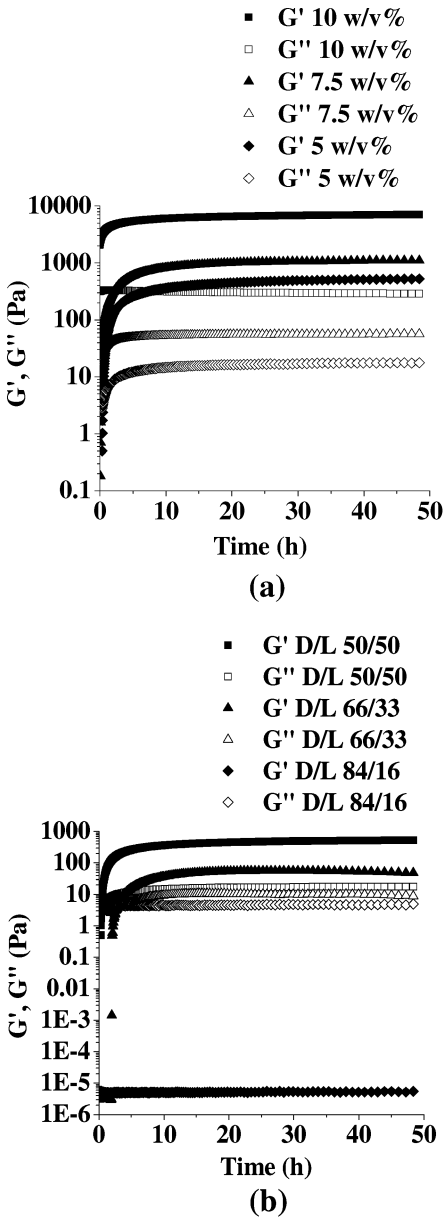

Figure 6. The storage modulus (*G′*) and loss modulus (*G″*) as a function of time after mixing solutions of PEG21800−(PLLA$_{14}$)$_8$ and PEG21800−(PDLA$_{14}$)$_8$ star block copolymers (a) with a D/L ratio of 50/50 in water at 20 °C at polymer concentrations of 10, 7.5, and 5 w/v %; and (b) at a polymer concentration of 5 w/v % in water at 20 °C with D/L ratios of 50/50, 66/33, and 84/16. Data for PEG21800−(PLA$_{14}$)$_8$ star block copolymers at 10 w/v % have been reported previously.[15]

In Figure 6a, the storage and loss moduli of PEG21800−(PLA$_{14}$)$_8$ stereocomplexed hydrogels at polymer concentrations of 5, 7.5, and 10 w/v % are presented. The results show that at higher concentrations a significantly higher storage modulus is obtained due to the formation of a denser cross-linked network. Moreover, gelation kinetics are highly dependent on the polymer concentration. At 5 w/v % concentration, the gelation time is approximately 25 min, giving a gel with a storage modulus of 0.5 kPa, but at 10 w/v % the gelation is instantaneous, affording a stereocomplexed hydrogel with a storage modulus of 7.0 kPa. The fast gelation is due to the higher probability of stereocomplex formation at higher concentrations. The highest storage moduli were obtained with stereocomplexed hydrogels of the PEG21800−(PLA$_{10}$)$_8$ copolymer at a relatively high polymer concentration of 25 w/v % (results not shown). Storage moduli of 27.2 and 14.0 kPa were obtained at 20 °C in water and at 37 °C in PBS, respectively. To further confirm the gelation by stereocomplexation, PEG21800−(PLA$_{14}$)$_8$ stereocomplexed hydrogels were prepared with mismatched D- and L-enantiomer ratios. Figure 6b shows that at a polymer concentration of 5 w/v % in water and at 20 °C the storage modulus of a 50/50 mixture is considerably higher than that of a 67/33 mixture (20 vs 9 Pa). Also, the gelation time increases at ratios other than 50/50. Eventually, at a ratio of 84/16, no gel formation was observed anymore. The influence of temperature and the presence of salts on the rheological properties of hydrogels is important considering the envisaged in-vivo application. We have previously shown that, independent of the PLA block length, enantiomeric mixtures of PEG21800−(PLA)$_8$ afforded strong hydrogels at 37 °C in PBS buffer at pH 7.4.[15] In summary, the rheology results show that the PEG−(PLA)$_8$ star block copolymers provide faster gelation kinetics, higher storage moduli, and a more perfect cross-linked network structure (as

indicated by the high storage to loss modulus ratio), as compared to the PLA−PEG−PLA triblock copolymers reported by our group and by Li et al.[15,18] and to the dextran−PLA graft copolymer stereocomplexed hydrogels prepared by De Jong et al.[26] Moreover, the results show that the gelation time and mechanical properties of the star block copolymer hydrogels can be tuned by simply varying the polymer concentration and/or the PLA block length.

Conclusions

Water-soluble 8-arm PEG−PLLA and PEG−PDLA star block copolymers were synthesized by ring-opening polymerization of L-lactide and D-lactide at room temperature using a single-site ethylzinc complex and 8-arm PEG as catalyst and initiator, respectively. Stereocomplexed hydrogels were formed by mixing aqueous solutions of PEG−$(PLLA)_8$ and PEG−$(PDLA)_8$ star block copolymers. Rheology on the stereocomplexed hydrogels showed that hydrogels with a range of storage moduli (up to 14 kPa in PBS at 37 °C) and gelation times (instant gelation up to ca. 1 h) can be designed by varying the PLA block length and polymer concentration. These stereocomplexed hydrogels are promising for use in biomedical applications, because they can be formed in situ under physiological conditions with suitable mechanical properties.

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References and Notes

(1) Sawhney, A. S.; Pathak, C. P.; Hubbell, J. A. *Macromolecules* **1993**, *26*, 581−587.
(2) Burdick, J. A.; Mason, M. N.; Hinman, A. D.; Thorne, K.; Anseth, K. S. *J. Controlled Release* **2002**, *83*, 53−63.
(3) West, J. L.; Hubbell, J. A. *Macromolecules* **1999**, *32*, 241−244.
(4) Nuttelman, C. R.; Henry, S. M.; Anseth, K. S. *Biomaterials* **2002**, *23*, 3617−3626.
(5) Goessl, A.; Tirelli, N.; Hubbell, J. A. *J. Biomater. Sci., Polym. Ed.* **2004**, *15*, 895−904.
(6) Pratt, A. B.; Weber, F. E.; Schmoekel, H. G.; Muller, R.; Hubbell, J. A. *Biotechnol. Bioeng.* **2004**, *86*, 27−36.
(7) Jeong, B.; Bae, Y. H.; Lee, D. S.; Kim, S. W. *Nature* **1997**, *388*, 860−862.
(8) Zhong, Z. Y.; Dijkstra, P. J.; Feijen, J.; Kwon, Y. M.; Bae, Y. H.; Kim, S. W. *Macromol. Chem. Phys.* **2002**, *203*, 1797−1803.
(9) Kim, S.; Healy, K. E. *Biomacromolecules* **2003**, *4*, 1214−1223.
(10) Collier, J. H.; Hu, B.-H.; Ruberti, J. W.; Zhang, J.; Shum, P.; Thompson, D. H.; Messersmith, P. B. *J. Am. Chem. Soc.* **2001**, *123*, 9463−9464.
(11) Yi, J. W.; Na, K.; Bae, Y. H. *Macromol. Symp.* **2004**, *207*, 131−138.
(12) Kisiday, J.; Jin, M.; Kurz, B.; H, H.; Semino, C.; Zhang, S.; Grodzinsky, A. J. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 9996−10001.
(13) Li, J.; Ni, X. P.; Leong, K. W. *J. Biomed. Mater. Res., Part A* **2003**, *65A*, 196−202.
(14) Van Tomme, S. R.; van Steenbergen, M. J.; De Smedt, S. C.; van Nostrum, C. F.; Hennink, W. E. *Biomaterials* **2005**, *26*, 2129−2135.
(15) Hiemstra, C.; Zhong, Z. Y.; Dijkstra, P. J.; Feijen, J. *Macromol. Symp.* **2005**, *224*, 119−132.
(16) De Jong, S. J.; De Smedt, S. C.; Demeester, J.; van Nostrum, C. F.; Kettenes-van den Bosch, J. J.; Hennink, W. E. *J. Controlled Release* **2001**, *72*, 47−56.
(17) Grijpma, D. W.; Feijen, J. *J. Controlled Release* **2001**, *72*, 247−249.
(18) Li, S.; El Ghzaoui, A.; Dewinck, E. *Macromol. Symp.* **2005**, *222*, 23−36.
(19) Mukose, T.; Fujiwara, T.; Nakano, J.; Taniguchi, I.; Miyamoto, M.; Kimura, Y.; Teraoka, I.; Lee, C. W. *Macromol. Biosci.* **2004**, *4*, 361−367.
(20) Bos, G. W.; Hennink, W. E.; Brouwer, L. A.; den Otter, W.; Veldhuis, T. F. J.; van Nostrum, C. F.; van Luyn, M. J. A. *Biomaterials* **2005**, *26*, 3901−3909.
(21) Bos, G.; Jacobs, J.; Koten, J.; Van Tomme, S.; Veldhuis, T.; van Nostrum, C.; Den Otter, W.; Hennink, W. *Eur. J. Pharm. Sci.* **2004**, *21*, 561−567.
(22) Alexandridis, P.; Holzwarth, J. F.; Hatton, T. A. *Macromolecules* **1994**, *27*, 2414−2425.
(23) Sarasua, J.-R.; Prud'homme, R. E.; Wisniewski, M.; Le Borgne, A.; Spassky, N. *Macromolecules* **1998**, *31*, 3895−3905.
(24) Li, S.; Vert, M. *Macromolecules* **2003**, *36*, 8008−8014.
(25) Chambon, F.; Winter, H. H. *J. Rheol.* **1987**, *31*, 683−696.
(26) De Jong, S. J.; De Smedt, S. C.; Wahls, M. W. C.; Demeester, J.; Kettenes-van den Bosch, J. J.; Hennink, W. E. *Macromolecules* **2000**, *33*, 3680−3686.

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