Thermo-sensitive and biodegradable hydrogels based on stereocomplexed Pluronic multi-block copolymers for controlled protein delivery

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Abstract

Injectable sol–gel transition hydrogels based on thermo-sensitive polymers are of great interest as potential biomaterials for sustained delivery of therapeutic molecules. A novel temperature-sensitive and in-situ forming hydrogel system based on Pluronic F127 was developed and evaluated. A series of multi-block Pluronic copolymers linked by D-lactide and L-lactide oligomers with different spacer lengths were synthesized. A pair of multi-block copolymers having the corresponding enantiomeric D- or L-lactide oligomer spacer was blended to form stereocomplexed hydrogels. The resultant physically crosslinked Pluronic hydrogels exhibited significantly altered sol–gel phase transition behaviors with much lower critical gelation concentrations and temperatures, compared to the uncomplexed multi-block or Pluronic homopolymer hydrogels. The stereocomplexed hydrogels also had far increased mechanical strength with high resistance to rapid dissolution in aqueous medium. When human growth hormone (hGH) was incorporated in the stereocomplexed multi-block Pluronic copolymers, hGH was released out in a sustained and zero-order fashion for 13 days by a diffusion/erosion coupled mechanism.

Keywords: Hydrogel; Multi-block; Stereocomplex; Injectable; Thermo-sensitive; Protein delivery

1. Introduction

Injectable and biodegradable hydrogels are of great interest as potential materials for protein delivery and tissue engineering [1]. Especially, thermo-sensitive and sol–gel transition water soluble polymers can provide in-situ forming hydrogels for delivery of therapeutic molecules upon injection into the body without an invasive surgical procedure [2]. Physically crosslinked hydrogels self-assembled from intermolecular ionic interaction, hydrogen bonding, and hydrophobic interaction are advantageous for macromolecular delivery systems, since they provide a more benign environment for encapsulation of proteins and cells, compared to chemically crosslinked hydrogels [3]. Various synthetic thermo-sensitive polymers such as poly(N-isopropylacrylamide) [4], polyphosphazenes [5,6], poly(ethylene oxide) (PEO)/poly(propylene oxide) (PPO) tri-block copolymers [7–9], and PEO/poly(α,β-lactide-co-glycolide) (PLGA) tri-block copolymers [10,11] have been extensively studied for use as injectable sol–gel transition hydrogel systems for pharmaceutical and biomedical applications.

Among these, a series of PEO–PPO–PEO tri-block copolymers (e.g. Pluronics and Poloxamers) has been widely exploited as in-situ forming drug delivery carriers because they exhibit unique sol–gel transition behaviors in response to temperature in aqueous solution [12]. These tri-block copolymers form spherical micelles in aqueous solution by hydrophobic interaction between the middle PPO segments [13]. Above a critical gelation temperature and concentration, the self-assembled micelles are closely packed to produce a physically crosslinked gel structure. However, the Pluronic hydrogels are very soft and easily disintegrated and dissolved out upon contact with excess amount of buffer solution. This is due to the fact that the concentration of Pluronic copolymers is immediately diluted to below the critical gelation concentration, resulting in the dis-assembling of the micellar structure [7,8]. Thus, when a highly concentrated aqueous solution of Pluronic copolymer is injected into the body tissue as a sol state, the in-situ formed gel structure cannot be maintained for the desired period to achieve sustained release of...
encapsulated proteins or support the proliferation of cells to induce new tissue formation.

Recently more robust physical hydrogels with improved gel stability have been prepared by introducing stereocomplexed crystalline domains in the hydrogel structure, which can crosslink water soluble polymers in a more stable manner than any other physical hydrogels prepared traditionally by the aforementioned noncovalent interactions [3,14–17]. Biodegradable poly(lactic acid) having a chiral carbon atom in the monomer structure exhibits such stereocomplex crystalline behavior. It is well known that a pair of enantiomeric poly(l-lactic acid) and poly(d-lactic acid) produces a stereocomplexed crystalline structure different from the crystalline structure of the respective homopolymer. Various water soluble polymers grafted with enantiomeric oligo d- and l-lactic acid chains can form more stable hydrogels based on stereocomplex formation. We have previously reported that a pair of poly(2-hydroxylethylmethacrylate, HEMA) grafted with enantiomeric oligo d- or l-lactic acid was stereocomplexed to form physically crosslinked hydrogels [18]. Similarly, de Jong et al. [16] reported that in-situ forming stereocomplexed dextran hydrogels could be prepared by grafting d-lactic acid and l-lactic acid oligomers to the backbone of dextran. The stereocomplexed dextran hydrogels exhibited superior rheological properties, compared to dextran or dextran grafted with a single enantiomeric oligo(lactic acid), and also demonstrated potential sustained release applications for therapeutic proteins [19,20]. Hydrogels and microparticles based on stereocomplex formation of tri-block copolymers of PEG end-capped with d-lactic acid and l-lactic acid oligomers have also been reported in various studies [21–23]. More recently, the incorporation of star-shaped PEG or crosslinking by photopolymerization has produced stereocomplexed hydrogels with enhanced gelation characteristics [24,25].

The synthesis of multi-block hydrogels has also been suggested as another approach to form more mechanically strong hydrogels. Cohn et al. [26] designed a series of multi-block copolymers based on Pluronic F127, which showed much enhanced stability and delayed degradation behaviors compared to the homopolymer. We and others also studied physically crosslinked multi-block copolymers by introducing stereocomplex crystalline domains [27,28]. Tri-block copolymers of poly(l-lactic acid)-PEG-poly(l-lactic acid) and poly(d-lactic acid)-PEG-poly(d-lactic acid) were able to form stereocomplexed multi-block hydrogels with improved mechanical properties even at low concentrations [28].

In this study, a novel type of thermo-sensitive and biodegradable stereocomplexed hydrogels composed of multi-block Pluronic copolymers was synthesized by combining the ideal hydrogel characteristics for sustained release of protein drugs: thermosensitivity, biodegradability, biocompatibility, and in-situ gel forming ability. A series of multi-block Pluronic copolymers linked by d-lactide and l-lactide oligomers was synthesized to form in-situ forming stereocomplexed hydrogels upon mixing the pair of two corresponding enantiomeric Pluronic multi-block copolymers (Fig. 1). It was hypothesized that the spontaneous formation of stereocomplex crystalline domains in the thermo-sensitive self-assembling hydrogel structure provided additional crosslinking points, thereby substantially increasing the gel strength of Pluronic based micellar hydrogels. The sol–gel transition behaviors and rheological properties of the hydrogels formed by stereocomplexation were examined. Furthermore, the stereocomplexed Pluronic

Fig. 1. Schematic illustration for molecular structure of hydrogels formed by PL copolymer end-capped with oligolactide (PL-Dn or PL-Ln), multi-blocks (M-Dn or M-Ln), and stereocomplexed multi-blocks (ST-n).
hydrogels were evaluated for sustained release of human growth hormone (hGH).

2. Materials and methods

2.1. Materials

Pluronic F127 ((PEG)99(PPG)69(PEG)99) (Mw 12,600), 1,6-hexamethylene diisocyanate, and stannous octoate (tin(II) bis(2-ethylhexanoate), were purchased from Sigma-Aldrich (Milwaukee, USA). D-lactide and L-lactide were obtained from Purac (Netherlands) and recrystallized using ethyl acetate before use. Recombinant human growth hormone (hGH) was kindly provided by Dong-A Pharmaceutical Co. (Seoul, Korea). All other chemicals were of analytical grade.

2.2. Synthesis of Pluronic copolymers end-capped with oligo(D- or L-lactic acid)

Pluronic F127 tri-block copolymers end-capped with oligo (D-lactic acid) or oligo(L-lactic acid) were synthesized by ring-opening polymerization of D-lactide or L-lactide using the two terminal hydroxyl groups of Pluronic F127 (PL) as initiators and stannous octoate as a catalyst (Fig. 2). Briefly, 3 g of PL was dissolved in 80 ml of anhydrous toluene and added with D- or L-lactide in 20 ml of anhydrous toluene at various molar ratios of −OH/D- or L-lactide (1/6, 1/12, 1/18) to produce PL end-capped with oligo(D- or L-lactic acid) with various chain lengths (PL-Dn and PL-Ln; n = degree of polymerization, DP). The solution mixture was heated to 130 °C, and the ring-opening polymerization reaction was initiated by adding 0.05 wt.% (w/w) stannous octoate and proceeded for 6 h. The product was purified by precipitation in cold diethyl ether (−20 °C), dried in vacuum, and stored in a deep freezer until use.

2.3. Synthesis of Pluronic multi-block copolymers with oligo (D- or L-lactic acid) spacers

Multi-block PL copolymers linked with the corresponding oligo(D- or L-lactic acid) chains (M-Dn and M-Ln) were synthesized using a diisocyanate compound as the cross-linker between the two terminal hydroxyl groups of PL-Dn or PL-Ln. Briefly, 5 g of PL-Dn or PL-Ln was dissolved in 120 ml of toluene. After heating the solution to 90 °C, an equimolar amount of 1-6-hexamethylene diisocyanate (HDI, 64.62 mg) was added to the reaction mixture, and the reaction was monitored every 4 h by gel permeation chromatography (GPC, Waters 600 S pump, Waters, USA) using a Phenogel column (Phenomenex, USA) having an effective molecular weight (Mw) range of 5000–70,000. The reaction was stopped after 20 h, purified by precipitation in cold diethyl ether (−20 °C), and stored in a deep freezer until use.

2.4. Stereocomplex formation and differential scanning calorimetry

Stereocomplexed PL multi-block copolymers (ST-n) were formed by mixing an equimolar amount of M-Dn and M-Ln having the same oligo(lactic acid) chain length in organic solvent. In brief, a total 1 g of M-Dn and M-Ln (50/50 weight ratio) was dissolved in 20 ml of chloroform and stirred for 12 h under stirring condition. The solvent was evaporated, and the formed stereocomplexed polymers were further dried in a vacuum oven for several days. Stereocomplex formation was assessed by examining the presence of crystallinity using differential scanning calorimetry (Exstar 6000, Seiko Instruments Inc.). Dry powders of PL, M-Ln, or ST-n were sealed in an aluminum pan, and the DSC thermograms were recorded based on calibration with indium standards. Samples were first cooled to −10 °C for 5 min and then heated to 250 °C at a heating rate of 5 °C/min.

2.5. Temperature-responsive sol–gel transition behavior of hydrogels

Temperature-responsive sol–gel transition behaviors for PL, PL-Ln, M-Ln, and ST-n were determined using a vial tilting method [29]. Hydrogel solutions were prepared in 2 ml-Eppendorf tubes at various concentrations by dissolving PL, M-Ln, or ST-n in 0.3 ml of phosphate buffered saline (PBS) solution and gently stirring overnight at 4 °C. To determine whether the hydrogel solutions were in a sol or gel state, the temperature was first set to 0 °C and increased 1 °C at a time using a temperature-controllable circulator. The samples were stabilized at each temperature point for at least 5 min and then the vial was tilted. At each temperature, an apparent flow of the solution was regarded as a sol, and otherwise a gel. Two different samples were used to determine the sol–gel phase transition curves. There was no significant deviation in the concentration- and temperature-dependent gelation behaviors between the two samples.
Table 1
Synthesized multi-block copolymers with various lengths of oligo(lactic acid) spacers

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Chemical formula</th>
<th>Mw</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-D6</td>
<td>[LA]<em>{k,5}PL-[LA]</em>{k,3}k_{18}</td>
<td>56,580</td>
</tr>
<tr>
<td>M-L6</td>
<td>[LA]<em>{k,2}PL-[LA]</em>{k,2}k_{03}k_{18}</td>
<td>54,373</td>
</tr>
<tr>
<td>M-D12</td>
<td>[LA]<em>{13,5}PL-[LA]</em>{13,5}k_{11}k_{11}</td>
<td>59,480</td>
</tr>
<tr>
<td>M-L12</td>
<td>[LA]<em>{12,5}PL-[LA]</em>{12,5}k_{18}k_{18}</td>
<td>57,312</td>
</tr>
<tr>
<td>M-D18</td>
<td>[LA]<em>{18,5}PL-[LA]</em>{18,5}k_{06}k_{06}</td>
<td>61,680</td>
</tr>
<tr>
<td>M-L18</td>
<td>[LA]<em>{17,5}PL-[LA]</em>{17,5}k_{91}k_{91}</td>
<td>59,477</td>
</tr>
</tbody>
</table>

2.6. Rheological measurements of hydrogels

The rheological properties of the hydrogels were investigated using the Gemini™ rheometer (Bohlin Instruments Inc, NJ, USA). The storage modulus (G') and loss modulus (G'') values were monitored according to oscillation frequency, PL, M-Ln, and ST-n samples at a concentration of 17.5 wt.% were prepared in PBS solution, applied to the rheometer, and gelated for 10 min prior to measurement. A parallel plate with a diameter of 20 mm was used and the gap size was assigned to 150 μm. Strain was controlled to 2%.

2.7. Protein release and degradation behaviors of hydrogels

Hydrogels encapsulating hGH were prepared by dissolving PL, M-Ln, ST-n at polymer concentrations of 17.5 wt.%, 12.5 wt.%, and 10 wt.%, respectively in 0.5 ml of 1 mg/ml hGH in PBS solution and stirring at 4 °C overnight. Since the four samples exhibited different sol–gel phase diagrams with different critical gelation concentrations and temperatures, the concentration for each sample was selected within the range where each hydrogel was in a sol state at room temperature (25 °C) but became a gel state at physiological temperature (37 °C). For gelation, the hydrogel samples were incubated at 37 °C for 10 min, added with 0.5 ml of PBS buffer solution, and then further incubated at 37 °C for release. The release conditions such as the volume of buffer solution were optimized by preliminary experiments with considering a sink condition. At determined time points, the release medium (0.5 ml) was collected for determining the amount of hGH, and the remaining hydrogel mass was freeze-dried for measuring dry weight. The collected volume was replenished with the same volume of PBS solution which was pre-equilibrated at 37 °C. After removing the insoluble precipitates by centrifugation, the released protein amount was quantified by the Micro-BCA assay (Pierce) based on a calibration curve obtained from a fresh hGH solution.

3. Results and discussion

3.1. Synthesis of Pluronic multi-block copolymers with oligo (D- or L-lactic acid) spacers

M-Dn and M-Ln copolymers with various lengths of oligo (D- or L-lactic acid) spacers were synthesized. At first, PL-Dn and PL-Ln were synthesized by ring-opening polymerization of D- or L-lactic acid monomers onto the terminal hydroxyl groups of PL. 

\[ ^{1}H \text{ NMR analysis showed that at } \text{−OH/(D- or L-lactide)} \]
molar ratios of 1/6, 1/12, 1/18, PL-Dn and PL-Ln with oligo (D- or L-lactic acid) units (n) of 6, 12, 18 (PL-D6, PL-D12, PL-D18 and PL-L6, PL-L12, PL-L18) were synthesized. Next, M-Dn and M-Ln were synthesized by chemical conjugation between each of the PL-Dn or PL-Ln copolymers with various chain lengths (M-D6, M-D12, M-D18 and M-L6, M-L12, M-L18) (Table 1). The molecular weight of the synthesized multi-block copolymers was determined using GPC. As shown in Fig. 3, M-D18 peak is eluted earlier than PL-D18 (M-D18: 6.338 min, PL-D18: 7.425 min). From the calculated weight average molecular weight (61,680), it was found that about 4.06 units of PL-D18 were conjugated to form an M-D18 multi-block chain. It should be also noticed that the GPC curve of M-Dn had a single peak with no detectable satellite peaks, implying that the conversion of PL-Dn into M-Dn was stoichiometrically complete with extremely low amount of unconjugated homopolymer.

3.2. Formation of stereocomplexed Pluronic multi-block copolymers

ST-n (ST-6, ST-12, ST-18) were produced by blending an equimolar pair of M-Dn and M-Ln in an organic solvent and inducing the formation of stereocomplex between the corresponding oligo(D-lactic acid) and oligo(L-lactic acid) spacers of M-Dn and M-Ln, respectively. The stereocomplexed crystallites produced from the complexation of enantiomeric oligo(D-lactic acid) and oligo(L-lactic acid) spacers should have physically crosslinked the M-Dn and M-Ln copolymers, resulting in more robust and stable PL based sol–gel transition hydrogels. DSC analysis showed that a 50/50 blending mixture of M-Dn and M-Ln with a spacer length of at least 12 lactide units resulted in stereocomplexed multi-block copolymers showing the typical stereocomplex crystallinities. As shown in Fig. 4, a crystalline melting temperature (Tm) peak for the PEO blocks of the PL copolymer first appears at 58 °C for all of the samples. Distinct Tm peaks can be observed for ST-12 at 163 °C, and for ST-18 at 165 °C and 171 °C, which can be attributed to the melting of crystallites for the stereocomplexed copolymers. In contrast,
3.3. Hydrogel characterization

Hydrogels were prepared by completely dissolving the synthesized copolymers in pH 7.4 PBS solution with a desired concentration at lower temperature (~4 °C) than the gelation temperature. Sol–gel phase transition behaviors were then assessed by a vial tilting method. All hydrogels, PL (control), PL-Ln, M-Ln, and ST-n where n = 6, 12, and 18, exhibited reversible sol–gel–sol transition behaviors in response to temperature, showing the typical sol–gel–sol transition curve similar to that of PL, as shown in Fig. 5. In a gel state, the M-Ln and ST-n hydrogels were observed to be turbid, while the PL hydrogels appeared transparent. When raising the temperature, a lower critical solution temperature (LCST) for sol-to-gel transition and an upper critical solution temperature (UCST) or precipitation temperature for gel-to-sol transition can be seen for each concentration, which also determines the critical gelation concentrations [12]. However, the critical gelation temperatures and concentrations were significantly altered depending on the chain length of oligo(lactic acid) spacer, and more importantly on the type of the synthesized copolymer. For n = 6, PL-L6, M-L6, and ST-6 showed increased critical gelation concentrations with more narrow gelation temperature windows, resulting in gradual right-hand shifts of the overall phase curves compared to that of PL (PL-L6 < M-L6 < ST-6). It was likely that for ST-6, the D- and L-oligo(lactic acid) chain lengths were not long enough for stereocomplexation (as shown in the DSC results), possibly perturbing the formation of a well-ordered Pluronic micelle packing structure. This might require higher polymer concentration for gelation [31]. For n = 12 and 18, however, the phase curves for the multi-blocks (M-L12 and M-L18) and stereocomplexed multi-blocks (ST-12 and ST-18) were significantly shifted to the left and broadened the gelation temperature window compared to that of PL, while PL-L12 and PL-L18 still showed right-hand shifts compared to PL. In particular, the stereocomplexed multi-block hydrogels (ST-12 and ST-18) were far more shifted to the left and exhibited broader gelation temperature ranges than the corresponding multi-block hydrogels (M-L12 and M-L18). Since the gradual left-hand shift of the phase curve means that the critical gelation concentration was decreased, it can be concluded that sol–gel transition of the M-Ln and ST-n hydrogels with a longer oligo (lactic acid) spacer occurred more easily at much lower polymer concentrations. This can be related to the structural stability of individual spherical PL micelles that self-assembled and were closely packed to form a three dimensional gel structure at a critical concentration and temperature. It was likely that multi-block hydrogels (M-L12 and M-L18) were formed by the packing of spherical PL micelles that were simply interconnected by oligo(l-lactic acid) spacers introduced between the PL copolymers as shown in Fig. 1 [32]. For stereocomplexed multi-block hydrogels (ST-12 and-18), stereocomplexed crystalline domains generated between the enantiomeric oligo(lactic acid) spacers might provide additional anchoring and inter-locking sites between the packed PL micelles. This would substantially stabilize the three dimensional gel structure maintained primarily by a well-ordered packed structure of

Fig. 4. DSC thermograms for multi-block and stereocomplexed multi-block copolymers: (A) M-L6, (B) M-L12, (C) M-L18, (D) ST-6, (E) ST-12, and (F) ST-18. Red circles indicate T_m peaks resulting from stereocomplex formation.

either M-Dn or M-Ln homopolymers did not show any noticeable crystalline melting behaviors in the DSC thermogram other than the one for the PEO block. This was due to the fact that stereocomplex crystallization of the enantiomeric oligo(lactic acid)s occurs more preferentially and readily than crystallization of the homopolymer alone [30]. For ST-18, two melting peaks can be seen, which was probably due to the formation of heterogeneous and imperfect stereocomplex crystalline structures upon blending the pair of M-Dn and M-Ln copolymers. Previous studies have shown that either D- or L-oligo(lactic acid) with at least 11 lactic acid units exhibit a crystalline melting temperature at 59 °C, while their blend mixture forms a stereocomplexed crystalline structure showing a melting temperature at 119 °C [30]. In this study, since D- or L-oligo(lactic acid) were introduced into PL multi-block copolymers, slightly increased chain lengths of oligo(lactic acid)s might be required for stereocomplexation probably due to the presence of the PEG chains adjacent to the D- and L-oligo(lactic acid) spacer. In addition, highly crystallizable PEG chains in a dry state might affect the crystallization behavior of very adjacently formed stereocomplexed structures, changing the extent of crystallinity and T_m values.
individually self-assembled PL micelles. Thus, the structural stabilization of packed micelles might decrease the critical gelation concentration and temperature. All hydrogels showed reversible sol–gel transition behaviors in response to temperature, meaning that the hydrogels can be potentially utilized as injectable material for sustained release of proteins, genes, and cells. Furthermore, stereocomplexed hydrogels could be formed at much lower concentration ranges than PL (12.5 wt.% for ST-12 and 8.75 wt.% for ST-18, compared to ~16 wt.% for Pluronic F127), suggesting additional advantages over the previously reported injectable matrices [27].

Rheological properties of multi-block (M-L18) and stereocomplexed multi-block (ST-18) hydrogels were examined as compared to those of PL hydrogel by measuring the storage modulus ($G'$) and loss modulus ($G''$) values as a function of oscillation frequency (Fig. 6). All three hydrogels, ST-18, M-L18, and PL, were observed as elastic gels throughout the whole frequency range at the determined polymer concentrations at 37 °C, as indicated by the tan δ values of over 1 and high $G'$ values of over 1000. Particularly, the ST-18 hydrogel exhibited much higher elasticity (e.g. $G' = 26,100$ at frequency of 10 Hz): about 1.6-fold higher $G'$ values than that of M-L18 ($G' = 16,400$ at 10 Hz) and 8.8-fold higher than PL ($G' = 2,980$ at 10 Hz) hydrogel. The M-L18 hydrogel showed much greater $G'$ values, about 5.5-fold higher, compared to that of the PL hydrogel. Therefore, the formation of stereocomplexed multi-block copolymer structures should have resulted in the greatly enhanced mechanical strength of the hydrogel network due to its increased crosslinking density.

3.4. Sustained release of hGH and mass erosion behaviors

Fig. 7 shows the hGH release and mass erosion behaviors of multi-block (M-L12 and M-L18) and stereocomplexed hydrogels (ST-12 and ST-18), as compared to those of PL hydrogels. PL hydrogels completely released out hGH within the first day of incubation. This was due to rapid dissolution and disintegration of the PL gel structure upon incubation in the PBS buffer solution. In contrast, M-L12, M-L18, ST-12, and ST-18 hydrogels exhibited much more sustained release patterns over an extended period of time. Particularly, ST-12 and ST-18 hydrogels showed sustained hGH release profiles for 13 days, while M-L12 and M-L18 hydrogels demonstrated similar constant release profiles for ~6 days. M-L12 and ST-18 hydrogels showed a near zero-order release pattern, while M-L12 and ST-12 hydrogels showed an initial burst followed by constant release. The release duration period of hGH for ST-12 and ST-18 hydrogels was almost doubled in comparison to that of the corresponding multi-block (M-L12 and M-L18) hydrogels. Increasing the chain length ($n$) of oligo(lactic acid) spacer from 12 to 18 enabled hGH release profiles to be more linear. It
plexed hydrogels exhibited delayed erosion behaviors through a

Further was significantly influenced by the dissolution/degradation rate of physically crosslinked hydrogels surface area of the gel was changed. This was because the above rhGH release profiles would be altered when the release saturation level, which indicates that a sink condition was hGH in the release buffer solution would be well below the solubility of over 10 mg/ml at pH 7.4. Since hGH has a benign microenvironment for the encapsulated proteins, as [33]. This suggests that the PL based hydrogels provided a more benign microenvironment for the encapsulated proteins, as demonstrated in a previous study [34]. Since hGH has a solubility of over 10 mg/ml at pH > 6.0, the concentration of hGH in the release buffer solution would be well below the saturation level, which indicates that a sink condition was maintained during the release period. It should be noted that the above rhGH release profiles would be altered when the release conditions such as the volume of buffer solution and exposed surface area of the gel was changed. This was because the dissolution/degradation rate of physically crosslinked hydrogels was significantly influenced by the in vitro release conditions [35]. Further in vivo release studies would be necessary to confirm the above in vitro release behaviors.

Mass erosion results revealed that multi-block and stereocomplexed hydrogels exhibited delayed erosion behaviors through a diffusion/erosion coupled mechanism. ST-12 and ST-18 hydrogels eroded more slowly than the M-L12 and M-L18 hydrogels, probably due to the presence of additional stereocomplex crystal-line domains that inter-locked and stabilized the closely packed PL micelles [36]. M-L12 and ST-12 hydrogels exhibited a bi-phasic erosion profile: a fast and linear erosion process in the early stage followed by slower erosion in the later stage, while for M-L18 and ST-18, mass erosion occurred in a near zero-order fashion. Within the hydrogel, a distinct swollen layer could be visually observed which should have developed during the incubation period. It was likely that this surface layer was preferentially degraded and eroded, rather than the interior region of the hydrogel. This was partly due to the fact that the surface layer was more directly exposed to a large excess amount of buffer media and more susceptible to decrease in polymer concentration to below the critical gelation concentration [37]. Additionally, multi-block and stereocomplexed hydrogels were also chemically degraded by gradual hydrolytic cleavage of the oligo(lactic acid) spacers linking the PL copolymers. The linear mass erosion rate could be attributed to a combined physical process of polymer chain disentanglement, disruption of single self-assembled Pluronic micelle structures, and overall disintegration of the closely packed micellar gel structure, resulting from dilution in polymer concentration upon incubation and chemical scission of the oligo(lactic acid) linkages. Since the mass erosion profiles were well matched with the hGH release patterns, it can be safely said that hGH was released out via a diffusion/erosion coupled mechanism, rather than just a diffusion controlled mechanism [38]. The entrapped hGH was likely to diffuse out from the enlarged and loosened pores in the eroding and swollen surface layer. This would result in the near zero-order hGH release profiles, similar to the surface eroding hydrophobic polymers such as polyanhydrides [39]. Concerning the stability and bioactivity of hGH released from the stereocomplexed Pluronic hydrogels, it was expected that there would be less severe structural deterioration compared to other reported delivery systems using PLGA polymers [40], because little or no acidic degradation products were generated during the erosion process. However, more detailed studies about the bioactivity of the released hGH would be desirable by measuring serum levels of hGH, insulin growth factor-1 (IGF-1), and IGF binding protein-3 (IGFBP-3) in in vivo studies. This is because conformational and structural analysis of the released hGH in vitro, using methods such as circular dichroism spectroscopy and gel electrophoresis, does not provide sufficient evidence for the inherent bioactivity of hGH in vivo.

Lastly, it should be noted that the current stereocomplexed multi-block Pluronic hydrogels are very different from other in-situ forming stereocomplexed hydrogels prepared from a pair of enantiomeric oligolactic acid) terminated PEG derivatives with respect to the loading of macromolecular drugs in the gel. Previously, two enantiomeric oligolactic acid) conjugated precursor polymers were mixed along with protein drugs by using a dual-barrel syringe to form a stereocomplexed hydrogel. This might require a short lag time for the formation of a stereocomplexed hydrogel network, during which the entrapped protein molecules were likely to release out rapidly. In contrast, the current stereocomplexed hydrogels, still exhibiting the thermo-sensitive sol–gel transition behavior, could be pre-fabricated and used for

Fig. 7. Release of hGH and mass erosion profiles for hydrogels of PL (17.5 wt.%) with (A) M-L12 (12.5 wt.%), ST-12 (10.0 wt.%), and (B) M-L18 (12.5 wt.%), ST-18 (10.0 wt.% ) copolymers.

is of particular interest to note that the encapsulated hGH within multi-block and stereocomplexed hydrogels was completely released out after 6 and 13 days, respectively. The complete and linear release of hGH suggests that the encapsulated hGH within the hydrogels might not undergo any deleterious physical aggregation and precipitation, often observed for biodegradable polymeric microspheres that normally show high initial bursts with slow release followed by subsequent incomplete releases due to serious protein instability problems [33]. This suggests that the PL based hydrogels provided a more benign microenvironment for the encapsulated proteins, as demonstrated in a previous study [34]. Since hGH has a solubility of over 10 mg/ml at pH > 6.0, the concentration of hGH in the release buffer solution would be well below the saturation level, which indicates that a sink condition was maintained during the release period. It should be noted that the above rhGH release profiles would be altered when the release conditions such as the volume of buffer solution and exposed surface area of the gel was changed. This was because the dissolution/degradation rate of physically crosslinked hydrogels was significantly influenced by the in vitro release conditions [35]. Further in vivo release studies would be necessary to confirm the above in vitro release behaviors. Mass erosion results revealed that multi-block and stereocomplexed hydrogels exhibited delayed erosion behaviors through a diffusion/erosion coupled mechanism. ST-12 and ST-18 hydrogels
homogeneous protein loading in a sol state below the gelation temperature. The protein containing sol solution could be directly injected into a desired tissue site to form an instantaneous, but robust thermo-gel at body temperature by using a single-barrel syringe. We are currently investigating in vivo studies of hGH delivery systems using the stereocomplexed multi-block Pluronic hydrogels.

4. Conclusions

A novel hydrogel delivery system based on stereocomplexed multi-block Pluronic copolymers was developed for sustained release of hGH. A series of multi-block Pluronic copolymers linked by t-lactide and l-lactide oligomers were synthesized and blended to form stereocomplexed hydrogels. The stereocomplexed multi-block hydrogels showed enhanced gel stability and mechanical strength, linear mass erosion profiles, and near zero-order hGH release patterns. Since the synthesized hydrogels are biodegradable, biocompatible, and thermosensitive, there would be a wide range of applications for delivering proteins, genes, and cells in an injectable manner.

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