European Polymer Journal 94 (2017) 152–161

Contents lists available at ScienceDirect

European Polymer Journal

journal homepage: www.elsevier.com/locate/europolj

In situ forming stereocomplexed and post-photocrosslinked acrylated star poly(ethylene glycol)-poly(lactide) hydrogels

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ARTICLE INFO

Keywords:
Tandem gelation
Stereocomplexation
Photocrosslinking
PEG-PLA
Star block copolymer

ABSTRACT

Biodegradable acrylate end-group functionalized poly(ethylene glycol)-poly(lactide) (PEG-PLA) star block copolymer hydrogels were formed by the consecutive physical gelation through stereocomplexation of star shaped PEG-$(PLLA)_8$ and PEG-$(PDLA)_8$ enantiomers and UV photopolymerization. The 8-armed PEG-PLA star block copolymers were prepared by ring opening polymerization of lactide onto an amine end-group functionalized PEG with a molecular weight of 20 kg/mol using stannous octoate as a catalyst. The degree of polymerization of the PLA blocks was 12 lactyl units and the end hydroxyl groups were reacted with acryloyl chloride to give the required acrylate end groups. Aqueous solutions of enantiomeric mixtures of the PEG-$(PLA)_8$ macromonomers formed physically crosslinked hydrogels above a critical gel concentration of 4 w/v%. Subsequent photopolymerization at 365 nm in the presence of Irgacure 2959 resulted in gels with improved mechanical properties and hydrolytic stability. With 40% polymer mass loss after 45 d *in vitro*, these hydrogels show excellent resistance against hydrolytic degradation and dissolution, which is believed to result from the combination of stable amide linkages between the PEG and PLA blocks and the high physical and chemical crosslink density owing to the star architecture.

1. Introduction

Hydrogels are highly water swollen polymer networks whose properties resemble those of natural soft tissues [1–3]. Biodegradable poly(ethylene glycol)-poly(lactide) (PEG-PLA) type hydrogels generally exhibit excellent biocompatibility and are accordingly widely investigated for their use in biomedical applications such as tissue engineering and systems for controlled delivery of biologically active agents. Thermo-sensitive amphiphilic block copolymers form hydrogels through physical crosslinking. Depending on their molecular architecture and molecular weight they provide a sol to gel transition upon a decrease or increase in temperature. Such systems offer the advantages of a simple injection method when the sol to gel transition is close to body temperature. In this way surgical procedures may be omitted, the shape can be properly adapted and cells or proteins can be easily incorporated [4–6]. Physical crosslinks, including stereocomplexation between enantiomeric PDLA and PLLA blocks in amphiphilic block copolymers [7,8], can be formed under mild conditions, but the resulting hydrogels are generally degraded and/or dissolved relatively fast. Alternatively, chemically crosslinked hydrogels have been prepared from various combinations of macromonomers

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http://dx.doi.org/10.1016/j.eurpolymj.2017.07.002
Received 18 January 2017; Received in revised form 17 March 2017; Accepted 5 July 2017
Available online 06 July 2017
0014-3057/

endcapped with reactive groups. Well-known examples are hydrogels formed by Michael addition between thiols and vinylic groups [9,10] or by reaction between activated esters and amines [11,12]. Most often, however, chemically crosslinked hydrogels are prepared by photocrosslinking of (meth)acrylate endcapped block copolymers with the aid of UV or visible light. Pioneering work was conducted by the group of Hubbell, who prepared photocrosslinked hydrogels from end acrylated PLA-PEG-PLA and PLGA-PEG-PLGA triblock copolymers [13]. The degradation times of the hydrogels could be tuned from 1 to 120 d by altering the molecular weight of the PEG and the length and composition of the hydrophobic polyester block. West et al. reported on a photocrosslinked hydrogel based on PEG diacrylate that was rendered biodegradable by incorporation of a collagenase sensitive peptide sequence in the network [14]. Fibroblasts were encapsulated successfully in the hydrogel by exposing a cell containing macromonomer solution briefly to UV light. An acrylated RGD (arginine-glycine-aspartic acid) cell adhesive peptide was incorporated by light-induced reaction with remaining acrylate groups in a partially crosslinked PEG diacrylate network. By using a photolithographic technique, the precise location of RGD could be dictated and cells exhibited guided three-dimensional migration only into the RGD-patterned regions of the hydrogels. The group of Anseth prepared various photocrosslinked hydrogels based on (meth)acrylate terminated PEG copolymers. It was demonstrated that the macroscopic properties and degradation of hydrogels prepared from PLA-PEG-PLA triblock copolymers endcapped with acrylate moieties could be tuned by altering the polymerization conditions [15]. Furthermore, they showed that a hydrogel composed of a mixture of degradable and non-degradable PEG diacrylate macromonomers can serve as a scaffold for the engineering of cartilage [16]. After implantation of the construct in mice, encapsulated chondrocytes were capable of survival and proliferation and newly formed tissue was integrated with surrounding native cartilage over time. Photocrosslinked hydrogels have also been used for the controlled release of various hydrophobic and hydrophilic drugs, including hydrogels based on methacrylate terminated PEG [17] and PEG-poly(ε-caprolactone) multiblock copolymers [18]. In both systems, the drug release could be regulated via the composition of the hydrogel.

It is important that during the gelation process no flow of macromonomers or collapse of the gel takes place [19]. This issue has been addressed by a few research groups by combining physical and chemical crosslinking. Most dual gelling systems reported to date are based on thermosensitive polymers, such as poly(N-isopropylacrylamide) (pNIPAAm), poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (commercially known as Pluronics) and poly(N-(2-hydroxypropyl)methacrylamide) (pHPMA) derivatives, which are modified with various chemically functional groups for post-gelation reactions [20]. For example, thermally induced physical crosslinking of an acrylated Pluronic was used to provide fast gelation [21]. The gel was then photocrosslinked to give a highly stable gel. More recently, Vermonden et al. combined temperature-induced physical gelation with native chemical ligation as a chemoselective crosslink strategy [22]. Triblock copolymers consisting of cysteine functionalities, thermo-responsive NIPAAm units and degradable moieties were mixed with thioester or N-hydroxysuccinimide functionalized PEG crosslinkers. The combined physical and chemical crosslinking resulted in fast network formation and mechanically strong hydrogels. These studies show that tandem gelation is a feasible concept, but the field is still in its infancy. For example, a controlled degradation is an important item that has yet received little attention in the design of dual gelling hydrogels.

Recently we showed that the mechanical stability and most importantly the degradation of star block copolymers of PEG and PLLA can be controlled by replacing the highly hydrolytically labile linking ester bond with an amide bond [23,24]. Furthermore it was demonstrated that stereocomplexation and photocrosslinking of methacrylate terminated enantiomeric PEG-PDLA and PEG-PLLA star block copolymers allows for respectively *in situ* formation of physical hydrogels and their subsequent chemical stabilization [25]. Although these stereocomplexed & photocrosslinked hydrogels were relatively stable with a degradation time of 3 weeks *in vitro*, for some applications, such as long-term drug delivery, a higher resistance against hydrolytic degradation is required. Inspired by the promising results of the two aforementioned papers, we hypothesized that stereocomplexation and photocrosslinking of PEG-PLA star block copolymers with amide groups between PEG and PLA may provide a tandem gelling system with a high resistance against hydrolytic degradation. In the present paper, we describe the synthesis and characterization of novel acrylate-terminated amide-linked PEG-PDLA and PEG-PLLA star block copolymers. The physical, mechanical and degradation properties of stereocomplexed and photocrosslinked hydrogels prepared from these macromonomers are presented with a particular focus on the concentration dependence of the gelation properties. Furthermore we propose a gelation mechanism for the formation and *in vitro* degradation of the stereocomplexed & photocrosslinked hydrogels starting from enantiomeric PEG-PDLA and PEG-PLLA star block copolymer solutions.

In summary, we show that stereocomplexation of these new macromonomers in aqueous solution and subsequent UV-initiated radical polymerization of the terminal acrylate groups result in hydrogels with excellent mechanical properties and a degradation time of several months, confirming our hypothesis that this system may be well applicable as an injectable, robust hydrogel with a high resistance against hydrolytic degradation.

2. Experimental section

2.1. Materials

Hydroxyl terminated 8-armed poly(ethylene glycol) (PEG-$(OH)_8$, $M_{n,NMR}$ = 21,400 g/mol) was purchased from Jenkem (Allen, Texas, USA) and purified before use by dissolution in dichloromethane and precipitation in cold diethyl ether. PEG-$(OH)_8$ was converted to PEG-$(NH_2)_8$ using a two-step procedure analogous to that described by Elbert et al. for linear hydroxyl terminated PEGs [26]. Eight-armed PEG-PDLA and PEG-PLLA star block copolymers with a PEG core and 12 lactyl units in each PLA block (PEG-$(PDLA_{12})_8$ and PEG-$(PLLA_{12})_8$ respectively) as well as their acrylate end functionalized analogs (PEG-$(PDLA_{12})_8$-AC and PEG-$(PLLA_{12})_8$-AC) were synthesized as described previously [23,27]. L-lactide and D-lactide were obtained from Corbion Purac

(Gorinchem, the Netherlands). Acryloyl chloride, methanesulfonyl chloride (mesyl chloride), tin(II) 2-ethylhexanoate (stannous octoate), triethylamine (TEA) and 25% aqueous ammonia solution were from Sigma-Aldrich (St Louis, Missouri, USA). Irgacure 2959 was obtained from Ciba (Basel, Switzerland). Toluene, diethyl ether, methanol and dichloromethane were all from Biosolve (Valkenswaard, the Netherlands). Dichloromethane, TEA and toluene were dried over calcium hydride, potassium hydroxide and sodium, respectively, and distilled prior to use.

2.2. Synthesis

Photocrosslinked PEG-PLA hydrogels were prepared by UV irradiation of aqueous solutions of acrylate terminated PEG-PLA star block copolymers. Typically, distilled water containing 10 mol% photoinitiator (Irgacure 2959) relative to acrylate groups was added to a mixture of PEG-$(PDLA_{12})_8$-AC and PEG-$(PLLA_{12})_8$-AC (D/L ratio 1/1). The system was heated repeatedly and subsequently equilibrated for 48 h at room temperature. The mixture was finally irradiated by UV light (~5 mW/cm^2) at 365 nm for 30 min in an inert atmosphere.

2.3. Characterization

^{1}H NMR (300 MHz) spectra were recorded on a Varian Inova 300 NMR spectrometer. Polymers were dissolved in $CDCl_3$ at a concentration of 15 mg/ml.

Thermal properties of polymers were determined using differential scanning calorimetry (DSC). Heating and cooling rates of 20 °C/min were applied. Samples were heated from 25 to 200 °C, kept at 200 °C for 1 min, cooled to −50 °C, kept at −50 °C for 1 min, and finally heated to 200 °C. Crystallization temperatures (T_c) and corresponding enthalpies (ΔH_c) were obtained from the cooling scan, while melting temperatures (T_m) and enthalpies (ΔH_m) were obtained from the second heating scan.

2.4. Self-assembly of PEG-PLA star block copolymers in water

Dynamic light scattering (DLS) of dilute (0.5 w/v%) solutions of PEG-$(PLLA_{12})_8$, PEG-$(PDLA_{12})_8$/PEG-$(PLLA_{12})_8$ and PEG-$(PDLA_{12})_8$-AC/PEG-$(PLLA_{12})_8$-AC (D/L ratios 1/1) in distilled water was performed to determine aggregate sizes. Repeated heating cycles were applied to dissolve the polymers. After dissolution, the samples were equilibrated for at least 24 h. Experiments were carried out at 25 °C using a Malvern Nano ZS, a laser wavelength of 633 nm and a scattering angle of 173°.

2.5. Gel properties

Oscillatory rheology experiments were performed to determine the mechanical properties of the photocrosslinked hydrogels. The storage (G′) and loss (G″) modulus of hydrogels were monitored for 30 min at 25 °C on an Anton-Paar Physica MCR 301 rheometer. Experiments were performed using a flat plate measuring geometry (diameter 25 mm, gap 0.3 mm) utilizing a strain of 1% and a frequency of 1 Hz. A strain of 1% was found to be in the linear visco-elastic range for closely related systems i.e. stereocomplexed and photocrosslinked PEG-(OCO)-$(PLA_{12})_8$-METHAC hydrogels [25] and stereocomplexed PEG-(NHCO)-$(PLA_{12})_8$ hydrogels [24]. To prevent water evaporation, a solvent trap was placed over the geometry.

Gravimetric degradation/dissolution experiments were performed to determine the stability of the hydrogels. Freshly prepared semi-spherical hydrogel samples (radius 5 mm) were dried in air, their initial weight W_0 was determined and the samples were immersed in PBS at 37 °C. To prevent bacterial growth, 0.02 w/v% NaN_3 was added to the buffer solution. At regular times, samples were taken out and their mass in swollen state (W_s) was measured after wiping the surface with tissue paper. Subsequently, the samples were allowed to dry in air overnight to yield the dry weight (W_D). The degree of swelling during degradation was calculated from:

$$\text{degree of swelling} = \frac{(W_S - W_D)}{W_D} \cdot 100\% \quad (1)$$

remaining relative polymer mass during degradation was calculated from:

$$\text{relative polymer mass} = \frac{W_D}{W_0} \cdot 100\% \quad (2)$$

3. Results and discussion

3.1. Synthesis and characterization of acrylate terminated macromonomers

PEG-$(PDLA_{12})_8$ and PEG-$(PLLA_{12})_8$ star block copolymers with an amide linkage between the PEG and PLA blocks were prepared by the stannous octoate catalyzed ring opening polymerization of D-lactide and L-lactide, respectively, initiated by PEG-$(NH_2)_8$ (Fig. 1). In the ^{1}H NMR spectrum (Fig. 2, top) a signal at 6.5 ppm confirms the presence of amide groups, whereas no peaks were found relating to unreacted amine groups. Since the signal relating to amine end groups (2.94 ppm) is well visible in the ^{1}H NMR spectrum of PEG-$(NH_2)_8$ in $CDCl_3$ [28], this demonstrates that each of the 8 amine groups initiated the ROP of lactide. An average

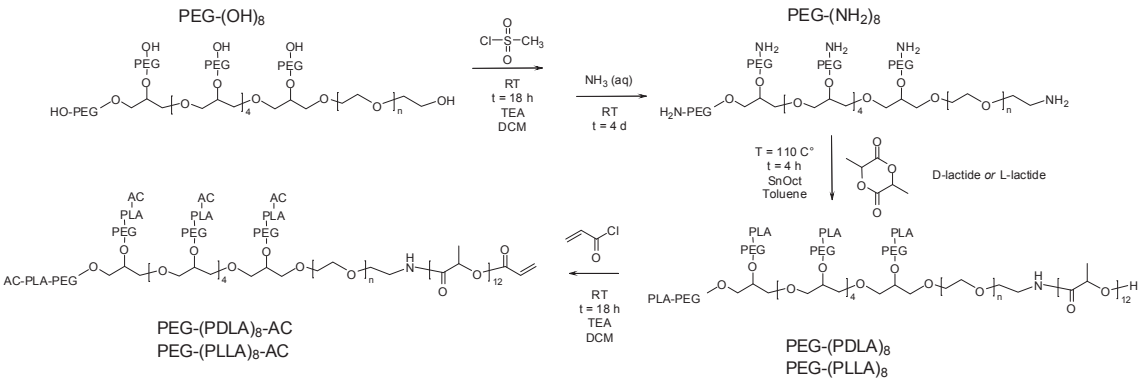

Fig. 1. Synthesis scheme for the preparation of PEG-$(PDLA_{12})_8$ and PEG-$(PLLA_{12})_8$ star block copolymers and their acrylate end group functionalized analogs PEG-$(PDLA_{12})_8$-AC and PEG-$(PLLA_{12})_8$-AC.

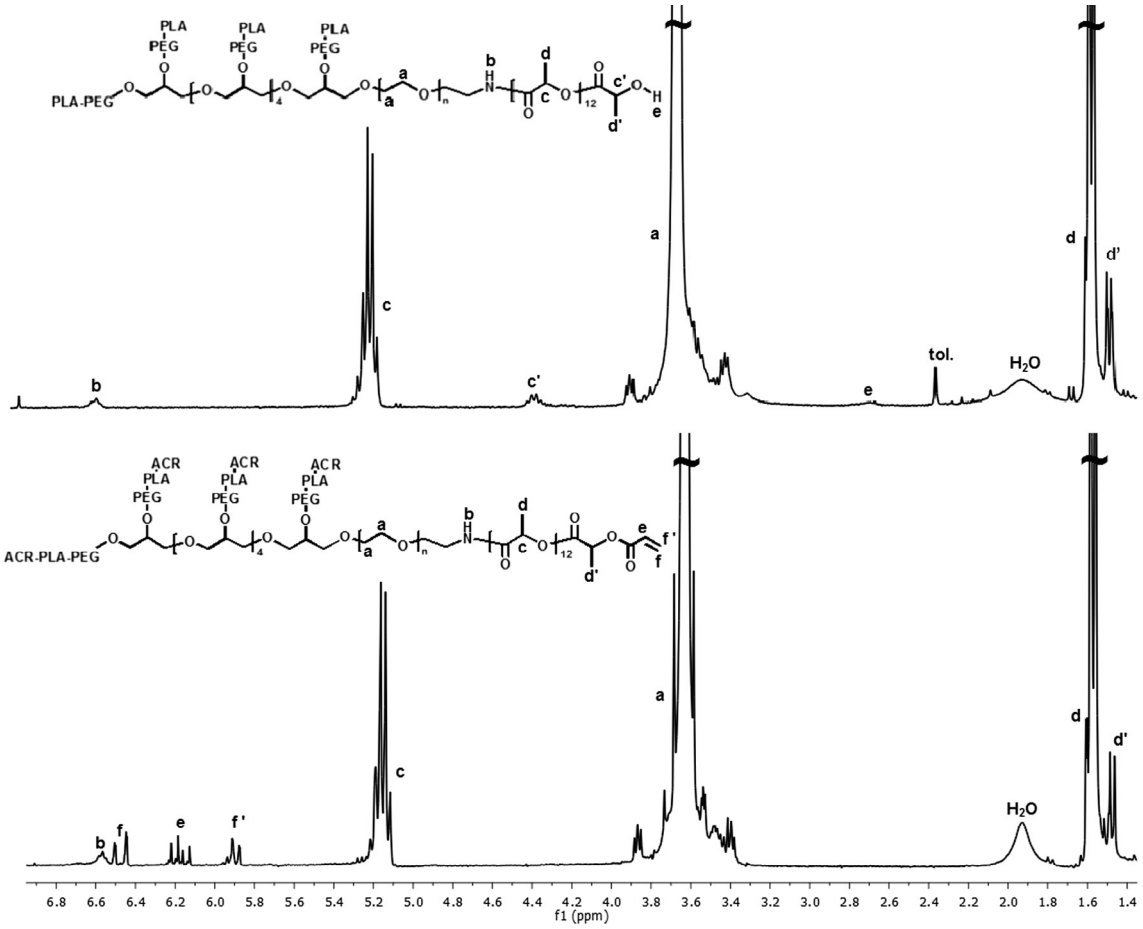

Fig. 2. ^{1}H NMR spectra of PEG-$(PLLA_{12})_8$ (top) and PEG-$(PLLA_{12})_8$-AC (bottom). Solvent: $CDCl_3$.

PLA block length of 12 lactyl units per arm was calculated using the integrals of peaks corresponding to the methine protons of the lactyl units and the main chain protons of PEG. Higher DPs led to a reduced solubility in water whereas lower DPs have been shown to hamper stereocomplex formation, with eleven lactyl units being the minimum required for gelation by stereocomplexation [29].

The PEG-$(PDLA_{12})_8$ and PEG-$(PLLA_{12})_8$ star block copolymers were functionalized with acrylate groups by reaction with acryloyl chloride in dichloromethane as reported previously [27]. Acrylate end groups were chosen over e.g. methacrylate groups because of their faster rate of free radical polymerization, allowing for shorter UV irradiation times. ^{1}H NMR spectroscopy confirmed the structure of PEG-$(PLA_{12})_8$-AC (Fig. 2). The signals at 2.68 and 4.35 ppm corresponding to the terminal hydroxyl and PLA methine protons, respectively, of the starting PEG-$(PLA_{12})_8$ completely disappeared, indicating quantitative end group conversion.

Table 1
Properties of PEG-$(OH)_8$, PEG-$(NH_2)_8$ macroinitiator and various PEG-PLA star block copolymers. Thermal properties and CGCs of copolymers refer to their enantiomeric mixtures.

Polymer	^{1}H NMR			T_m (°C)	ΔH_m (J/g)	T_c (°C)	ΔH_c (J/g)	CGC (w/v %)
	M_n (kg/mol)	DP[a]	Degree of functionalization (% acrylate end groups)					
PEG-$(OH)_8$	21.4			54	126	28	118	
PEG-$(NH_2)_8$	21.4			53	126	28	126	
PEG-$(PDLA_{12})_8$ + PEG-$(PLLA_{12})_8$	27.7	10.9		41	56	13	57	5
	28.3	11.9						
PEG-$(PDLA_{12})_8$-AC + PEG-$(PLLA_{12})_8$-AC	28.1	10.9	> 95	39	44	21	50	4
	28.7	11.9	> 95					

[a] Degree of polymerization of the PLA blocks, expressed in lactyl units per arm.

The thermal properties of enantiomeric mixtures of the block copolymers as determined with differential scanning calorimetry (DSC) revealed major transitions in the second heating and cooling scans, corresponding to melting and crystallization of the PEG domains, respectively (Table 1). The PEG-PLA star block copolymers exhibit lower melting transitions and accompanying enthalpies than their PEG precursors indicating that the crystallization of PEG is hampered by the presence of the PLA blocks. Functionalization of PEG-PLA with acrylate groups further impedes PEG crystallization as shown by the lower T_m and ΔH_m values of the enantiomeric PEG-$(PLA_{12})_8$-AC mixture. A melting transition of the PLA phase in the single enantiomer block copolymers was not observed. Occasionally, for the stereocomplexed PLA phase we could observe a melting transition at ~160 °C with a very small ΔH_m value depending on the heating and cooling rates [24].

3.2. Self-assembly and physical gelation of PEG-PLA star block copolymers in water

We previously demonstrated that 8-armed PEG-PLA star block copolymers self-assemble in water to form micelles and small aggregates with a hydrophobic PLA core and a hydrophilic PEG corona at concentrations above the critical association concentration (CAC) [23]. ^{1}H NMR analysis revealed that also PLA domains and free dangling PLA chains are present in the periphery of the aggregates [30]. The CACs of mixed enantiomers of PEG-$(PLA_n)_8$ are approximately 2–3 times lower than the values of the corresponding single enantiomers (~0.4 w/v%) due to the stronger interactions in the PEG-PLA stereocomplexes [24]. Using dynamic light scattering (DLS) the influence of stereocomplexation and end group acrylation on aggregate sizes and aggregate size distributions of PEG-PLA star block copolymers in water was investigated at a concentration of 0.5 w/v% (Fig. 3).

Above the CAC small aggregates with diameters ranging from 40 to 50 nm, indicative of micellar type aggregates, as well as larger aggregates with a diameter ranging from 100 to 300 nm were observed. Such bimodal distributions were also observed in previous investigations concerning single and mixed enantiomer PEG-$(PLA)_8$ star block copolymers [23,24]. It should be noted that the intensity of scattered light cannot directly be related to the number or volume of aggregates, because larger aggregates scatter light more intensely than smaller aggregates. The distributions presented in Fig. 3 show that stereocomplexation between enantiomeric block copolymers results in larger aggregates, similar to previous observations on stereocomplexed systems [24,31]. The volume fraction of these larger aggregates (0.4%, 17% and ~0.1% for PEG-$(PLLA_{12})_8$, PEG-$(PDLA_{12})_8$/PEG-$(PLLA_{12})_8$ and PEG-$(PDLA_{12})_8$-AC/PEG-$(PLLA_{12})_8$-AC, respectively) is still small compared to the small aggregates. Upon acrylation the size distribution of the aggregates shifts back to lower values, which is likely due to hampering of stereocomplexation caused by the modification of the PLA chain ends, as proposed earlier by our group [32]. In this paper we showed that the modification of the hydroxyl terminus lowered the number of lactic acid units within a PLA chain that can effectively participate in crystallization. Both the T_m and ΔH_m values for these polymers were lower compared to those of PLAs of comparable chain length with no modification of the hydroxyl end group. The suggested hampering of crystallization caused by the modification of both PLA chain ends was observed for both single

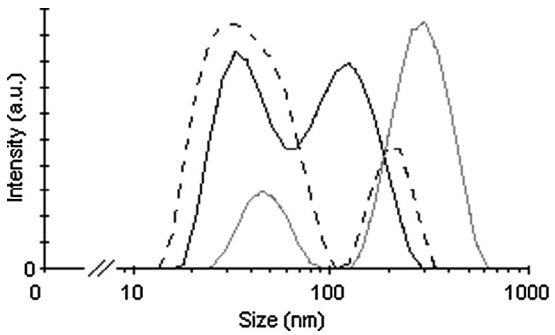

Fig. 3. Aggregate size distributions in 0.5 w/v% aqueous solutions at room temperature. PEG-$(PLLA_{12})_8$ (dashed line), PEG-$(PDLA_{12})_8$/PEG-$(PLLA_{12})_8$ (gray line), PEG-$(PDLA_{12})_8$-AC/PEG-$(PLLA_{12})_8$-AC (black line).

enantiomer and stereocomplexed PLAs.

The physical gelation of the 8-armed block copolymers was studied by dissolving an appropriate amount of PEG-$(PLLA_{12})_8$, PEG-$(PLLA_{12})_8$-ACR, PEG-$(PDLA_{12})_8$/PEG-$(PLLA_{12})_8$ (1:1 wt ratio) or PEG-$(PDLA_{12})_8$-AC/PEG-$(PLLA_{12})_8$-AC (1:1 wt ratio) in distilled water. A critical gelation concentration is observed for the PEG-$(PLLA_{12})_8$ and PEG-$(PLLA_{12})_8$-AC single enantiomers of 12 and 6 w/v %, respectively. The lower CGC after end group modification is due to increased hydrophobic interactions resulting from the acrylate groups. At these concentrations still a thermo-reversible gel-sol transition is observed (e.g. ~45 °C for 12 w/v% PEG-$(PLLA_{12})_8$). The stereocomplexed PEG-$(PDLA_{12})_8$/PEG-$(PLLA_{12})_8$ and PEG-$(PDLA_{12})_8$-AC/PEG-$(PLLA_{12})_8$-AC behave differently. Previously, it was shown that formation of stereocomplexed domains can be enhanced when the temperature is elevated [24,33]. Upon heating, the increased mobility of the PLA chains facilitates intermicellar and interaggregate bridging to a greater extent. The enhanced formation of stereocomplexes and the high stability of the stereocomplexed domains prohibit gel to sol transitions at elevated temperatures making these thermo-irreversible. Therefore, in the current research multiple heating cycles were applied, followed by an equilibration time of 48 h at room temperature, to allow an optimal formation of stereocomplexes. The minimum concentrations to form a hydrogel are 5 and 4 w/v% for the PEG-$(PDLA_{12})_8$/PEG-$(PLLA_{12})_8$ and PEG-$(PDLA_{12})_8$-AC/PEG-$(PLLA_{12})_8$-AC systems, respectively (Table 1). Below these concentrations the number and/or volume of micelles and aggregates is too low for efficient intermicellar and interaggregate bridging and no gels are formed. Consequently, an operation window exists in which *in situ* hydrogel formation is facilitated by mixing aqueous solutions of single enantiomers (steps 1 and 2 in Fig. 4).

Oscillatory rheology measurements revealed that 7 w/v% PEG-$(PDLA_{12})_8$/PEG-$(PLLA_{12})_8$ and 7 w/v% PEG-$(PDLA_{12})_8$-AC/PEG-$(PLLA_{12})_8$-AC hydrogels exhibit G′ values of 630 and 710 Pa and G″ values of 45 and 70 Pa, respectively. Although end group modification impairs stereocomplexation, as observed for dilute solutions in e.g. the DLS experiments (vide supra), at these higher, gel-forming concentrations the increased hydrophobicity of the PLA arms resulting from the acrylate groups prevails over the hampered stereocomplexation and lowers the CGC (Table 1) and enhances the gel stiffness at a given concentration.

The 7 w/v% PEG-$(PDLA_{12})_8$-AC/PEG-$(PLLA_{12})_8$-AC hydrogels furthermore showed a higher damping factor (tan δ = G″/G′) compared to the 7 w/v% PEG-$(PDLA_{12})_8$/PEG-$(PLLA_{12})_8$ hydrogels (0.10 vs. 0.07), in accordance with the more hydrophobic character upon acrylation. Similar results were observed previously for hydrogels based on methacrylated PEG and poly(2-hydroxyethylmethacrylate) (PHEMA), which exhibited higher damping factors upon increasing the hydrophobic i.e. PHEMA content [34]. This was ascribed to the high friction that occurs when water moves through hydrophobic nanopores within the gel upon application of an external mechanical pressure.

3.3. Photocrosslinked PEG-PLA hydrogels

Physically crosslinked stereocomplexed hydrogels of PEG-$(PDLA_{12})_8$-AC and PEG-$(PLLA_{12})_8$-AC were prepared at different concentrations in distilled water containing Irgacure 2959 as a photoinitiator. To stabilize the initially formed stereocomplexed hydrogel subsequent crosslinking by photopolymerization of the acrylate groups using UV irradiation was carried out (step 3 in Fig. 4). In preliminary experiments the effects of UV wavelength, reaction time and initiator concentration on the mechanical properties of the resulting hydrogels were determined by oscillatory rheology measurements. It followed that an initiator concentration of 10 mol% relative to acrylate groups, an UV irradiation time of 30 min and an UV irradiation wavelength of 365 nm resulted in the highest storage modulus. An initiator concentration of 10 mol% with respect to the acrylate groups corresponds to 0.06 wt% in case of a 10 w/v% hydrogel. In previous research a similar concentration of 0.05 wt% was shown to be cytocompatible [35]. An UV irradiation intensity of 5 mW/cm^2 was used, which is low compared to commonly used intensities for photopolymerized hydrogel systems. Although the highest storage modulus was obtained after 30 min, a substantial increase in G′ was already observed after a short irradiation time of 5 min.

Fig. 5 clearly demonstrates the improved mechanical properties resulting from photocrosslinking under the optimized conditions. The effect of the additional chemical crosslinks is more noticeable at lower concentrations where the physical crosslink density is relatively low. The increase in G′ with concentration can be ascribed to the formation of a more densely crosslinked network at higher concentrations. Photopolymerization of stereocomplexed 7 w/v% PEG-$(PDLA_{12})_8$-AC/PEG-$(PLLA_{12})_8$-AC hydrogels resulted in a decrease in tan δ from 0.10 to 0.07, indicating a more elastic (i.e. gel-like) behavior in accordance with the increased crosslink density. 7 w/v% photocrosslinked single enantiomer PEG-$(PLLA_{12})_8$-AC hydrogels exhibited a lower G′ than 7 w/v% PEG-$(PDLA_{12})_8$-AC/PEG-$(PLLA_{12})_8$-AC (4.3 vs. 10.3 kPa), confirming that the stabilizing stereocomplexes persist after photocrosslinking. These rheological experiments show that the mechanical properties of the photocrosslinked PEG-PLA hydrogels can be tuned within a broad range by altering the polymer concentration.

3.4. Hydrogel degradation

The *in vitro* degradation of stereocomplexed and photocrosslinked PEG-PLA hydrogels was investigated by a gravimetrical procedure. The polymer mass loss, as well as the swelling of the hydrogels during degradation was monitored up to 9 weeks (Fig. 6). PEG-$(PDLA_{12})_8$-AC/PEG-$(PLLA_{12})_8$-AC hydrogels (20 w/v% in water) were prepared as described above, photocrosslinked and the gels were immersed in PBS at 37 °C. At regular time intervals, hydrogels were taken out and their mass in the swollen state was determined. Subsequently, the hydrogels were allowed to dry overnight to yield the dry polymer weight after degradation. It follows from Fig. 6 that the stereocomplexed and photocrosslinked PEG-$(PLA_{12})_8$-AC hydrogels show excellent stability with approximately 40% polymer mass loss after 45 d *in vitro*. In sharp contrast, 14 w/v% PEG-$(PLA_{12})_8$ hydrogels crosslinked only by stereocomplexation completely dissolved after 30 d *in vitro* [24]. For closely related systems, i.e. PEG-(OCO)-$(PLA_{12})_8$-METHAC hydrogels, it was found

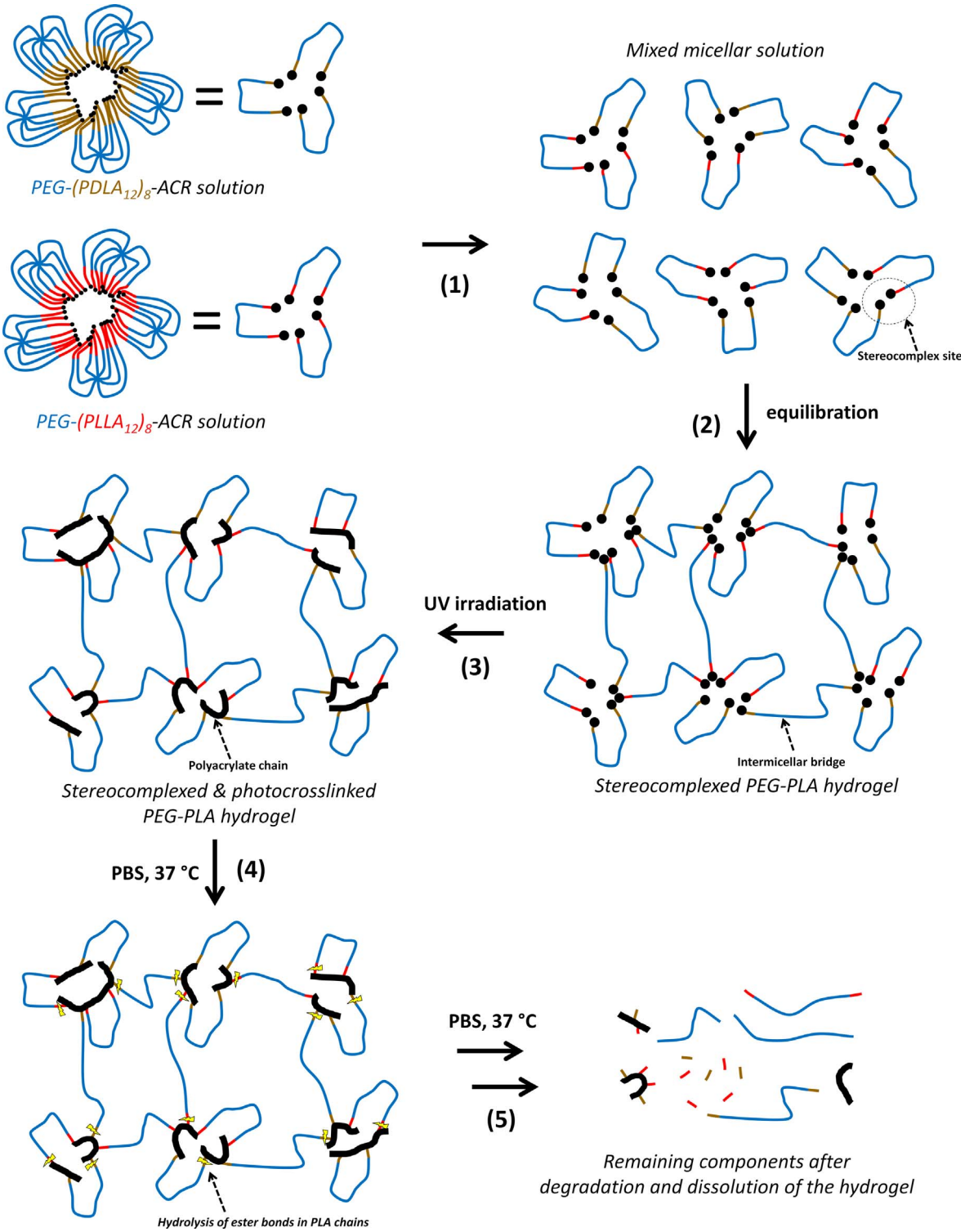

Fig. 4. Proposed mechanisms for the formation and *in vitro* degradation/dissolution of a stereocomplexed & photocrosslinked PEG-PLA hydrogel starting from separate PEG-$(PDLA_{12})_8$-AC and PEG-$(PLLA_{12})_8$-AC solutions. The photoinitiator (Irgacure 2959), which is dissolved in the initial solutions, is not included for the sake of clarity.

that the degradation time of 15 w/v% stereocomplexed and photocrosslinked PEG-(OCO)-$(PLA_{12})_8$-METHAC hydrogels was twice as high as compared to 15 w/v% single enantiomer, photocrosslinked PEG-(OCO)-$(PLLA_{12})_8$-METHAC hydrogels due to a higher crosslink density [25]. Although not investigated in the present work, stereocomplexed and photocrosslinked PEG-$(PLA_{12})_8$ hydrogels

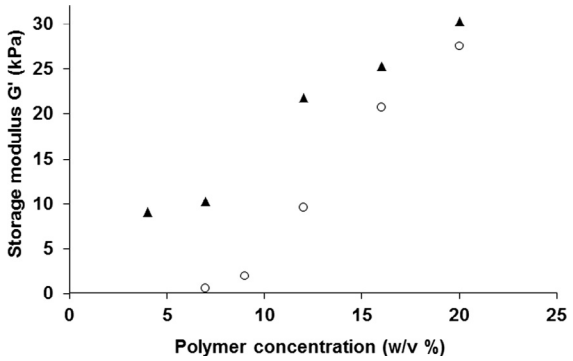

Fig. 5. Storage modulus (G′) as a function of the initial polymer concentration for stereocomplexed and photocrosslinked PEG-$(PLA_{12})_8$-AC hydrogels (closed triangles) and previously reported [24] stereocomplexed PEG-$(PLA_{12})_8$ hydrogels (open circles).

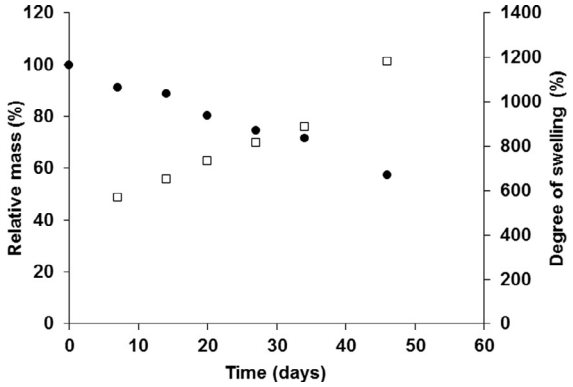

Fig. 6. Polymer mass (closed circles) and degree of swelling (open squares) versus time for 20 w/v% stereocomplexed and photopolymerized PEG-$(PLA_{12})_8$-AC hydrogels in PBS at 37 °C.

are therefore expected to be more stable than single enantiomer photocrosslinked PEG-$(PLLA_{12})_8$ hydrogels.

The degradation of the networks is accompanied by an increase in swelling (Fig. 6). These results are likely due to the loss of physical and chemical crosslinks as a result of hydrolysis of ester bonds in the PLA blocks (step 4 in Fig. 4), which disrupts the PDLA/PLLA stereocomplex crystallites acting as physical crosslinks. Consequently lactic acid, PLA oligomers as well as poly(ethylene glycol) and poly(acrylate) macromolecules will diffuse out of the network as a result of the concentration difference between the hydrogel and the surrounding medium. Eventually, the system will dissolve completely (step 5 in Fig. 4). It cannot be excluded that some of the weight loss, most notably in the initial stages, is due to the release of a small amount of non-photocrosslinked material resulting from non-quantitative conversion of the acrylate groups. The number of remaining double bonds may be quantified via HR-MAS NMR spectroscopy, which will be the subject of future investigations. Previously Censi et al. reported 95% conversion of vinylic groups upon UV irradiation of PEG-poly(N-(2-hydroxypropyl)methacrylamide lactate) block copolymers for the preparation of photocrosslinked hydrogels [36].

Oscillatory rheology measurements showed that the storage modulus of the stereocomplexed and photocrosslinked PEG-$(PLA_{12})_8$-AC hydrogels decreased from 21.8 kPa at the start of the degradation experiments to 0.04 kPa after 75 d. This was an expected behavior because G′ is directly related to the crosslink density. Altering the polymer concentration prior to photopolymerization did not significantly influence the relative mass loss rate, as 8 w/v% and 12 w/v% stereocomplexed and photocrosslinked PEG-$(PLA_{12})_8$-AC hydrogels lost 33 and 39% of polymer mass after 45 d, respectively. The stereocomplexed and photocrosslinked PEG-$(PLA_{12})_8$-AC hydrogels are remarkably more stable than stereocomplexed and photocrosslinked PEG-(OCO)-$(PLA_{12})_8$-METHAC hydrogels, which completely dissolved within 4 weeks [25]. This is partly due to the higher degree of end group functionalization on the PEG-$(PLA_{12})_8$-AC star block copolymers compared to PEG-(OCO)-$(PLA_{12})_8$-METHAC (95 vs 40%), resulting in a higher number of chemical crosslinks. Furthermore the PEG-PLA linking groups likely play an important role in the degradation profiles of both systems. Previously we showed that the degradation of the PEG-(OCO)-$(PLA)_8$ block copolymer proceeds through preferential hydrolysis of the linking ester group resulting in rapid loss of PLA arms [23]. In contrast, the stable amide linking unit in stereocomplexed PEG-$(PLA)_8$ hydrogels only allows degradation to take place via hydrolysis of ester groups in the PLA chains, affording materials that are more slowly degrading. In comparison with e.g. linear block copolymers of the same molecular weight and hydrophilic/hydrophobic balance, star block copolymers offer the advantages of a higher concentration of functional end groups as well as an additional branching point. The results show that our rationally designed PEG-$(PLA_{12})_8$-AC macromonomer, containing i) stable amide linkages at the hydrolytically sensitive connection between PEG and PLA ii) an eight-armed star architecture and iii) a high end group functionalization with reactive acrylate groups, facilitates tandem gelation via stereocomplexation and subsequent photocrosslinking,

yielding an injectable, robust hydrogel with a high resistance against hydrolytic degradation. This system may, for example, be used for controlled, local drug delivery after *in situ* application of the hydrogel via tandem gelation. It is envisioned that stereocomplexation provides fast initial gelation *in vivo*, so that photopolymerization through the skin or via a minimally invasive device (e.g. an optical fiber) can be performed with lower photopolymerization rates, limiting the local temperature rise and potentiating the use of low initiator concentrations and low light intensities. When the UV irradiation conditions (such as initiator type, wavelength and irradiation time) are optimized, photopolymerizations under mild circumstances may still proceed quickly to high conversions, limiting the exposure of the organism to free acrylate groups, UV irradiation and photoinitiator. *In vivo* application of our system raises the relevant issue of acrylate toxicity, but hydrogels based on vinyl group terminated PEG macromonomers have already been successfully used in hydrogel research. As an example, Qiu et al. showed that application of an *in situ* forming hydrogel based on PEG-vinylsulfone in the back of rats resulted in a limited adverse tissue response such as minimal inflammatory cell response and a mild number of macrophages associated with the gel [37].

4. Conclusions

PEG-PLA star block copolymer hydrogels were prepared by physical crosslinking in combination with photopolymerization. Stereocomplexation of enantiomeric PEG-PLA star block copolymers was used for facile mixing of polymers resulting in gelation *in situ*. Photopolymerization of acrylate end group functionalized polymers improved the mechanical properties and stability of the formed gels. The PEG-$(PLA_{12})_8$-AC polymers could be physically crosslinked by stereocomplexation at very low concentrations and, upon photopolymerization, afforded highly robust and stable hydrogels. These PEG-$(PLA_{12})_8$-AC gels degraded by hydrolysis of ester groups in the PLA chains resulting in a loss of physical and chemical crosslinks. This study shows that injectable, photocrosslinked PEG-PLA star block copolymer hydrogels are promising materials for biomedical applications such as long-term controlled drug delivery.

Acknowledgements

This research was supported by the Dutch Program for Tissue Engineering (DPTE; Project Number 6732).

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