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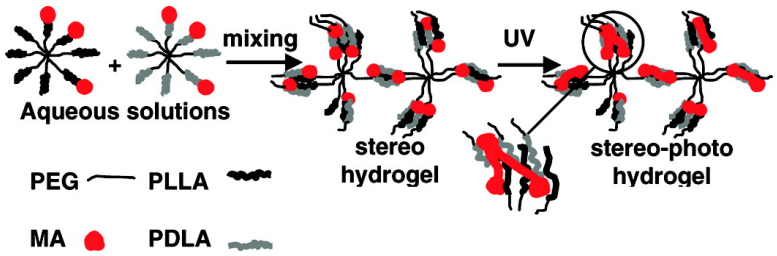

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Rapidly in Situ Forming Biodegradable Robust Hydrogels by Combining Stereocomplexation and Photopolymerization

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Abstract: Our previous studies have shown that stereocomplexed hydrogels can be rapidly formed in vitro as well as in vivo upon mixing aqueous solutions of eight-arm poly(ethylene glycol)−poly(L-lactide) (PEG−PLLA) and poly(ethylene glycol)−poly(D-lactide) (PEG−PDLA) star block copolymers. In this study, stereocomplexation and photopolymerization are combined to yield rapidly in situ forming robust hydrogels. Two types of methacrylate-functionalized PEG−PLLA and PEG−PDLA star block copolymers, PEG−PLLA−MA and PEG−PDLA−MA, which have methacrylate groups at the PLA chain ends and PEG−MA/PLLA and PEG−MA/PDLA, which have methacrylate groups at the PEG chain ends, were designed and prepared. Results showed that stereocomplexed hydrogels could be rapidly formed (within 1−2 min) in a polymer concentration range of 12.5−17.5% (w/v), in which the methacrylate group hardly interfered with the stereocomplexation. When subsequently photopolymerized, these hydrogels showed largely increased storage moduli as compared to the corresponding hydrogels that were cross-linked by stereocomplexation or photopolymerization only. Interestingly, the storage modulus of stereocomplexed−photopolymerized PEG−PLA−MA hydrogels increased linearly with increasing stereocomplexation equilibration time prior to photopolymerization (from ca. 6 to 32 kPa), indicating that stereocomplexation aids in photopolymerization. Importantly, photopolymerization of stereocomplexed hydrogels could take place at very low initiator concentrations (0.003 wt %). Swelling/degradation studies showed that combining stereocomplexation and photopolymerization yielded hydrogels with prolonged degradation times as compared to corresponding hydrogels cross-linked by photopolymerization only (3 vs 1.5 weeks). Stereocomplexed−photopolymerized PEG−MA/PLA hydrogels degraded much slower than corresponding PEG−PLA−MA hydrogels, with degradation times ranging from 7 to more than 16 weeks. Therefore, combining stereocomplexation and photopolymerization is a novel approach to obtain rapidly in situ forming robust hydrogels.

Introduction

Hydrogels have been widely used for biomedical applications, such as tissue engineering and drug delivery, due to their favorable characteristics.[1−3] Hydrogels are water-swollen networks of cross-linked hydrophilic polymers. Their high water content renders them highly biocompatible and also leads to minimal adsorption of proteins. The mechanical properties of hydrogels parallel those of soft tissues, making them particularly interesting for tissue engineering. Hydrogels may be formed in situ, thus allowing easy mixing of cells and bioactive molecules, such as proteins, with the polymer solutions prior to gelation.[4−6] Moreover, in situ hydrogel formation enables the preparation of complex shapes and use of minimally invasive surgery. In situ forming hydrogels have been prepared by physical and chemical cross-linking methods. Physically cross-linked hydrogels include those based on hydrophobic interactions between thermosensitive block or graft polymers,[7−11] stereocomplexation between poly(L-lactide) (PLLA) and poly(D-lactide) (PDLA) graft[12] and block copolymers,[13−15] inclusion complexation using

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α-dextrin polymers,[16−20] and ionic interactions between oppositely charged microparticles[21] or peptides.[22] The cross-linking conditions for these gels are generally very mild, thus allowing the entrapment of labile compounds, such as proteins. Stereocomplexation, i.e., cocrystallization, of the PLLA and PDLA blocks offers an attractive method for in situ preparation of physically cross-linked hydrogels, since the gelation is fast and the hydrogels are degradable through degradation of the polylactide (PLA) sequences.[12,13] We have previously reported stereocomplexed hydrogels that were rapidly formed in situ by mixing aqueous solutions of PEG−PLLA and PEG−PDLA (PEG = poly(ethylene glycol) star block copolymers via stereocomplexation. The presence of the stereocomplex crystals was confirmed by wide-angle X-ray scattering (WAXS) experiments.[13] A major disadvantage of physically cross-linked hydrogels is that they are mechanically weak compared to chemically cross-linked hydrogels, and changes in the external environment (e.g., ionic strength, pH, temperature) may give rise to disruption of the network.

Chemically cross-linked hydrogels have been formed in situ by Michael addition between thiols and acrylates or vinyl sulfones,[23−29] reaction between aldehydes and dihydrazides[30] or amines,[31] reaction between activated esters and amines,[32] and redox-initiated radical chain polymerization of (meth)acrylates.[33−37] Photopolymerization of (meth)acrylates[5] using UV light[38−41] or visible light[42−44] has been mostly used for in situ formation of chemically cross-linked hydrogels. Biodegradable hydrogels prepared by photopolymerization of PEG−PLA diacrylate derivatives were first reported by the group of Hubbell.[42] More recently, this group has prepared degradable hydrogels by the incorporation of plasmin degradable peptide sequences.[39,43] When modified with cell-adhesive RGD peptide sequences, these hydrogels supported three-dimensional outgrowth of human fibroblasts embedded as a cluster within the hydrogel. Another type of degradable hydrogel was prepared by copolymerization of a hyaluronic acid methacrylate derivative and PEG diacrylate.[44] Fibroblasts adhered and proliferated when cultured on the RGD-functionalized hydrogels. The group of Anseth has done much work on degradable hydrogels based on PEG−PLA dimethacrylates.[40] It was shown that by using combinations of PEG and PEG−PLA dimethacrylates and/or by changing the PLA block length, the hydrogel degradation rate, compressive modulus, and cross-linking density could be tuned to provide suitable scaffolds for cartilage tissue engineering.[41] The major advantage of photopolymerization is the spatial and temporal control over the polymerization. However, photopolymerization in vivo is hampered by the absorption of UV light by the skin (>99%). In clinical applications, fast gelation is desired to prevent diffusion of hydrogel precursors or bioactive molecules to the surrounding tissue. Elisseeff et al. have reported on transdermal photopolymerization of a 20 wt % PEG dimethacrylate aqueous solution injected subcutaneously into nude mice by UV irradiation for 3 min at 2 mW/cm^2 incident light intensity.[45] In this study, high molecular weight PEG (100 000) was used as an additive to prevent rapid diffusion of the gel precursors after injection and to increase the mechanical properties of the photopolymerized hydrogel. A drawback is that it is very difficult to excrete high molecular weight PEG by the kidneys.[46] Elisseeff et al. have studied the UV light attenuation by the skin using swine skin as a model.[47] The incident light intensity of 100 mW/cm^2 was attenuated by the skin to ca. 0.05 mW/cm^2. After 3 min of UV irradiation of a 20 wt % PEG dimethacrylate aqueous solution with 0.04 wt % photoinitiator concentration, a conversion of ca. 10% was reached. The remaining unsaturated bonds may cause toxicity problems, and the incomplete conversion may result in hydrogels with weak mechanical properties.[48] The polymerization rate may be increased by increasing the photoinitiator concentration or the intensity of the incident light. However, due to their toxicity photoinitiators can only be used at low concentrations (ca. 0.01−0.05 wt %),[49] and the intensity of the UV light is limited to ca. 5−10 mW/cm^2 to prevent cell damage. Visible light is less attenuated by the skin, but efficient initiators with less cytotoxicity are required.[49,50] Another problem of photopolymerization is that fast polymerization is generally accompanied by substantial heat effects.[48] The resulting temperature rise may cause local cell morbidity and tissue necrosis surrounding the implant.

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In this study, we have combined two cross-linking methods, i.e., stereocomplexation and photopolymerization, to achieve fast in situ forming, robust hydrogels. Stereocomplexation provides fast gelation in vitro and in vivo,[13,51,52] allowing for lower photopolymerization rates, providing easier handling, limiting the local temperature rise, and potentiating the use of low initiator concentrations and low light intensities. Moreover, photopolymerization provides robust hydrogels, with increased mechanical properties and prolonged degradation times compared to hydrogels cross-linked by stereocomplexation only.[51] Interestingly, our results show that stereocomplexation aids in the photopolymerization of methacrylate groups, resulting in hydrogels with increased storage moduli and degradation times compared to the corresponding hydrogels that were formed by photopolymerization only.

Materials and Methods

Materials. L-Lactide and D-lactide were obtained from Purac and recrystallized from dry toluene. Eight-arm star PEG ($M_{n,NMR}$ = 21 800) was supplied by Nektar and used as received. The single-site Zn complex catalyst (Zn(Et)[$OC_6H_4(CH_2N(Me)_2)$-2-Me-4]) was kindly provided by Professor G. van Koten of the University of Utrecht (The Netherlands). Methacrylic anhydride was purchased from Merck and Irgacure 2959 from Ciba Specialty Chemicals. Both were used as received. Dichloromethane and triethylamine (TEA) were dried over calcium hydride and potassium hydroxide, respectively, and distilled prior to use. Eight-arm poly(ethylene glycol)−poly(L-lactide) and poly(ethylene glycol)−poly(D-lactide) star block copolymers with 12 lactyl units per PLA block (PEG−$PLLA_{12}$ and PEG−$PDLA_{12}$, respectively) were prepared as reported previously ($M_{n,PEG}$ = 21 800).[53]

Synthesis. PEG−$PLLA_{12}$−MA and PEG−$PDLA_{12}$−MA (MA = methacrylate) were synthesized by partial methacrylation of the hydroxyl groups of PEG−$PLLA_{12}$ and PEG−$PDLA_{12}$, respectively, according to the procedure reported by Lin-Gibson et al.[54] Typically, PEG−$PLLA_{12}$ (5.0 g, 0.174 mmol, dried overnight under vacuum over phosphorus pentoxide) was dissolved in 18 mL of dichloromethane. A solution of TEA (0.171 g, 1.690 mmol) in 1 mL of dichloromethane was added, and the reaction mixture was cooled in an ice bath. Subsequently, a solution of methacrylic anhydride (0.244 g, 1.583 mmol) in 2 mL of dichloromethane was added dropwise. The reaction mixture was stirred for 2 days at 30 °C, and the product was recovered by precipitation in a mixture of cold diethyl ether/hexane/methanol (10/1/1, v/v). Degree of methacrylation: 40%. Yield: 88%. ^{1}H NMR ($CDCl_3$): δ 1.4 (m, CH(CH_3)OH end group PLA), 1.5 (m, CHCH_3), 1.9 (s, C(CH_3)=CH_2), 3.6 (m, PEG methylene protons), 4.2−4.3 (m, CH_2OCO, linking unit PEG−PLA), 4.3−4.4 (q, CH(CH_3)OH end group PLA), 5.1 (m, $CH$$CH_3$), 5.6 and 6.2 (C($CH_3$)=$CH_2$).

PEG−MA/PLLA and PEG−MA/PDLA, in which both MA and PLA blocks are directly linked to PEG, were synthesized by ring-opening polymerization of lactide using partially methacrylate-functionalized eight-arm star PEG (PEG−MA). For the synthesis of PEG−MA, typically, PEG (16.0 g, 0.734 mmol) was dissolved in 33 mL of dichloromethane. A solution of TEA (0.442 g, 4.368 mmol) in 1 mL of dichloromethane was added, and the reaction mixture was cooled in an ice bath. Subsequently, a solution of methacrylic anhydride (0.654 g, 4.242 mmol) in 2 mL of dichloromethane was added dropwise. The reaction mixture was stirred for 2 days at 30 °C, and the product was recovered by precipitation in a mixture of cold diethyl ether/hexane/methanol (10/1/1, v/v). Degree of methacrylation: 42%. Yield: 90%. ^{1}H NMR ($CDCl_3$): δ 1.9 (s, C(CH_3)=CH_2), 3.6 (m, PEG methylene protons), 4.2 (m, CH_2OCO, linking unit PEG−MA), 5.6 and 6.2 (C(CH_3)=CH_2).

PEG−MA/PLLA and PEG−MA/PDLA were synthesized by ring-opening polymerization of L-lactide and D-lactide, respectively, in dichloromethane at room temperature, initiated by the remaining hydroxyl groups of PEG−MA (dried overnight under vacuum over phosphorus pentoxide). The single-site Zn complex Zn(Et)[$OC_6H_3(CH_2Me_2)$-2-Me-4] was used as a catalyst. Typically, PEG−MA (3.0 g, 0.136 mmol) (degree of methacrylation 42%) and L-lactide (0.532 g, 3.694 mmol) were dissolved in 14 mL of dichloromethane ($[LA]_0$ = 0.25 M). A solution of single-site Zn complex catalyst (0.064 g, 0.247 mmol) was added in 1 mL of dichloromethane, and the reaction mixture was stirred for 1 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). Lactide conversion: 95%. Yield: 85%. ^{1}H NMR ($CDCl_3$): δ 1.4 (m, CH(CH_3)OH end group PLA), 1.5 (m, CHCH_3), 1.9 (s, C(CH_3)=CH_2), 3.6 (m, PEG methylene protons), 4.2 (m, CH_2OCO, linking unit PEG−MA), 4.2−4.3 (m, CH_2OCO, linking unit PEG−PLA), 4.3−4.4 (q, CH(CH_3)OH end group PLA), 5.1 (m, $CH$$CH_3$), 5.6 and 6.2 (C($CH_3$)=$CH_2$).

Characterization. ^{1}H NMR spectra ($CDCl_3$) were recorded on a Varian Inova spectrometer (Varian, Palo Alto, CA) operating at 300 MHz. The number of lactyl units per PLA block was calculated on the basis of the methyl protons of lactyl units (δ 1.4−1.5) and the methylene protons of PEG (δ 3.6). The number of methacrylate groups per PEG molecule was determined on the basis of the methylene protons of PEG (δ 3.6) and the methylene protons of the methacrylate group (δ 5.6 and 6.2).

Critical gel concentrations (CGCs) were determined as described before.[53] Briefly, polymer solutions were prepared by dissolving the polymers in deionized water overnight. Subsequently, polymer solutions of equimolar amounts of PEG−PLLA−MA and PEG−PDLA−MA or PEG−MA/PLLA and PEG−MA/PDLA star block copolymers were mixed and equilibrated overnight. The CGCs were determined at room temperature by inverting the vials. When the sample showed no flow within 20 s, it was regarded as a gel.

Rheology experiments were performed on a US 200 rheometer (Anton Paar), as described previously.[53] Briefly, a parallel plate measuring geometry (25 mm diameter, gap 0.5 mm), a frequency of 1 Hz, and a strain of 1% were used. Polymer solutions in HEPES-buffered saline (pH 7.0, 100 mM, adjusted to 300 mOsm with NaCl) containing equimolar amounts of PEG−PLLA−MA and PEG−PDLA−MA or PEG−MA/PLLA and PEG−MA/PDLA star block copolymers were mixed, homogenized, quickly applied to the rheometer, and measured at 37 °C.

In situ UV irradiation and rheology experiments were performed on a US 200 rheometer (Anton Paar) equipped with a UV light source (Bluepoint 4, Dr. Hönle, intensity of 16 mW/cm^2 in the 350−400 nm range). The samples were irradiated from above. A parallel plate measuring geometry made of quartz glass (10 mm diameter, gap 0.1 mm) was used in an oscillatory measurement with a frequency of 1 Hz and a strain of 1% or 5%. Both strains are within the linear viscoelastic region. Both PEG−PLA−MA and PEG−MA/PLA stereocomplexed hydrogels (stereohydrogels) and solutions of PEG−PLLA−MA or PEG−MA/PLLA single enantiomers in HEPES-buffered saline were UV-irradiated and at the same time measured at 37 °C. Irgacure 2959 was used as the photoinitiator. The stereohydrogels were measured 10 min after the enantiomeric solutions were mixed, unless mentioned otherwise.

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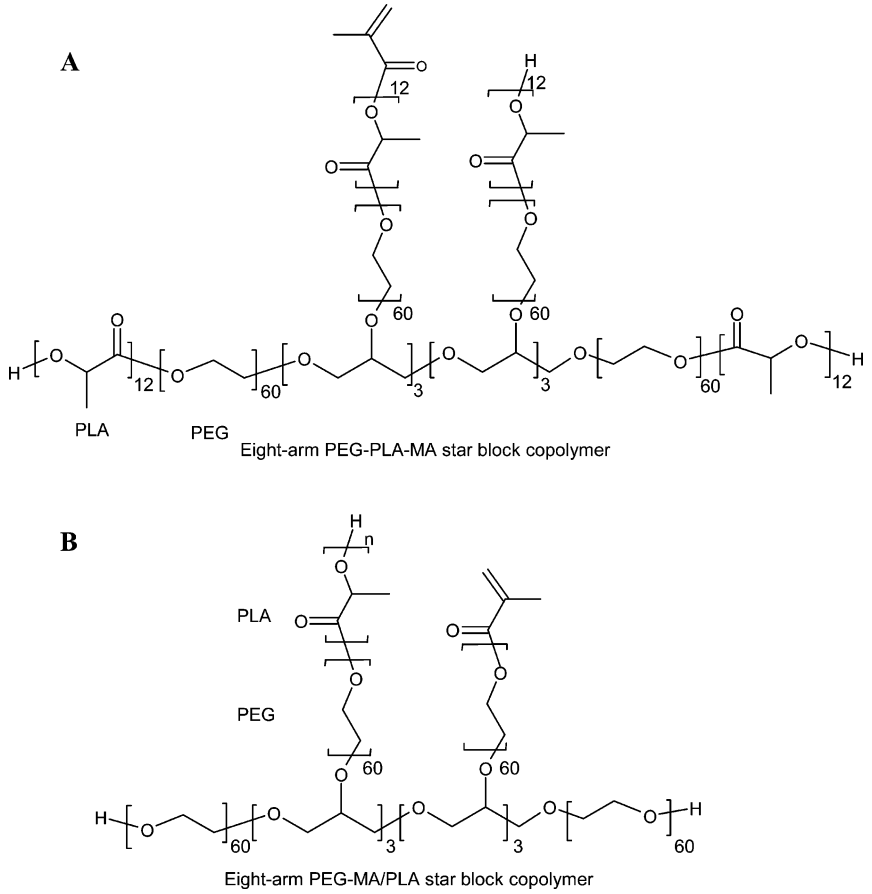

Figure 1. Molecular structures of (A) eight-arm PEG−PLA_{12}−MA star block copolymers and (B) PEG−MA/PLA_n (n = 12 or 16) star block copolymers. As an example three methacrylate groups per molecule are drawn.

Hydrogels for scanning electron microscopy (SEM) experiments and swelling/degradation tests were prepared similarly in a 96-well plate with sample volumes of 125 μL, resulting in cylinders of ca. 4 mm in height and 6 mm in diameter. PEG−PLA_{12}−MA or PEG−PLA_{16}−MA stereo−photohydrogels were prepared by UVA irradiation (320−400 nm, with 250 mW/cm^2 at 365 nm) for 10 min of the stereohydrogels (equilibrated for ca. 15 min after mixing of the enantiomeric solutions) with 8 mol % initiator concentration (with respect to the methacrylate groups) prepared in HEPES-buffered saline. Photohydrogels were formed similarly by UVA irradiation of PEG−$PLLA_{12}$−MA or PEG−MA_{16}/PLLA single-enantiomer solutions in HEPES-buffered saline.

SEM experiments were performed on freeze-dried hydrogels using a LEO Gemini 1550 FEG-SEM instrument, fitted with a field emission gun, and a voltage of 2 kV. Freeze-dried hydrogels were prepared by freezing in liquid nitrogen and subsequent freeze-drying at −50 °C and 5×10^{-7} bar overnight.

For the swelling/degradation tests, the hydrogel cylinders were placed in vials, and after addition of 1 mL of HEPES-buffered saline the hydrogels were allowed to swell at 37 °C. The swelling experiment was performed in duplicate or triplicate. The swollen hydrogels were weighed at regular intervals after removal of the buffer. After each weighing the buffer was refreshed. The swelling ratio of the hydrogels was calculated from the initial hydrogel weight after hydrogel preparation (W_0) and the swollen hydrogel weight after exposure to buffer (W_t):

$$\text{swelling ratio} = W_t/W_0$$

Results and Discussion

Synthesis. Two types of methacrylate-functionalized PEG−PLA star block copolymers, PEG−PLLA−MA and PEG−PDLA−MA (Figure 1A) and PEG−MA/PLLA and PEG−MA/PDLA (Figure 1B), were designed. PEG−PLLA−MA and PEG−PDLA−MA copolymers were prepared by a two-step synthesis procedure. First, eight-arm PEG−PLLA and PEG−PDLA star block copolymers with 12 lactyl units per PLA block (PEG−$PLLA_{12}$ and PEG−$PDLA_{12}$, $M_{n,PEG}$ = 21 800) were synthesized, as reported previously (Table 1, entries 1 and 2).[53] Subsequently, the PLA hydroxyl end groups were reacted with methacrylic anhydride using TEA as a catalyst and dichloromethane as a solvent at 30 °C. The PEG−$PLLA_{12}$−MA and PEG−$PDLA_{12}$−MA copolymers were recovered by precipitation in a diethyl ether/hexane/methanol mixture (10/1/1, v/v) (Table 1, entries 3 and 4). ^{1}H NMR showed a degree of methacrylation of ca. 40%, determined by comparing the integrals of the peaks corresponding to the methylene protons of the methacrylate group (δ 5.6 and 6.2) and the methylene protons of PEG (δ 3.6).

PEG−MA/PLA copolymers were prepared by a two-step synthesis procedure. First ca. 40% of the hydroxyl end groups of an eight-arm star PEG (M_n = 21 800) were methacrylated. Subsequently, the ring-opening polymerization of L-lactide or D-lactide was initiated by the remaining hydroxyl groups of methacrylate-functionalized PEG, using a single-site Zn complex as a catalyst and dichloromethane as a solvent at room temperature. PEG−MA/PLLA and PEG−MA/PDLA copolymers were obtained by precipitation in a diethyl ether/methanol mixture (20/1, v/v). PEG−MA/PLA copolymers with 12 and 16 lactyl units per PLA block were prepared by varying the

Table 1. Synthesis of PEG−PLLA−MA and PEG−PDLA−MA and PEG−MA/PLLA and PEG−MA/PDLA Star Block Copolymers[a]

entry	polymer	lactide conversion (%)	N_{LA}[b] theory[c]	N_{LA}[b] ^{1}H NMR	degree of methacrylation (%)	$M_n \times 10^{-3}$, ^{1}H NMR
1	PEG−$PLLA_{12}$	94	12	12		28.7
2	PEG−$PDLA_{12}$	96	12	12		28.7
3	PEG−$PLLA_{12}$−MA	94	12	12	40	28.8
4	PEG−$PDLA_{12}$−MA	96	12	12	42	28.9
5	PEG−MA/$PLLA_{12}$	95	12	12	46	25.6
6	PEG−MA/$PDLA_{12}$	94	12	12	46	25.6
7	PEG−MA/$PLLA_{16}$	99	17	16	42	27.4
8	PEG−MA/$PDLA_{16}$	95	16	16	42	27.4

[a] The ring-opening polymerization of lactide was performed in dichloromethane for 1 h at room temperature using PEG or partially methacrylate-functionalized PEG as an initiator and the single-site Zn complex Zn(Et)[$OC_6H_3(CH_2Me_2)$-2-Me-4] as a catalyst, ($[LA]_0 = 0.25$ M, PEG hydroxyl groups/Zn catalyst = 2/1). The methacrylation was performed in dichloromethane for 2 days at 30 °C ($[OH]_0 \approx 5$ mM, MA/OH/TEA = 1/1.5/1.1). [b] Number of lactyl units per PLA block. [c] Based on feed composition and conversion.

Table 2. CGCs of Solutions Containing PEG−PLLA, PEG−PLLA−MA, and PEG−MA/PLLA Single-Enantiomer Star Block Copolymers or Equimolar Amounts of PEG−PLLA and PEG−PDLA, PEG−PLLA−MA and PEG−PDLA−MA, or PEG−MA/PLLA and PEG−MA/PDLA Star Block Copolymers in Deionized Water at Room Temperature

polymer	CGC(single enantiomer) (%, w/v)	CGC(mixed enantiomers) (%, w/v)
PEG−PLA_{12}	20	7.5
PEG−PLA_{12}−MA	17.5	7.5
PEG−MA/PLA_{12}	30	22.5
PEG−MA/PLA_{16}	20	12.5

feeding ratio of lactide to PEG (Table 1, entries 5−8). The use of the single-site Zn catalyst allowed excellent control over the degree of polymerization of the PLA blocks, and the methacrylation reaction was reproducible, giving similar degrees of methacrylation (Table 1).

Gelation by Stereocomplexation. The influence of the methacrylate groups and the PLA block length on stereocomplex hydrogel (denoted as stereohydrogel) formation was studied at room temperature. Aqueous solutions of equimolar amounts of PEG−PLLA−MA and PEG−PDLA−MA or PEG−MA/PLLA and PEG−MA/PDLA star block copolymers were mixed, and after equilibration it was tested whether the sample had turned into a gel by the vial tilting method. Table 2 shows that the CGCs for stereocomplexation of PEG−PLA_{12}−MA and PEG−PLA_{12} are equal, indicating that the methacrylate end groups do not influence the stereocomplexation. PEG−PLLA, PEG−PLLA−MA, and PEG−MA/PLLA single enantiomers were also able to form gels at relatively high polymer concentrations. The CGC of the PEG−$PLLA_{12}$−MA single enantiomer is somewhat lower compared to that of the PEG−$PLLA_{12}$ single enantiomer, which is attributed to the increased hydrophobicity of PEG−$PLLA_{12}$−MA. Aqueous solutions of the PEG−MA/$PLLA_{12}$ single enantiomer could be prepared up to much higher polymer concentrations compared to those of the PEG−$PLLA_{12}$−MA single enantiomer. Stereohydrogels could also be formed from PEG−MA/$PLLA_{12}$ and PEG−MA/$PDLA_{12}$ copolymers, but at much higher polymer concentrations compared to PEG−$PLLA_{12}$−MA and PEG−$PDLA_{12}$−MA copolymers. The higher CGC for stereocomplexation of PEG−MA/PLA_{12} compared to PEG−PLA_{12}−MA is due to the lower cross-linking functionality (i.e., number of PLA blocks per molecule) and lower hydrophobicity of PEG−MA/PLA_{12} compared to PEG−PLA_{12}−MA. Previously we have shown that the CGCs for stereocomplexation of PLA−PEG−PLA triblock copolymers are higher compared to the CGCs of eight-arm PEG−PLA star block copolymers.[13] PEG−MA/PLA_{16} copolymers showed lower CGC values for stereocomplexation compared to PEG−MA/PLA_{12} copolymers, due to the increased PLA block length.

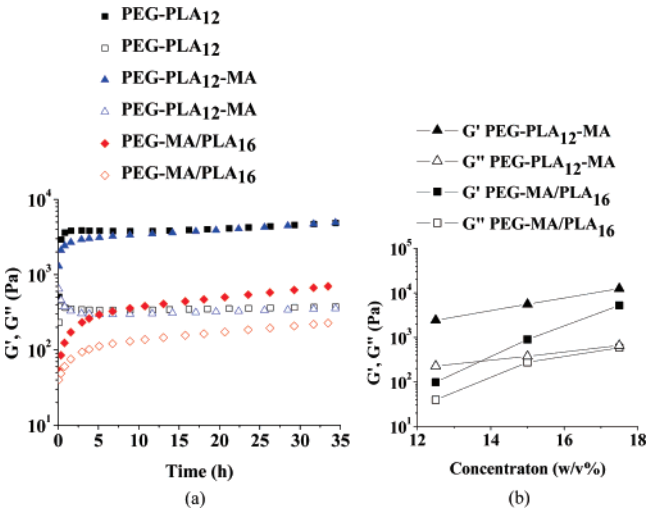

Figure 2. Storage modulus (G', closed symbols) and loss modulus (G'', open symbols) of stereohydrogels containing equimolar amounts of PEG−$PLLA_{12}$ and PEG−$PDLA_{12}$, PEG−$PLLA_{12}$−MA and PEG−$PDLA_{12}$−MA, or PEG−MA/$PLLA_{12}$ and PEG−MA/$PDLA_{12}$ star block copolymers in HEPES-buffered saline (pH 7) at 37 °C: (a) PEG−PLA_{12}, PEG−PLA_{12}−MA, and PEG−MA/PLA_{16} at 15% (w/v) polymer concentration as a function of time; (b) PEG−PLA_{12}−MA and PEG−MA/PLA_{16}, 48 h after mixing as a function of the polymer concentration.

Rheology. The mechanical properties of stereohydrogels were studied by rheological experiments at 37 °C. Stereohydrogels were prepared by mixing aqueous solutions of equimolar amounts of PEG−$PLLA_{12}$ and PEG−$PDLA_{12}$, PEG−$PLLA_{12}$−MA and PEG−$PDLA_{12}$−MA, or PEG−MA/$PLLA_{12}$ and PEG−MA/$PDLA_{12}$ star block copolymers in HEPES-buffered saline (pH 7) in a polymer concentration range of 12.5−17.5% (w/v). After mixing, the solutions were quickly applied to the rheometer, and the evolution of the storage modulus (G') and loss modulus (G'') was recorded (Figure 2a). Due to fast gelation, the gelation point of PEG−PLA_{12}, PEG−PLA_{12}−MA, and PEG−MA/PLA_{16} in a polymer concentration range of 12.5−17.5% (w/v) could not be determined by rheology. After application of the sample on the rheometer, ca. 1−2 min was needed to set the instrument before the measurement was started. This shows that stereohydrogels of PEG−PLA_{12}, PEG−PLA_{12}−MA, and PEG−MA/PLA_{16} were formed within 1−2 min.

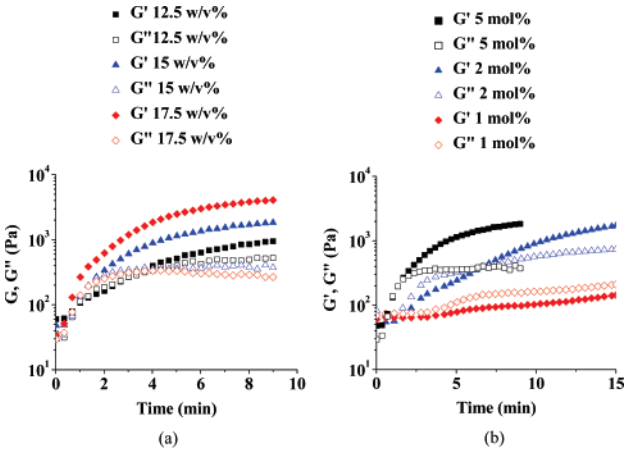

Figure 3. Storage modulus (G') and loss modulus (G'') as a function of UV irradiation time (350−400 nm, 16 mW/cm^2) of PEG−PLLA$_{12}$−MA solutions in HEPES-buffered saline (pH 7) at 37 °C: (a) 12.5%, 15%, and 17.5% (w/v) polymer concentration and 5 mol % initiator concentration (with respect to the methacrylate groups); (b) 1, 2, and 5 mol % initiator concentration and 15% (w/v) polymer concentration.

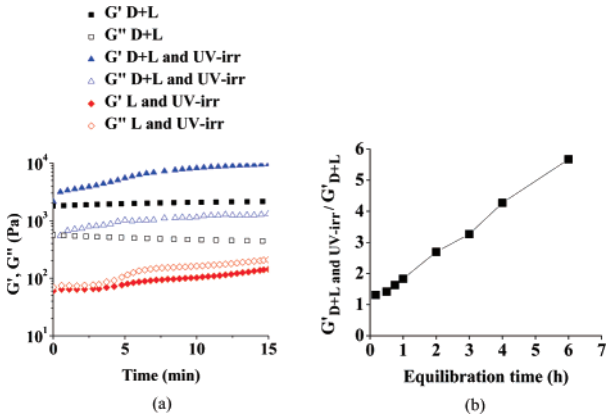

Figure 4. Rheology of UV-irradiated (350−400 nm, 16 mW/cm^2) PEG−PLA$_{12}$−MA in HEPES-buffered saline (pH 7) at 15% (w/v) polymer concentration and 37 °C: (a) storage modulus (G') and loss modulus (G'') as a function of time of a stereohydrogel (D + L) and a stereo−photohydrogel (D + L and UV-irradiated) after 10 min of stereocomplex equilibration and a UV-irradiated PEG−PLLA$_{12}$−MA solution (L) at 1 mol % initiator concentration (with respect to the methacrylate groups); (b) ratio of the storage modulus plateau value of a stereo−photohydrogel ($G'_{\text{D+L and UV−irradiated}}$) and the storage modulus plateau value of a stereohydrogel ($G'_{\text{D+L}}$) after 8 min of UV irradiation as a function of the stereocomplexation equilibration time.

The storage modulus increased in time due to the ongoing stereocomplexation, until reaching a plateau value, marking the end of the cross-linking process (Figure 2a). Figure 2a shows that the storage modulus evolutions and plateau values of PEG−PLA$_{12}$−MA and PEG−PLA$_{12}$ copolymers were similar, which agrees well with the vial tilting tests, indicating that the methacrylate groups hardly influence the stereocomplexation (Table 2). For PEG−PLA$_{12}$ and PEG−PLA$_{12}$−MA copolymers the storage modulus plateau value was reached within ca. 5 h after mixing (Figure 2a). In contrast, the storage moduli of PEG−MA/PLA$_{16}$ stereohydrogels continuously increased over 48 h. The storage moduli of the stereohydrogels increased from 2.4 to 12.5 kPa for PEG−PLA$_{12}$−MA and from 0.1 to 5.2 kPa for PEG−MA/PLA$_{16}$, upon increasing the polymer concentration from 12.5% to 15% (w/v) (Figure 2b). The PEG−PLA$_{12}$−MA stereohydrogels showed lower damping factors (tan $\delta = G''/G'$) compared to the PEG−MA/PLA$_{16}$ stereohydrogels (Figure 2b), indicating a higher network perfection (i.e., lower contribution of viscous components, such as dangling ends and loops).[56]

In Situ Monitoring of Mechanical Properties during Photopolymerization. The mechanical properties of photopolymerized hydrogels were determined by in situ rheology and UV irradiation (350−400 nm, 16 mW/cm^2) of PEG−PLA$_{12}$−MA or PEG−MA/PLA$_{16}$ stereohydrogels (yielding stereo−photohydrogels) or solutions of PEG−PLLA$_{12}$−MA or PEG−MA/PLLA$_{16}$ single enantiomers (yielding photohydrogels) in HEPES-buffered saline (pH 7) at 37 °C (Figures 3 and 4).

Figure 3a shows that the gelation time of the PEG−PLLA$_{12}$−MA single enantiomer decreased from ca. 3 to 0.5 min upon increasing the polymer concentration from 12.5% to 17.5% (w/v) at 5 mol % initiator concentration (with respect to the methacrylate groups). The storage modulus plateau value was reached within ca. 8 min and increased from 0.9 to 4.1 kPa upon increasing the polymer concentration from 12.5% to 17.5% (w/v) (Figure 3a). Figure 3b shows that the gelation time of the PEG−PLLA$_{12}$−MA single enantiomer at 15% (w/v) polymer concentration decreased rapidly with increasing initiator concentration. At initiator concentrations of 2 and 5 mol % (with respect to the methacrylate groups) the gelation times of the PEG−PLLA$_{12}$−MA single enantiomer were 6.5 and 1.7 min, respectively. At 1 mol % initiator concentration the 15% (w/v) PEG−PLLA$_{12}$−MA single enantiomer-solution did not gelate within 15 min (Figure 3b).

As shown earlier, a stereohydrogel was formed within 1−2 min after aqueous solutions of equimolar amounts of PEG−PLLA$_{12}$−MA and PEG−PDLA$_{12}$−MA copolymers were mixed. UV irradiation of the stereohydrogel at 1 mol % initiator and 15% (w/v) polymer concentration 10 min after mixing increased the storage modulus from 5.6 to 9.6 kPa within 15 min due to photopolymerization (Figure 4a). Here, an initiator concentration of 1 mol % (with respect to the methacrylate groups) corresponds to 0.003 wt %, which is very low compared to the commonly used concentration of 0.05 wt %.[49] Low initiator concentrations are preferred, due to toxicity of the initiator. The photopolymerization at this low initiator concentration implies in turn that low light intensities may be used to obtain stereo−photohydrogels.

The storage modulus of the stereo−photohydrogel is highly dependent on the stereocomplex equilibration time before UV irradiation. Figure 4b shows a plot of the ratio of the storage modulus of a PEG−PLA$_{12}$−MA stereo−photohydrogel and the storage modulus plateau value of the corresponding stereohydrogel (reached after ca. 5 h, Figure 2a) as a function of the stereocomplex equilibration time. The storage modulus plateau value of the stereo−photohydrogel (after 8 min of UV irradiation) increased linearly with increasing stereocomplex equilibration time at 15% (w/v) polymer concentration and 5 mol % initiator concentration (corresponding to 0.015 wt %). This initiator concentration is low compared to the generally used concentration of 0.05 wt %.[49] UV irradiation after 6 h of equilibration resulted in an almost 6-fold increase in the storage modulus of the PEG−PLA$_{12}$−MA stereo−photohydrogel compared to the corresponding PEG−PLA$_{12}$−MA stereohydrogel (31.6 vs 5.6 kPa) and a 17-fold increase compared to the

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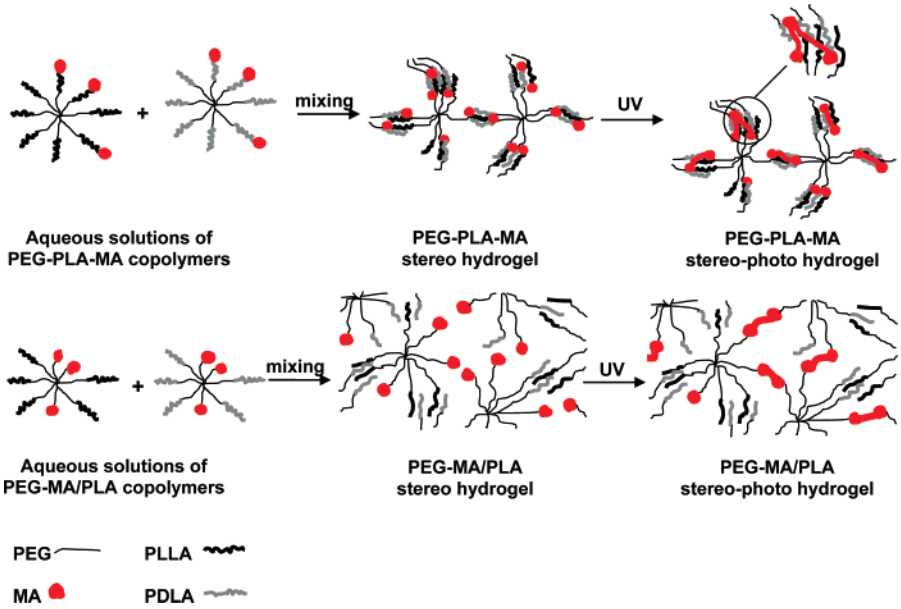

Figure 5. Schematic representation of the preparation of stereo and stereo−photohydrogels based on PEG−PLA−MA or PEG−MA/PLA star block copolymers.

corresponding PEG−$PLLA_{12}$−MA photohydrogel (31.6 vs 1.8 kPa). It should also be noted that the storage modulus of the stereo−photohydrogel (31.6 kPa) is much higher than the added value of the corresponding stereohydrogel (5.6 kPa) and the photohydrogel (1.8 kPa). Since the hydrophobic methacrylate groups are at the PLA chain ends, the chemical cross-links are most probably formed in the PLA domains. A schematic representation of the stereo- and stereo−photohydrogel preparation for PEG−PLA−MA and PEG−MA/PLA copolymers is shown in Figure 5. Furthermore, the photoinitiator used, Irgacure 2959, is rather hydrophobic (the maximum concentration in water is 0.7 wt %[49]) and may therefore preferably partition into the hydrophobic PLA domains, thereby increasing the local initiator concentration and thus photopolymerization rate in these domains. Therefore, the increased storage modulus upon increased stereocomplex equilibration time may be due to the formation of more PLA domains, resulting in a more densely cross-linked network and increased photopolymerization conversion.

PEG−MA/PLA_{16} stereo−photohydrogels also showed much higher storage moduli compared to the corresponding PEG−$MA/PLLA_{16}$ stereo- or photohydrogels (results not shown). Therefore, combining stereocomplexation and photopolymerization may provide fast gelation in vitro and in vivo,[55] yielding hydrogels with good mechanical properties.

Morphology of Photopolymerized Hydrogels. To study the influence of stereocomplexation on the morphology of photopolymerized hydrogels, SEM measurements were performed on freeze-dried PEG−PLA_{12}−MA and PEG−MA/PLA_{16} stereo−photo- and photohydrogels. The stereo−photo- and photohydrogels were prepared by UVA irradiation (250 mW/cm^2) of PEG−PLA_{12}−MA or PEG−MA/PLA_{16} stereohydrogels (equilibrated for ca. 15 min after the enantiomeric solutions were mixed) and solutions of PEG−$PLLA_{12}$−MA or PEG−$MA/PLLA_{16}$ single enantiomers, respectively, in HEPES-buffered saline (pH 7) at 8 mol % initiator and 15% (w/v) polymer concentration. Parts A and B of Figure 6 show that PEG−PLA_{12}−MA stereo−photohydrogels have pore sizes of ca. 5 μm, while PEG−$PLLA_{12}$−MA photohydrogels have pore sizes of ca. 10 μm, indicating that stereocomplexation has a significant influence on the pore size of the freeze-dried PEG−PLA−MA hydrogels. In contrast, PEG−MA/PLA_{16} stereo−photohydrogels and PEG−$MA/PLLA_{16}$ photohydrogels showed similar pore sizes (ca. 10 μm, Figure 6C,D). Apparently, the position of the cross-linking group has much influence on the pore size of freeze-dried stereo−photohydrogels.

Hydrogel Swelling and Degradation. Hydrogels based on PEG−PLA−MA or PEG−MA/PLA copolymers were degradable under physiological conditions. To study the rate of degradation, stereo−photo- and photohydrogels were prepared by UVA irradiation (250 mW/cm^2) of PEG−PLA_{12}−MA or PEG−MA/PLA_{16} stereohydrogels (equilibrated for ca. 15 min after the enantiomeric solutions were mixed) and solutions containing PEG−$PLLA_{12}$−MA or PEG−$MA/PLLA_{16}$ single enantiomers, respectively, in HEPES-buffered saline (pH 7) at 8 mol % initiator concentration. After the hydrogels were formed, HEPES-buffered saline was applied on top, and the gels were allowed to swell at 37 °C. At regular time intervals, the swelling ratio was calculated by ratioing the swollen hydrogel weight after exposure to buffer to the initial hydrogel weight after preparation (W_t/W_0). Figure 7a shows that the PEG−PLA_{12}−MA stereo−photohydrogels swelled to ca. twice their initial weight within 1 day, independent of the polymer concentration. The swelling ratio of PEG−$PLLA_{12}$−MA photohydrogels also doubled after 1 day at 15% (w/v) polymer concentration (Figure 7a). After the initial swelling, the swelling ratio remained constant for the PEG−PLA_{12}−MA stereo−photohydrogels, while the swelling ratio of PEG−$PLLA_{12}$−MA photohydrogels continued to increase. In time, both hydrogels disintegrated, as shown by the decreasing swelling ratio, until they finally dissolved completely. The degradation time is defined as the time required to completely dissolve at least one of the two or three hydrogels used for testing one type of hydrogel. Figure 7a shows that the PEG−PLA_{12}−MA stereo−photohydrogels were completely degraded after ca. 3 weeks and increasing the polymer concentration from 12.5% to 17.5% (w/v) hardly affected the degradation time. Interestingly, the degradation time of the PEG−PLA_{12}−MA stereohydrogels was

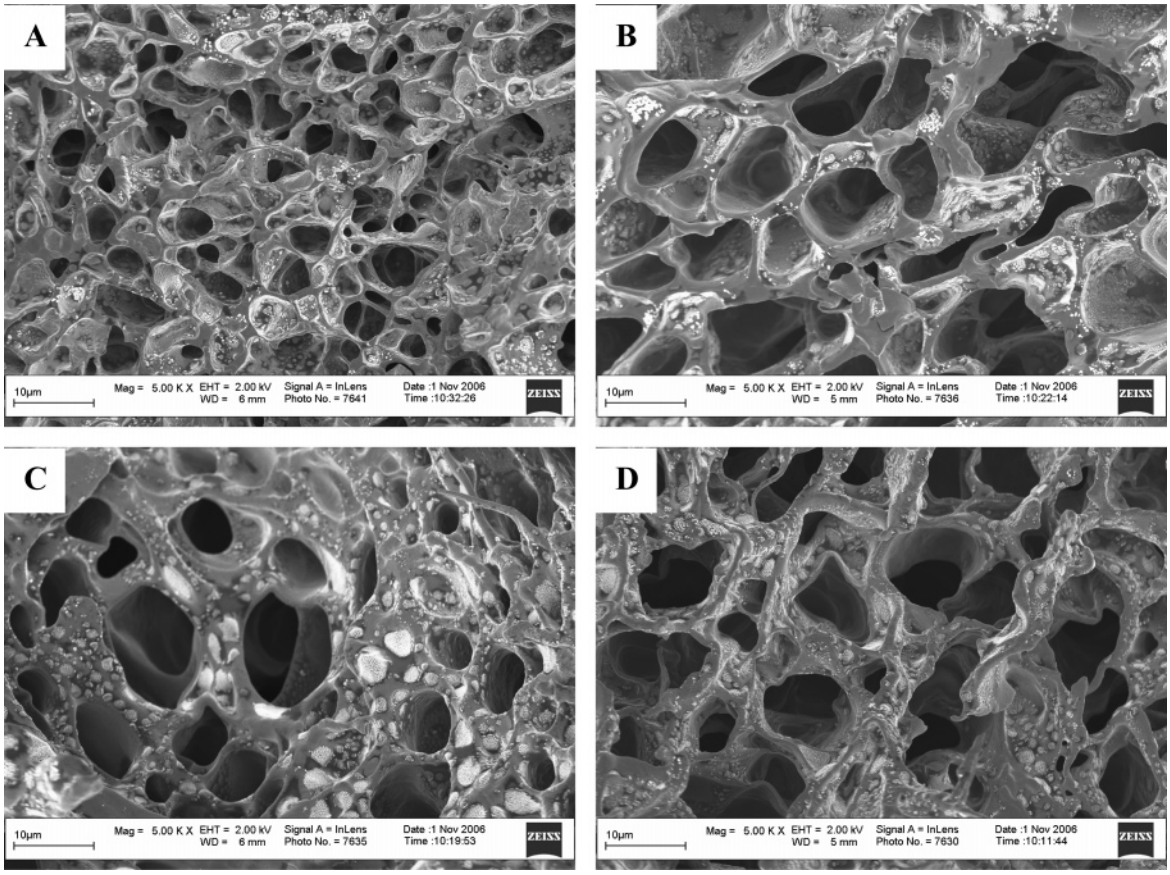

Figure 6. SEM photos of freeze-dried photopolymerized hydrogels prepared in HEPES-buffered saline (pH 7) at 15% (w/v) polymer concentration and 8 mol % initiator concentration (with respect to the methacrylate groups) by UVA irradiation for 10 min (stereohydrogels were equilibrated for ca. 15 min after mixing of the enantiomeric solutions): (A) PEG−PLA_{12}−MA stereo−photohydrogel; (B) PEG−$PLLA_{12}$−MA photohydrogel; (C) PEG−MA/PLA_{16} stereo−photohydrogel; (D) PEG−MA/$PLLA_{16}$ photohydrogel.

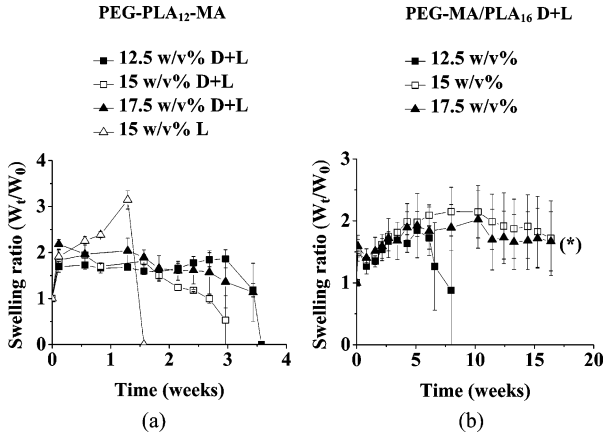

Figure 7. Swelling ratio (W_t/W_0) profiles of photopolymerized hydrogels prepared in HEPES-buffered saline (pH 7) at 8 mol % initiator concentration (with respect to the methacrylate groups) and 37 °C by UVA irradiation for 10 min (stereohydrogels were equilibrated for ca. 15 min after mixing of the enantiomeric solutions): (a) PEG−PLA_{12}−MA stereo−photohydrogels at 12.5%, 15%, and 17.5% (w/v) polymer concentration and PEG−$PLLA_{12}$−MA photohydrogels at 15% (w/v) polymer concentration; (b) PEG−MA/PLA_{16} stereo−photohydrogels at 12.5%, 15%, and 17.5% (w/v) polymer concentration. (*) PEG−MA/PLA_{16} stereo−photohydrogels at 15% and 17.5% (w/v) polymer concentration retained their integrity after 16 weeks.

twice as high as that of the PEG−$PLLA_{12}$−MA photohydrogels (ca. 3 vs 1.5 weeks, Figure 7a). This may be due to a higher cross-linking density of PEG−PLA_{12}−MA stereo−photohydrogels compared to PEG−$PLLA_{12}$−MA photohydrogels, as was also shown by the rheology measurements. The PEG−MA/PLA_{16} stereo−photohydrogels swelled over a period of ca. 5 weeks until reaching ca. twice their initial weight, independent of the polymer concentration (Figure 7b). The ongoing swelling is most likely due to PLA degradation, upon which the physical cross-links are lost, resulting in a less densely cross-linked network held together by only chemical cross-links (Figure 8). PEG−MA/PLA_{16} stereo−photohydrogels with 12.5% (w/v) polymer concentration completely degraded after 7 weeks, while at 15% and 17.5% (w/v) polymer concentration the stereo−photohydrogels retained their integrity after 16 weeks.

The much slower degradation of the PEG−MA/PLA_{16} stereo−photohydrogels compared to the PEG−PLA_{12}−MA stereo−photohydrogels is attributed to the slower hydrolysis of ester bonds of the polymerized methacrylate groups with a hydrolytically stable PEG chain compared to the ester bonds of the PLA blocks, which correlates well with the results obtained by Bryant et al. for photopolymerized PEG dimethacrylate and PEG−PLA dimethacrylate hydrogels.[57] PEG−PLA−MA stereo−photohydrogels degrade mainly through hydrolysis of the ester bonds in the PLA block, upon which both physical and chemical cross-links are lost (Figure 8). In contrast, PLA degradation in the PEG−MA/PLA stereo−photohydrogels leads to the formation of a less densely, chemically cross-linked network with increased swelling (Figure 8). The swollen PEG−MA/PLA stereo−photohydrogels finally degrade through hy-

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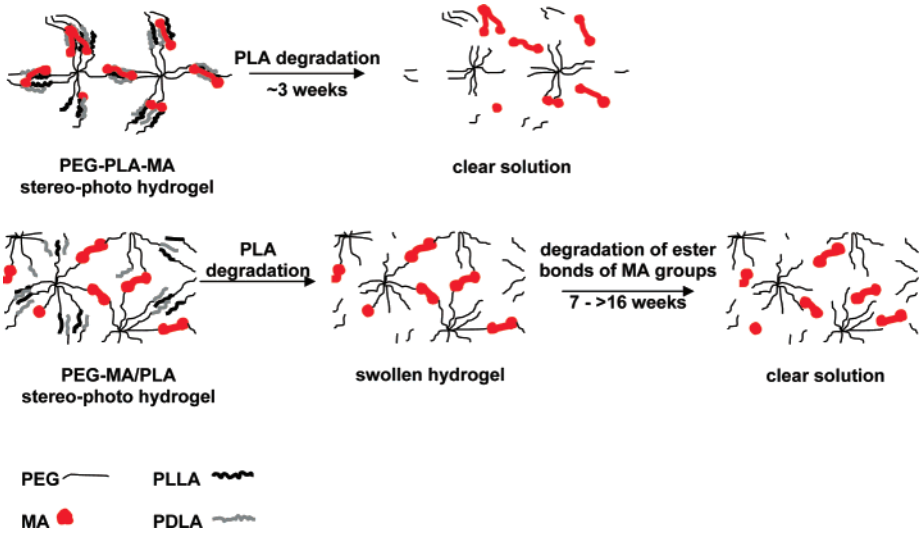

Figure 8. Schematic representation of the degradation of stereo−photohydrogels based on PEG−PLA−MA or PEG−MA/PLA star block copolymers.

drolysis of the ester bonds of the polymerized methacrylate groups. It is possible to combine PEG−PLA−MA and PEG−MA/PLA copolymers to vary the degradation time.

Conclusions

PEG−PLA−MA copolymers were prepared by methacrylation of ca. 40% of the PLA hydroxyl end groups of eight-arm PEG−PLA star block copolymers. PEG−MA/PLA copolymers were prepared by ring-opening polymerization of lactide initiated by eight-arm star PEG with 40% of its hydroxyl end groups methacrylated. PEG−PLA−MA and PEG−MA/PLA stereocomplexed hydrogels could be rapidly formed in situ upon mixing aqueous solutions containing equimolar amounts of PEG−PLLA−MA and PEG−PDLA−MA or PEG−MA/PLLA and PEG−MA/PDLA copolymers. Interestingly, stereocomplexation aided in the photopolymerization of the methacrylate groups. Photopolymerization of stereohydrogels, yielding stereo−photohydrogels, resulted in increased hydrogel storage moduli, compared to those of the hydrogels cross-linked by only stereocomplexation (stereohydrogels) or only photopolymerization (photohydrogels). Moreover, photopolymerization of stereohydrogels already took place at very low initiator concentrations. The degradation time of PEG−PLA−MA stereo−photohydrogels was doubled compared to that of PEG−PLLA−MA photohydrogels (ca. 3 vs 1.5 weeks). PEG−MA/PLA stereo−photohydrogels degraded within ca. 7 to over 16 weeks, depending on the polymer concentration. In principle, PEG−PLA−MA and PEG−MA/PLA may be combined to vary the hydrogel degradation rate. To our knowledge, this is the first paper on fast in situ forming hydrogels by combined cross-linking via photopolymerization and physical interactions. The fast gelation in vitro and in vivo due to stereocomplexation circumvents the need for fast photopolymerization, thus preventing substantial heat effects due to the photopolymerization and potentiating the use of low initiator concentrations and low light intensities. Moreover, the fast gelation allows for easy handling. The combination of stereocomplexation and photopolymerization is a novel approach to obtain fast in situ forming and robust hydrogels, which have a high potential for in vivo applications, including tissue engineering and drug delivery.

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