Thermo-Responsive Biodegradable Hydrogels from Stereocomplexed Poly(lactide)s

Tomoko Fujiwara, Tetsuji Yamaoka, and Yoshiharu Kimura

Abstract Hydrogels that form by responding to temperature changes are used for injectable biomaterials with many potential applications. Numerous techniques have been used to prepare biodegradable polymers for bioapplications. Specifically, biocompatible hydrogels that can be safely injected without surgery and sustained/disintegrated in a controlled manner are of interest. Poly(lactide), PLA, is the most studied and utilized biodegradable polymer, and its block copolymers provide a great variety of structures and properties. Utilizing stereocomplexation technology of enantiomeric PLAs on thermo-sensitive hydrogels of PLA–PEG block copolymers is an important aspect of bioapplications of hydrogels.

Introduction

Stimuli-sensitive hydrogels have the ability to respond to changes in the environment. Temperature is one of typical stimuli and can produce physically crosslinked gels. The physical gels are established by various interactions, such as van der Waals, hydrogen bonding, hydrophobic interaction, and molecular entanglement. Poly(*N*-isopropylacrylamide) (PNIPAM), which is the most widely known thermo-responsive physical gels, has a low critical solution temperature (LCST) around 32°C and forms a gels above the LCST as a result of dehydration of the hydrophobic isopropyl groups and hydrogen bonding to the carbonyl groups [1–4]. Triblock copolymers of poly(ethylene oxide) and poly(propylene oxide) (PEO–PPO–PEO), are nonionic surfactants known as Pluronic® and Poloxamer®, also exhibit sol–gel phase transition in water. The gelation mechanism [5–9] involves the formation and packing of micelles to induce sol-to-gels transition near LCST; the PEO corona blocks shrink to lead gel-to-sol transition at the higher temperature. In other cases, such as gelatin and agarose, helix formation is responsible for the gels formation in a cooled aqueous medium [10], while the hydration of poly(oxyethylene) grafted onto a substrate forms a gels [11].

Polymer gels, applied as biomedical materials, have achieved remarkable advances in medical science and biotechnology [12]. These applications include cell culture, tissue engineering, drug delivery system (DDS), and medical sensing. The biocompatibility, biodegradability, and safety of the gels are extremely important as well as the physicochemical properties for these applications. Hydrogels biodegradability, in particular, is essential for in vivo use; accordingly,

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they are prepared from degradable polymers with good biocompatibility. Polylactides (PLA) are among the commonly used biodegradable polymers that are of special interest not only as eco-plastic materials [13] but also as biomedical materials as well [14]. Since lactic acid, the monomer for PLA, can be derived from renewable natural resources such as cornstarch, it is regarded as one of the sustainable materials.

There is a great need for the polymer systems that can respond to temperature changes and biodegrade safely in the body. Biodegradable hydrogels based on block copolymer systems consisting of PLAs and poly(ethylene glycol) (PEG), as well as thermo-responsive gels, that utilize stereocomplexation of enantiomeric PLAs are very important.

Micelles and Hydrogels with Various Block, Graft, and Armed PLA Copolymers

One of the approaches to these hydrogels is to synthesize copolymers that consist of block components; for example, PLA hydrophobic "hard" A-blocks and PEG hydrophilic "soft" B-blocks. Typical chemical structures of ABA, BAB, and AB block copolymers are shown in Fig. 1. Since both PLA and PEG are biocompatible and bioresorbable, the PLA–PEG block copolymers have many biomedical applications, such as temporary devices for clinical and pharmaceutical purposes.

The diblock copolymer, poly(DL-lactide)-*block*-poly(ethylene glycol) (PDLLA–PEG), was first used as a drug carrier in the early 1980s [15, 16]. The ABA triblock copolymer, PLA–PEG–PLA, was prepared in the late 1980s [17–24] and the properties were studied [25, 26], such as degradability [27–31] and drug release [32, 33]. In the ABA system, PEG acts as an intermolecular plasticizer for processing implant pastes, films, and scaffolds. This system has been used for biomedical application since early 1980s [23, 34–38]. Later, Vert et al. reported the utilization of hydrogels for protein release [39]. Microspheres prepared from the ABA block copolymers by emulsion technique [40–42] are used to encapsulate hydrophilic macromolecular drugs. Nanoparticles with much smaller diameters (10–1,000 nm) are used for the drug targeting [43] and as practical biodegradable materials [32, 44–46].

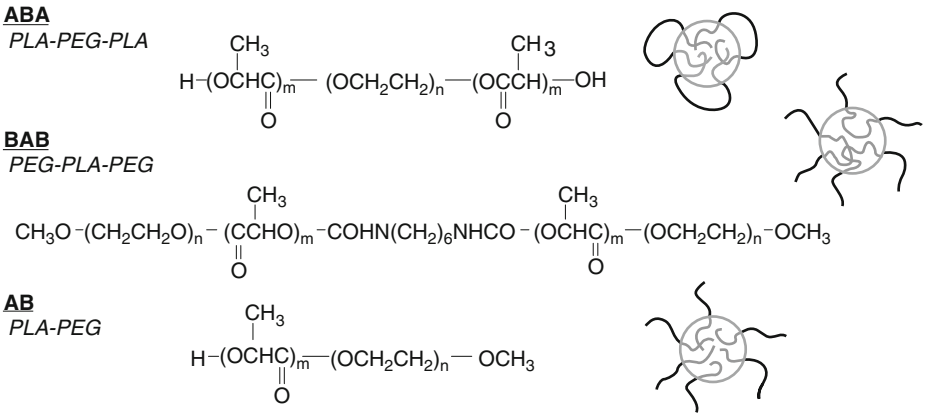

Fig. 1. Typical polymer structures and schematics of the micelles in aqueous medium.

Injectable microparticles for DDS were prepared from a BAB type triblock copolymer, PEG–PLLA–PEG [47, 48]. The aqueous micellar solution containing BAB copolymers exhibited a sol-to-gels phase transition with decreasing temperature from higher temperatures to the body temperature. The transition temperature is variable depending on the block lengths and polymer concentration. The mechanism of this hydrogels is a simple swelling and hydration of the PEG layers. Other BAB and ABA type thermo-sensitive hydrogels were made by incorporating poly(L-lactide-coglycolide) (PLGA) as the A-block and PEG as the B-block; copolymer has a complicated phase diagram with both sol-to-gels and gels-to-sol transitions with increasing temperature, similar to PEO–PPO–PEO. From static (SLS) and dynamic light scattering (DLS) studies it appears that an increase in the aggregation number of the micelles causes the gelation [49, 50]. The in vitro drug release behavior of the PEG–PLGA–PEG hydrogels was evaluated by using both hydrophilic and hydrophobic model drugs [51, 52]. ABA or BAB block copolymer gels based on PLGA and PEG have also been studied [53–59]. AB diblock copolymers have been used for many years to prepare micelles to encapsulate drugs and DNA [41, 60–62]. These AB diblock copolymers form hydrogels by temperature change similar to PEG–PLLA–PEG or other simple BAB triblock copolymers [63].

The rheological properties of hydrogels are strongly influenced by polymer structures, i.e., the block length and type [64] and the crystallinity [65]. The latter was systematically studied by mixing ABA type and AB type copolymers and mixing crystalline PLLA–PEG and amorphous PDLLA–PEG copolymer systems. The effect of the synthesis method on rheological properties is important [66] as well as polymer structural effects [67].

There are several techniques for photo-crosslinking hydrogels of PLA–PEG block copolymer systems. For example, methacrylate capped triblock copolymer MA–PLLA–PEG–PLLA–MA and its' degradation properties were studied [68, 69] and the degradability of this high modulus gels can be controlled by tailoring the composition. Tissue-adhesive hydrogels were prepared using crosslinkable polymeric micelles of aldehyde-terminated PEG-*block*-PLA with a Schiff base polymer [70]. When the polymeric micelle solution and a polyallylamine solution are mixed, hydrogels are formed either in vivo or in vitro almost instantaneously.

Stereocomplexation of Enantiomeric PLAs, and the Hydrogels Applications

PLAs consisting of enantiomeric L- and D-lactic acids are generally differentiated as PLLA and PDLA, respectively. PLLA is now produced by ring-opening polymerization of L-lactide that is made from L-lactic acid manufactured by large-scale fermentation. A polymer blend of PLLA and PDLA forms a stereocomplex with a melting temperature (Tm) of 230°C which is approximately 50°C higher than that of single crystal of PLLA or PDLA, due to the differences in crystal formation (Fig. 2) [71–76]. Therefore, the improved properties are expected with the stereocomplex of PLLA and PDLA. Based on these backgrounds many attempts have been made to obtain polymer gels from PLA derivatives.

The use of stereocomplexation of PLLA and PDLA was first applied to form hydrogels in 2000 to form a system based on polymer of hydroexyethylmethacrylate P(HEMA) with grafts of lactide (LA) oligomers [78]. In 2001, a dextran-graft-oligo(LA) system was prepared [79–81]. Both systems involve P(HEMA) stereocomplexation of the lactide oligomers in the hydrogels. The hydrogels made with grafted oligo(LLA) and oligo(DLA) degraded more slowly than the gels made from the single p(HEMA)-graft-oligo(LA). The gelation mechanism of the stereocomplex interaction of oligo-LAs act as crosslinkers between main polymers as illustrated in Fig. 3.

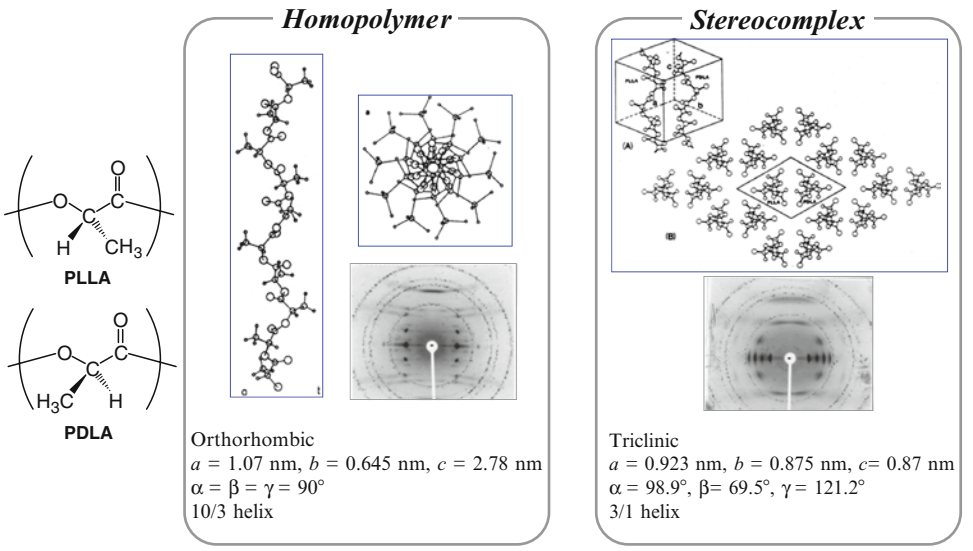

Fig. 2. Stereochemistry of PLLA and PDLA, and crystal forms of the homopolymer and stereocomplex. Reproduced from [77] with permission from ACS.

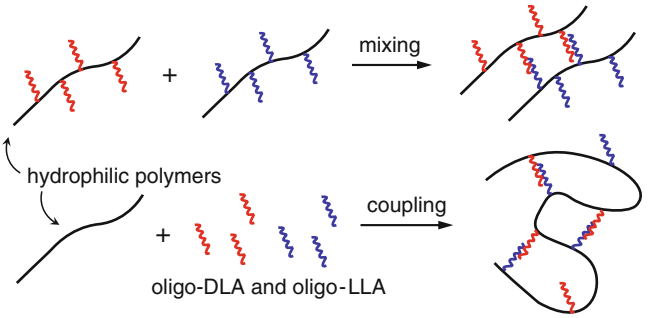

Fig. 3. Typical mechanisms of hydrogels formation using enantiomeric LA oligomers.

Fujiwara and Kimura, on the other hand, first reported the temperature-dependent, injectable hydrogels by stereocomplex formation of the enantiomeric micelle mixture of PLA–PEG–PLA triblock copolymers in 2001 [82]. Wide Angle X-ray Scattering (WAXS) and rheological analyses was used to monitor the increase of stereocomplex crystals and gelation process. The mechanism of this system was new and unique (vide infra) along with studies on BAB and AB block copolymers [83]. Similar hydrogels formations from enantiomeric PLA–PEG di- and triblock copolymers were studied using Raman spectroscopy in addition to WAXS and rheology measurements to confirm the stereocomplex crystals [84].

Another in-situ hydrogels system by stereocomplexation was developed from PEG-(PLLA)(8) and PEG-(PDLA)(8) star block copolymers [85]. Relatively short chain PLA

(9–17 lactate units per PLA block) was prepared by ring-opening polymerization of L- or D-lactide onto 8-arms PEG (Mw 22,000 and 44,000) as an initiator. Hydrogels were formed by mixing of solutions of L-star and D-star copolymers. With increasing PLA block length, water solubility and critical gels concentration (CGC) decreased. The protein delivery using these injectable hydrogels was evaluated in vitro and in vivo [86]. The relatively small protein lysozyme followed first order kinetics, wherein a high cumulative release of approximately 90% was obtained in 10 days. The larger protein IgG was released in vitro with nearly zero order kinetics for 16 days. The release of the therapeutic protein rhIL-2 followed almost zero order kinetics for 7 days, wherein up to 45% was released. To prepare robust hydrogels of stereocomplexed PLA copolymers, co-crosslinking systems by photo reactive groups are used by adding methacrylate groups on to star-block PLA–PEG chain ends [87]. Stereocomplexation from the enantiomeric PLA still occurred. After UV-polymerization, the hydrogels showed significantly higher storage modulus and prolonged degradation times. Biodegradability of these stereocomplexed-photopolymerized hydrogels varied depends on the design and procedures.

A thermo-sensitive and biodegradable stereocomplexed hydrogels composed of multiblock Pluronic copolymers were developed by linking oligo-LLA and oligo-DLA [88]. A scheme of the gelation mechanism is shown in Fig. 4. The stereocomplexed multiblock hydrogels showed enhanced gels stability and mechanical strength, linear mass erosion profiles, and near zero-order hGH release patterns.

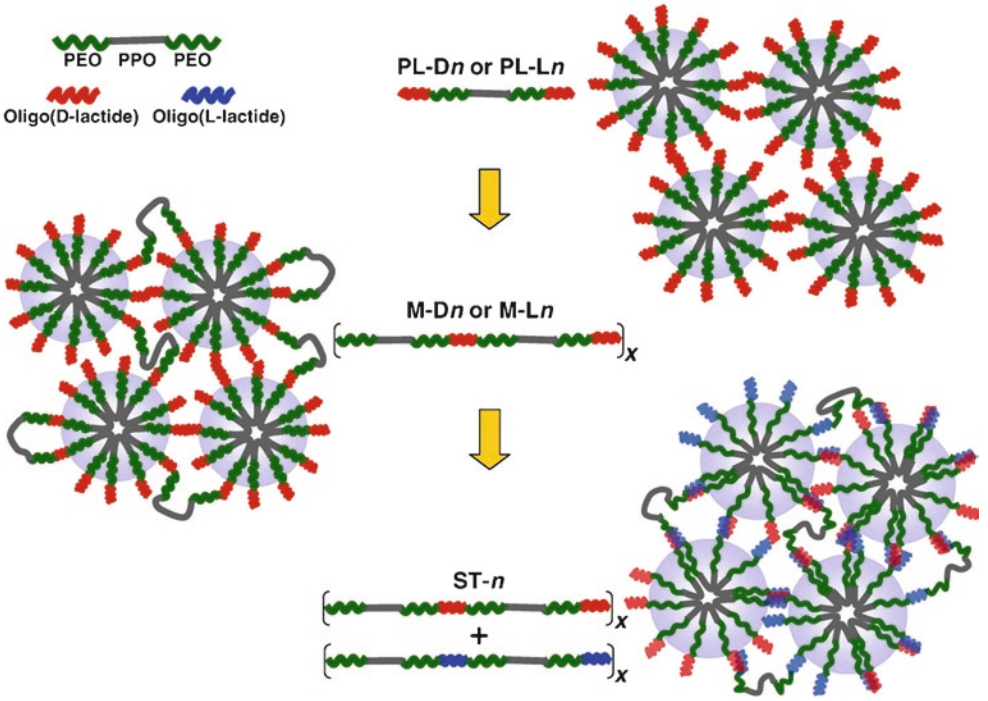

Fig. 4. Schematic illustration for molecular structure of hydrogels formed by PN-multi-oligo(LLA) and PN-multi-oligo(DLA) developed by Park et al. With permission from [88]. Copyright Elsevier.

Hydrogels Study on Enantiomeric PLA–PEG Linear Block Copolymers

Hereafter, the systematic studies on ABA, BAB, and AB type enantiomeric PLA–PEG block copolymers by the authors are described.

Motivation for the Study of Stereocomplexed Micellar Hydrogels

In late 1990s, the authors discovered interesting band morphology formed from the micellar nanoparticles of PLLA–PEG diblock and PLLA–PEG–PLLA triblock copolymers that were placed on a flat substrate surface [89–91]. The nanoparticles on the mica surface were self-organized into different structures by mild thermal treatment. It has been verified that the band morphology is directed by crystallization of the PLLA segments and that the PLLA chains take a doubly twisted structure in it with the ordinary 10/3 helical conformation preserved. Prior to this PLLA band formation, PEG blocks phase-separate and plays an important role. The two-dimensional network formed by the PLLA–PEG–PLLA bands on the surface well simulates the structure of the three-dimensional network systems observed in melt, concentrated solution, and hydrogels. These studies directed us to thermo-responsive hydrogels formation from PLA–PEG block copolymers.

The atomic force microscopic (AFM) images of the bicontinuous network structure (for high concentration sample) and nanofibers (for low concentration sample) formed from a mixture of PLLA–PEG (5,000–5,000) and PDLA–PEG (5,000–5,000) micellar solutions are shown in Fig. 5. Aqueous solutions (0.2 and 0.01 wt%) of both enantiomeric micelles were mixed at room temperature, cast on the mica surface, and then heated at 60°C for 1 h. For the high concentration samples of the enantiomeric mixture, gel-type network formation was clearly seen, which was totally different from the structure of a single polymer system of PLLA–PEG high concentration micelles [90]. As previously observed from single PLLA–PEG of low concentration samples, the mixed system also formed similar crystal nanofibers (Fig. 5). An interesting phenomenon observed only in this enantiomeric mixture is that the nanofibers are aligned in pairs. Analysis by TEM diffraction indicated that the two bands in pair consist of the single PLLA and PDLA crystals. These facts suggest that the PEG blocks connected with PLLA and PDLA interact so strongly prior to the band formation and guide the separate crystallization of the PLLA and PDLA blocks with opposite helical sense. Under different conditions, the enantiomeric micelle mixture was found to reorganize in different ways by thermal treatment, which inspired us to the temperature responsive network formation by PLA stereocomplexation.

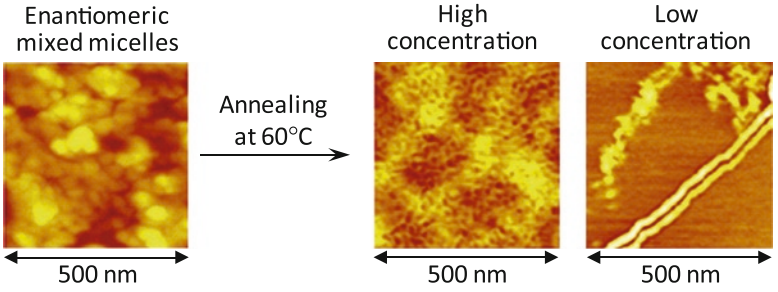

Fig. 5. AFM height images of thermal reorganization of micelles casted from the enantiomeric mixture of PLLA–PEG (5,000–5,000) and PDLA–PEG (5,000–5,000) micellar solutions.

Table 1. Typical block copolymers and the molecular weight

Type	Copolymers	PLA block (Mn)	PEG block (Mn)	Total (Mn)	PLA/PEG (wt/wt)
ABA	PLLA–PEG–PLLA	1,300	4,600	7,200	0.56
	PDLA–PEG–PDLA	1,100	4,600	6,800	0.48
BAB	PEG–PLLA–PEG	2,000	2,000	6,000	0.50
	PEG–PDLA–PEG	2,000	2,000	6,000	0.50
AB	PLLA–PEG	1,100	2,000	3,100	0.55
	PDLA–PEG	900	2,000	2,900	0.45

Copolymer Synthesis and Gels Formation

A number of PLA–PEG block copolymers with various molecular weights (Mn) and block ratios have been synthesized. Summarized in Table 1 are copolymers of ABA, BAB, and AB types that induce thermo-sensitive gelation when L- and D-copolymers are mixed. Interestingly, the PLA/PEG ratio of all the different types is near 0.5. The ordinary ring-opening polymerization of L- or D-lactide, initiated with PEG and MePEG, generated the ABA and AB block copolymers, respectively, in high yields [82, 83]. The BAB triblock copolymers were obtained by the coupling the AB diblock copolymers with hexamethylene diisocyanate (HMDI) (Fig. 1) [47]. These copolymers readily formed the core-shell type amphiphilic micelles in water as illustrated in Fig. 1. The average hydrodynamic diameters of the micelles measured by DLS were in the range of 20–30 nm for 1 wt% solutions of all these copolymers. To obtain sol-to-gels or gels-to-sol transition, micellar solutions were prepared at various concentrations, and both solutions of L- and D-copolymers were mixed together at low temperature (typically at 4°C). Then the temperature was increased up to 75°C to observe the sol–gel behavior. All solutions were prepared in water.

The molecular weights of ABA, BAB, and AB copolymers listed in Table 1 showed the best performance as a thermo-sensitive hydrogels. A variety of length and composition of copolymers for each block type was prepared and it was found that the PLA/PEG composition ratio and the thickness of appearance of PEG shell layer (since the PEG of ABA micelle makes a loop) were similar for the three types of micelles rather than the molecular weights of PLA blocks [83].

Hydrogels from Micellar Solutions of ABA Triblock Copolymers

Spontaneous gels formation occurs when a micellar solution of the enantiomeric ABA triblock copolymers, PLLA–PEG–PLLA and PDLA–PEG–PDLA, are mixed. This system is characterized by an interesting temperature-dependent sol-to-gels transition that is induced around 37°C by the stereocomplexation of the PLLA and PDLA block segments [82]. As seen in Fig. 6, only enantiomeric mixture of micellar solutions formed hydrogels. The gels formation was successfully monitored by the rheological change of a micellar solution, and the stereocomplex formation was confirmed by wide-angle X-ray scattering (WAXS).

The sol–gel transition diagram of the mixed micellar solutions of PLLA–PEG–PLLA and PDLA–PEG–PDLA with respect to temperature and polymer concentration is shown in Fig. 7.

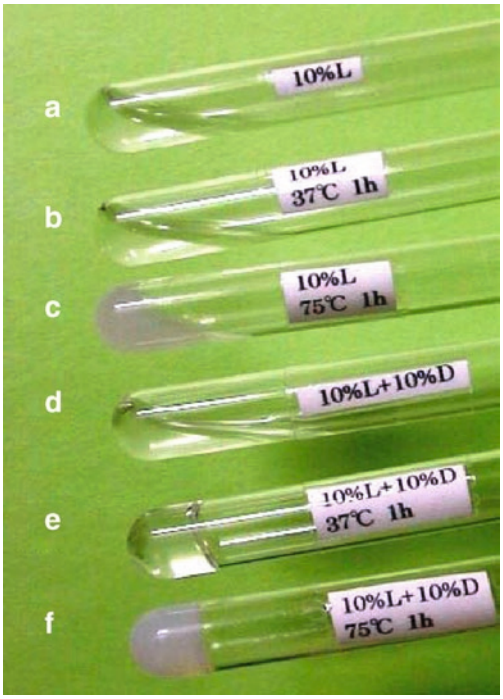

Fig. 6. The appearances of 10 wt% of ABA micellar solutions; PLLA–PEG–PLLA at room temperature (a), 37°C (b), 75°C (c), and enantiomeric mixture of L- and D-triblock copolymers at room temperature (d), 37°C (e), 75°C (f). Reproduced from [82]. Copyright 2001 Wiley-VCH.

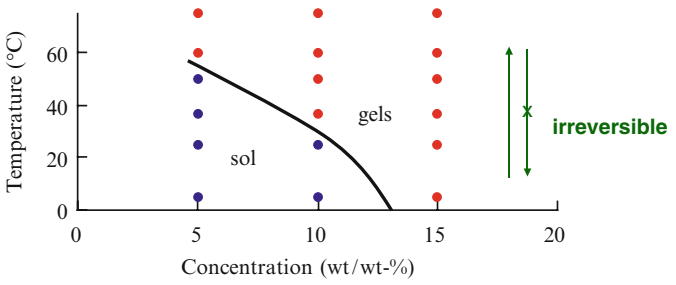

Fig. 7. The phase diagram of mixed micellar solutions of PLLA–PEG–PLLA and PDLA–PEG–PDLA.

The single PLLA–PEG–PLLA micellar solution had only the solution state at all temperatures and concentrations plotted in Fig. 7 and all later phase diagrams. With the 10 wt% enantiomeric mixture, the sol-to-gels transition was between room temperature (25°C) and the body temperature (37°C) as shown in Fig. 6d, e. While the single micellar solution of PLLA–PEG–PLLA (control) turned white fluid after heating to 75°C (c) by the crystallization of homocrystals, the mixture became a white gels at 75°C (f).

The responsibility for the stereocomplex formation of the enantiomeric polylactide blocks on the gelation was confirmed by synchrotron Wide Angle X-ray Scattering (WAXS) measurements.

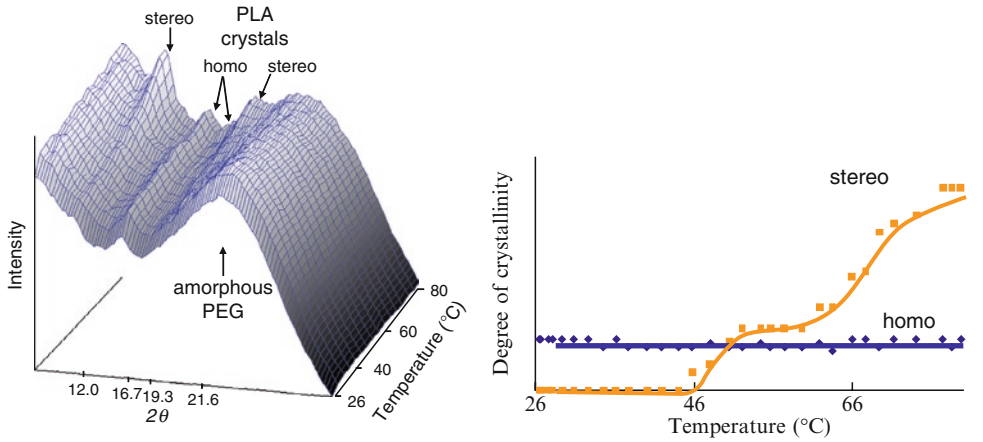

Fig. 8. The temperature-dependent WAXS measurement of the 10% enantiomeric mixture at a heating rate 2°C/min. Two-dimensional WAXS data were collected every 30 s with a synchrotron X-ray at the BL-15A beamline (PF, Tsukuba, Japan).

The temperature-dependent WAXS profiles for the mixed solution are shown in Fig. 8. The measurement was started immediately after the 10 wt% micellar solutions of PLLA–PEG–PLLA and PDLA–PEG–PDLA were mixed at room temperature, and the data was collected every half minute by in situ heating at a rate of 2°C/min. Small diffraction peaks are confirmed at $2\theta = 16.8°$ and 19.4° in the starting mixture, which means that the small amount of hexagonal crystals of PLLA and PDLA exists in the core of the micelles at room temperature. These diffractions are attributed to the (200) or (110) plane and the (203) or (113) plane of the hexagonal crystal lattice comprising the PLLA or PDLA 10/3-helices [92]. With increasing temperature, the WAXS data exhibits two different reflections, at $2\theta = 12.1$ and 21.7°, in addition to the small reflections of the hexagonal crystals. These new peaks can be reasonably ascribed to the crystals of the stereocomplex of PLLA and PDLA [82, 83]. Around 37°C, these reflections are still weak, indicating that both the PLLA and PDLA chains may be mixed into a complexation state prior to the crystallization. At 75°C, the significant crystal growth of the stereocomplex is clearly seen.

In the single PLLA–PEG–PLLA micellar solution (control experiment), the hexagonal crystal growth was also observed with increasing temperature despite its continuous sol nature. The total degree of crystallinity estimated from the WAXS was almost identical with that observed in the mixed solution at each temperature. Since the single and mixed solutions have same degree of crystallinity but have different major crystal forms which are hexagonal and stereocomplex, respectively, it is suggested that the gels formation in the mixed solution is closely related with the stereocomplexation of the enantiomeric PLA blocks.

In the micellar solutions of the enantiomeric ABA triblock copolymers, the hydrophobic PLLA or PDLA segments aggregate to form a core region, around which, the hydrophilic PEG segments settle to form a shell when the micelles are prepared separately. Consequently, the PLLA and PDLA segments can be isolated from each other when the micellar solutions of the enantiomeric block copolymers are mixed. Illustrated in Fig. 9 is a schematic of an ABA system gelation. When heated, the aggregation of the PLLA and PDLA segments in the core/shell interface is weakened to allow the segments to mix outside of the core. The shorter block length of PLLA and

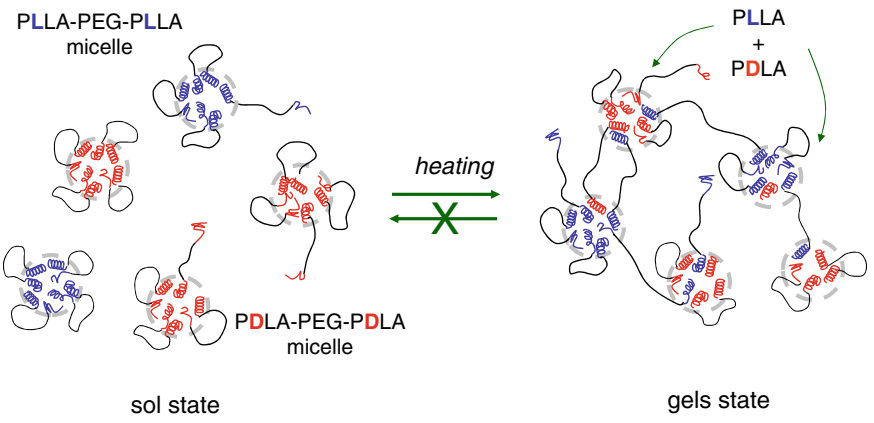

Fig. 9. Proposed gelation mechanism of enantiomeric mixture of ABA triblock copolymers.

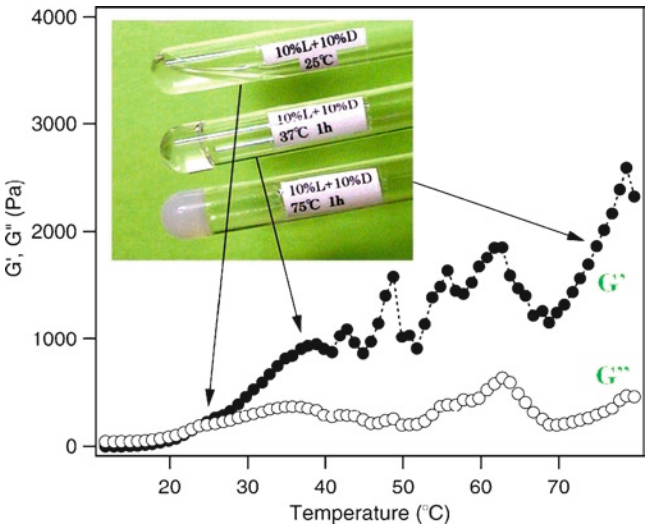

Fig. 10. Rheological changes of the mixed dispersion of ABA copolymers in regard to temperature increase; slit-shear mode with frequency 128 Hz.

PDLA is favorable for this chain scrambling and mixing. Consequently, the stereocomplexation starts as indicated by the WAXS data (Fig. 8), and the micelles are wholly crosslinked with each other at 37°C to form a gels. With increasing temperature, the crosslinking state is changed by reorganization of the hydrophobic cores and increased crystallization of the stereocomplex.

This process was also supported by fluctuations in the storage modulus (G′) curve in the rheology measurement [83]. Plotted in Fig. 10 are the temperature-dependent rheological changes with the gelation of the mixed dispersion of ABA-type block copolymers. A dramatic increase in storage modulus (G′) was observed from 20 to 37°C. The crossing of the G′ and loss modulus (G″) curves is detectable around 23°C. This change corresponds to the cross-linking reaction that leads to gels formation. Above 37°C, G′ fluctuates around 10^3 Pa, which

is an ordinary G′ level for physically crosslinked gels. It starts to elevate again above 70°C, corresponding to the turbidity of the gels. Since this turbid gels regains its transparency to some degree when cooled, this turbidity change is mainly attributed to the clouding phenomenon resulting from the desolubilization of nonionic surfactants (such as PEG). This data supports the gels formation of the mixed dispersion at around 37°C. Because the stereocomplex formation depresses the mobility of the PLA chains and stabilizes the PEG crosslinkers, gels formation is only possible for the enantiomeric mixture. This sol–gel transition is irreversible though the crosslinking is performed by an ordinary physical mechanism.

Hydrogels from BAB Triblock Copolymers

The second gels system consists of the enantiomeric BAB type triblock copolymers, PEG–PLLA–PEG and PEG–PDLA–PEG. The sol–gel transition of this system should be induced by the stereo interaction of L- and D-copolymers, being much different from that of the single BAB (PEG–PLLA–PEG) system that has previously been described to undergo gelation by the ordinary hydrophobic/hydrophilic interaction [47].

The aqueous micellar solution of single enantiomer, PEG–PLLA–PEG (2,000–2,000–2,000) or PEG–PDLA–PEG (2,000–2,000–2,000), remains a "sol" phase at all temperatures in the concentration range of 10–40 wt%. Shown in Fig. 11 is a sol–gel transition diagram plotted for 1:1 (v/v) mixed micellar solutions of PEG–PLLA–PEG and PEG–PDLA–PEG with respect to temperature and polymer concentration. It is found that the gels state cannot be formed at concentrations lower than 30 wt% and that the gels state is preferentially kept below 75°C at concentrations higher than 40 wt%. The gels-to-sol transition temperature increases up to 75°C at higher concentrations. It should be noted here that in the present BAB system the gels and sol are formed, respectively, at low and high temperatures in a reversible manner. This is opposite to the above ABA system where gelation is induced with increasing temperature in an irreversible manner.

Shown in Fig. 12a are the typical changes in the mixed solution at 35 wt% concentration before and after the heat treatment from 37°C (c) to 75°C (d). This gels is formed immediately after the L- and D-solutions are mixed at room temperature. It is observed that the mixed solution is in gels and sol states at 37 and 75°C, respectively, while the single solution remained fluid irrespective of the temperature (a, b). Since gels-to-sol transformation of the mixed solutions is reversible with the temperature change, sample (d) returns to gels after cooling to room temperature.

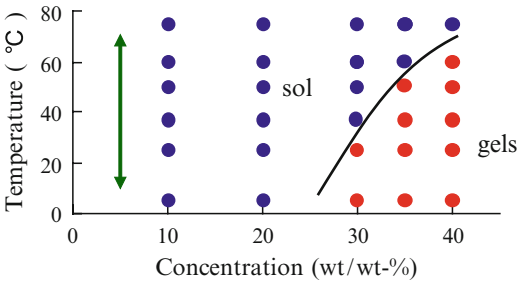

Fig. 11. The phase diagram of mixed micellar solutions of PEG–PLLA–PEG and PEG–PDLA–PEG. Reproduced from [83]. Copyright 2004 Wiley-VCH.

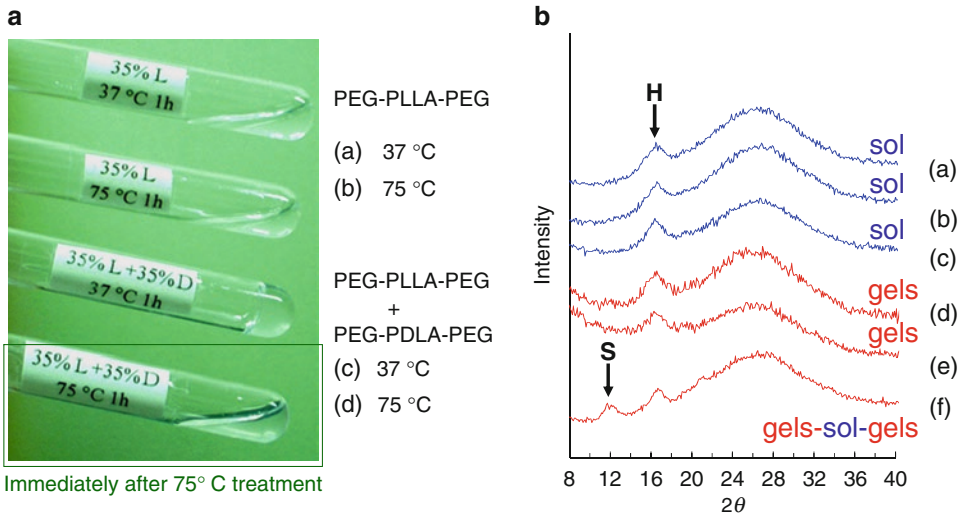

Fig. 12. The appearance of 35 wt% of BAB micellar solutions (**a**); PEG–PLLA–PEG at 37°C (a), 75°C (b), and enantio-mixture at 37°C (c), 75°C (d). The WAXS profiles of BAB micellar solutions (**b**); PEG–PLLA–PEG at room temperature (a), after 37°C (b), after 75°C (c), and enantio-mixture at room temperature (d), after 37°C (e), and after 75°C (f). Reproduced from [83]. Copyright 2004 Wiley-VCH.

The WAXS profiles of the single and mixed solutions at different temperatures are shown in Fig. 12b. The mixed solution gave very small reflections at $2\theta = 12.1$ and 21.7° in addition to the reflections of the hexagonal crystals ($2\theta = 16.8°$) of PLLA or PDLA only when heated at 75°C. This indicates that the stereocomplexation of the PLLA and PDLA blocks is induced even in the mixed solution heated at high temperatures where the sol state is achieved. When this BAB sol is cooled, the gelation is restored without significant change in the WAXS profile. Furthermore, the degree of crystallinity which was estimated by peak separation of each crystal and amorphous peaks does not increase by the heat treatment, being obviously different from the ABA gels system. It is, therefore, concluded that the stereocomplexation is not directly related with the gels formation mechanism for the BAB system.

Hydrogels from AB Diblock Copolymers

Mixed micellar solutions of enantiomeric ABA and BAB block copolymers exhibit very different gelation behavior and crystal structure. As a third system, the micellar solutions of AB block copolymers, PLLA–PEG and PDLA–PEG, were examined for the hydrogels formation. The AB system is similar to ABA in that the A-blocks associate in the core of the micelles as illustrated in Fig. 10, while being similar to BAB because the mobility of the corona B-blocks is comparable to each other.

A typical sol–gel phase diagram, plotted for the mixed solutions of the enantiomeric AB diblock copolymers, is illustrated in Fig. 13. It resembles the BAB system diagram shown in Fig. 11, where the gels-to-sol transition occurs with increasing temperature. The typical phase changes of a mixed solution of the PLLA–PEG and PDLA–PEG (total 30 wt%) are shown in Fig. 14a at 37°C (c) and 75°C (d) as compared with those of the corresponding single

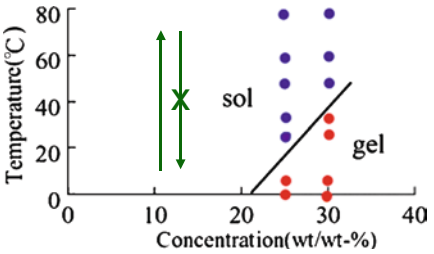

Fig. 13. The phase diagram of mixed micelle solution of PLLA–PEG and PDLA–PEG.

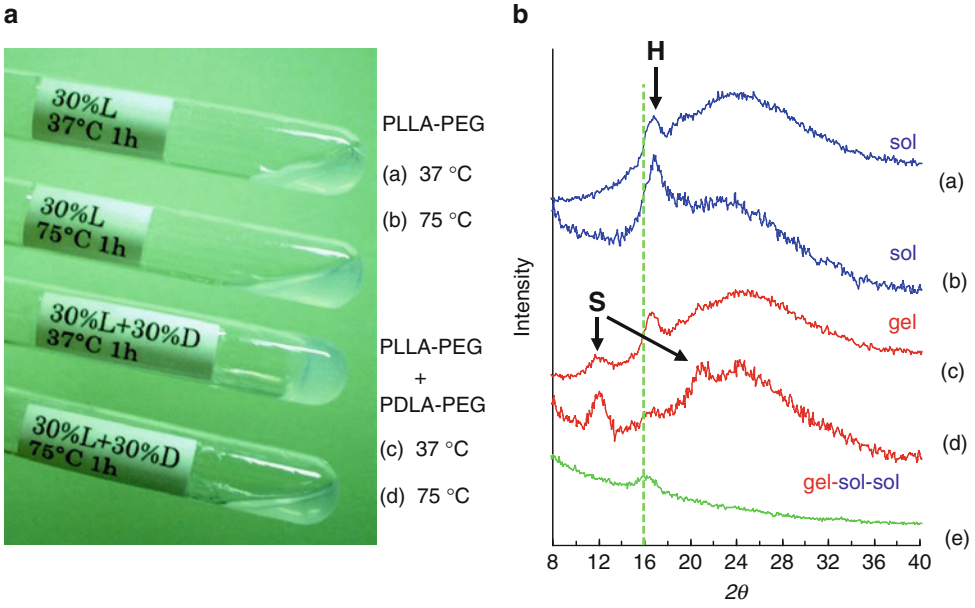

Fig. 14. The appearance of 30 wt% of AB micellar solutions (**a**); PLLA–PEG at 37°C (a), 75°C (b), and enantio-mixture at 37°C (c), 75°C (d). The WAXS profiles of AB micellar solutions (**b**); PLLA–PEG after 37°C (a), after 75°C (b), and enantio-mixture after 37°C (c), and after 75°C (d), PET film (e). Reproduced from [83]. Copyright 2004 Wiley-VCH.

micellar solution of PLLA–PEG (30 wt%) (a, b). Although the latter solution does not form a gels at any temperature, the mixed solution forms a gels on mixing at room temperature. The difference from the BAB system is that the sol formed at 75°C never returns to a gels again when cooled. This irreversible nature suggests that the interaction of the micelles formed in the hydrogels at lower temperature may be changed after turning to sol state by heating. Note that the normally synthesized AB diblock copolymer should involve a small amount of ABA triblock copolymer because MePEG is contaminated with dihydroxyterminated PEG [93, 94]. This impurity can be eliminated by high osmotic pressure chromatography; the same hydrogels formation for the pure enantiomeric AB system is also confirmed.

The WAXS profiles of the AB systems are shown in Fig. 14b, which are significantly different from those of the BAB system. The single micellar solution of PLLA–PEG shows

an increase in the crystallinity (a, b) with increasing temperature, while that of PEG–PLLA–PEG does not have this behavior. In the mixed AB solution, the gels state formed at the lower temperature involves the hexagonal crystals, as shown by the reflection at $2\theta = 16.8°$ (c); the sol state attained by heating at 75°C produces the stereocomplex crystals ($2\theta = 12.1$ and 21.7°) with most of the hexagonal crystals being lost (d). This feature is similar to that of the ABA system rather than the BAB system.

Gelation mechanism of the mixtures of enantiomeric BAB and AB block polymers may be much different from that of ABA system since the PEG blocks cannot act as direct cross-linkers between the micelles. The sol–gel transition diagrams and WAXS data suggest that the stereocomplexation between the PLLA and PDLA blocks is not directly correlated with the gelation of the mixed solution. Since, in the BAB triblock copolymers the hydrophobic PLLA and PDLA are confined in the core of the micelles and surrounded by the hydrophilic PEG shell, a sort of macromolecular reorganization is needed to grow the PLLA stereocomplexes and PDLA blocks in the micelles. Even when heated at high temperatures, the block chains are not easily exchanged among the micelles in the BAB system since PLLA and PDLA blocks are in the middle of copolymers (Fig. 15a), so that the degree of stereocomplexation is limited. Even if the stereocomplex crystals could be formed, they are confined to the micelle core and cannot achieve an interaction between the particles strong enough as to induce gelation.

The WAXS data (Fig. 12b) revealed that both PLLA and PDLA blocks of the BAB block copolymers form the hexagonal crystals in the micellar cores at room temperature. The IR spectra of the micellar solutions both in gels and sol states also have absorption bands at 921 and 1,210 cm^{-1}, supporting the presence of the 10/3 helical structure of the

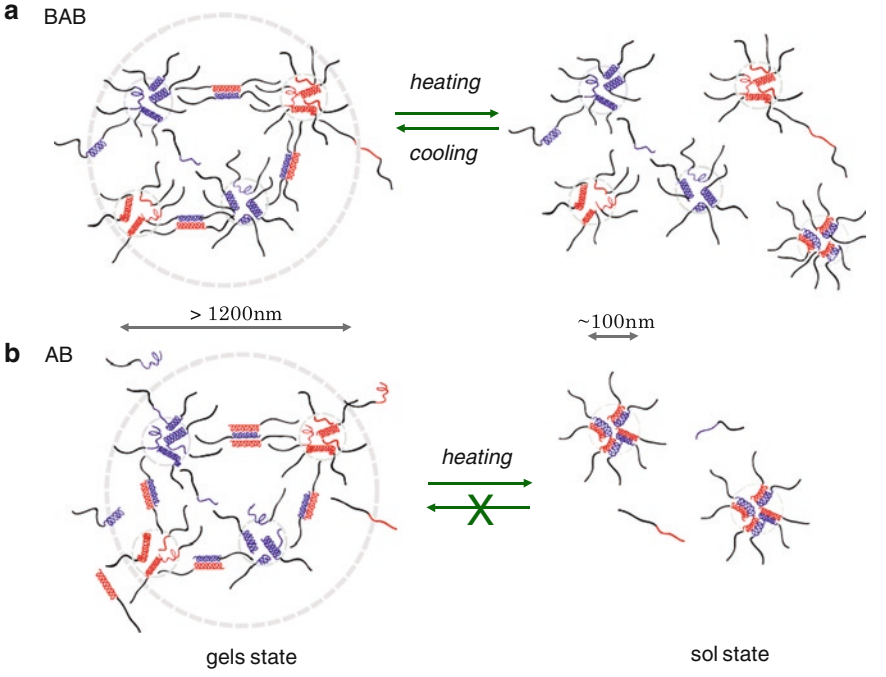

Fig. 15. Proposed gelation mechanisms of enantiomeric mixture of BAB triblock (**a**) and AB diblock (**b**) copolymers.

PLLA and PDLA blocks [95]. One possible explanation of this gel-to-sol behavior is that PEG blocks between micelles act as crosslinkers at low temperature even though they are free to move. This helix formation of PLLA and PDLA blocks is possibly transmitted to the PEG chains through the block-linking bonds, because the PEG chain can readily take on a similar helical conformation. In fact, ordinary monoclinic crystals of PEG are known to consist of 7/2-helical chains.

Since the helical senses of PLLA and PDLA are opposite to each other, the induced helices of the PEG chains should be right- and left-handed depending on the connecting PLA chains. Maybe, the helical chains of PEG, with opposite senses, aggregate through the chain interdigitation mechanism and change the hydrophilic/hydrophobic balance that leads to the interchain cohesion of the PEG blocks even in an aqueous environment. With the helical conformation, the hydrophilic ether linkages are surrounded by the hydrophobic alkylene chains to make the whole chain hydrophobic. With a single BAB copolymer, the helices have an identical sense, and the chain interdigitation to cause gelation is impossible. The interaction of the PEG chains in opposite helical senses was supported by the gelation behavior of the mixed micellar solution of the enantiomeric AB diblock copolymers (Fig. 15b). In this case, the exchange of the core PLA blocks between micelles is much faster than that of the BAB system; in fact, the stereocomplex crystals grow with increasing temperatures of the mixture. At 75°C, most of the PLA crystals were replaced by the stereocomplex crystals. Therefore, most of the micelles comprise both PLLA and PDLA blocks in their core due to the exchange of PLA blocks at high temperature and intermicelle interaction through the PEG is weakened even after cooling. The PEG interaction changed to intramicellar instead of intermicellar. This is why in the AB system the gels is irreversible.

Another effect on these BAB and AB-type hydrogels formation is that micelles are aggregating into microgel size as depicted in Fig. 15; a negligible amount of stereocomplex crystals of PLLA and PDLA blocks is still responsible for the gels formation. Dynamic light scattering revealed a bimodal size distribution of the AB-type gels of 120 and 1,200 nm (Fig. 16). Upon heating, the microparticles at 1,200 nm disappear. The difference in reversibility for the BAB and AB gels is due to the rate of micelle reformation. If the core of all micelles becomes stereocomplexed PLA as seen in WAXS data of AB micelles (Fig. 14b(d)), no more intermicelle interaction occurs.

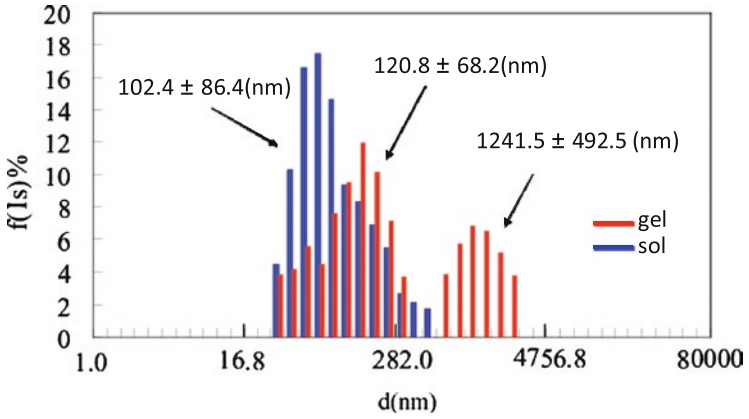

Fig. 16. Particle sizes of the micelles (30 wt%) in the mixed gels and sol (*after heating*) states of a mixture of PLLA–PEG (1,100–2,000) and PDLA–PEG (900–2,000) determined by DLS.

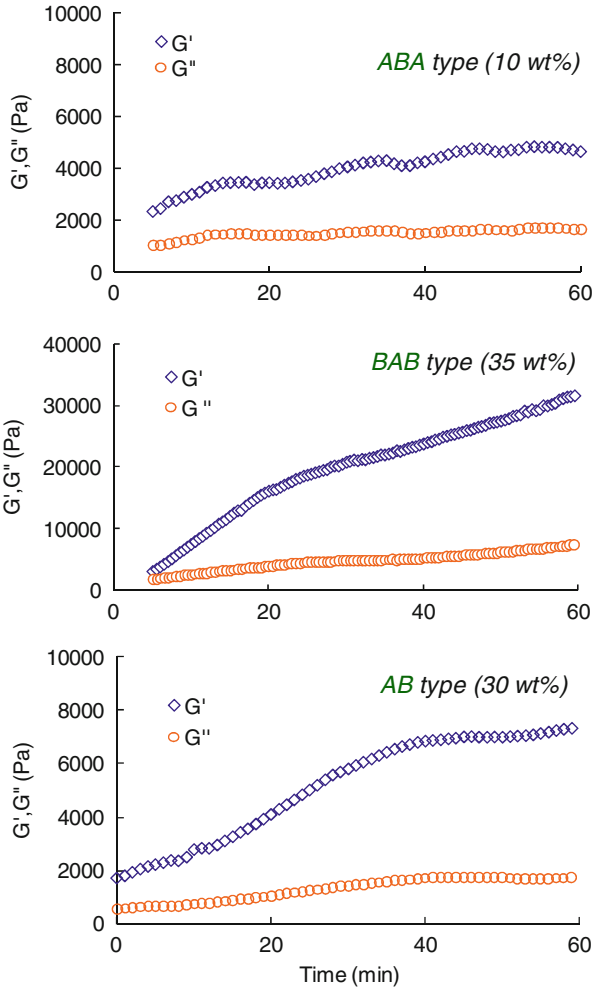

Fig. 17. Rheological change of the hydrogels of enentiomeric micelle mixtures at 37°C, 128 Hz.

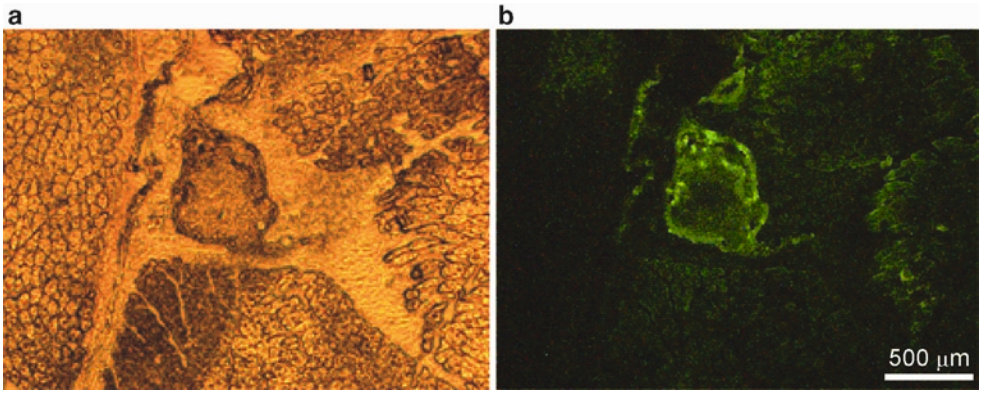

Fig. 18. The sectioned tissue from the femoral region of mouse; (**a**) optical and (**b**) fluorescent microscopes.

Hydrogels Properties and Applications

The time-dependent rheological changes in the mixed micellar solutions of ABA, BAB, and AB copolymers at 37°C are shown in Fig. 17. The mechanical properties of the BAB system are dramatically greater than those of the ABA system [82, 83]. For the BAB system, the storage modulus (G′) gradually rises to 31 kPa after 60 min with gelation. The physical crosslinking through the PEG interaction provides the mechanical properties of the gels. The G′ value of AB-type hydrogels from PLLA–PEG (1,100–2,000) and PDLA–PEG (900–2,000) reaches 7 kPa, which is lower than the BAB triblock system.

The mechanical properties of ABA thermo-responsive gels, copolymers with different molecular weight and different block ratios examined for improvements [84]. It is still a challenge to find better systems with sol-to-gels transitions between room temperature and body temperature as well as with superior mechanical properties, that do not cause other crosslinking mechanisms to occur, such as photoreactions with other low LCST polymers.

The biodegradable thermo-sensitive hydrogels formed by the enantiomeric PLA–PEG block copolymers can potentially be used as an injectable biomedical matrix. The injection of ABA mixed micellar solutions results successful gels formation in the body of mice, and the hydrogels is absorbed within 3 days after implantation. A suspension of mouse embrionic fibroblast (MEF) cells, with 9% PLLA–PEG–PLLA and 9% PDLA–PEG–PDLA micelles, was injected into the femoral region of an inbred mouse. After 3 days, the femoral regions were removed and examined; the microscopic images of a section of the femoral region are shown in Fig. 18. The tissues contain some domains that are different from the muscular tissue seen in the optical image. The observed fluorescent cells indicate that the injected cells survived among the muscular cells. In contrast, no living cells were observed when the cells were injected as a PBS solution without enantiomeric micelles.

Summary

The combination of “thermo-responsive” and “biodegradable” properties is the grail that is being sought by scientists for biomedical use. Consequently, the hydrogels formation via block copolymer micelles consisting of enantiomeric PLA and PEG has an important role in many hydrogels bioapplications. The mixture of ABA-type block copolymers, PLLA–PEG–PLLA and PDLA–PEG–PDLA, with specific molecular weight and block ratio exhibits attractive sol-to-gels transition between room and body temperature, can be used as an injectable biodegradable scaffold. The reversible gels–sol transitions occur in the mixed micellar solution of the enantiomeric BAB triblock copolymers, PEG–PLLA–PEG and PEG–PDLA–PEG, depending on the polymer concentration and temperature. An understanding of the gelation mechanisms for ABA, BAB, and AB systems involving stereocomplex crystals is critical to achieve their potential for bioapplications. There are many hydrogels formation theories and understanding gelation mechanisms will provide new prospectives for biomaterial applications.

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