Carbohydrate Polymers 108 (2014) 26–33

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Thermosensitive *in situ* hydrogel based on the hybrid of hyaluronic acid and modified PCL/PEG triblock copolymer

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ARTICLE INFO

Article history:
Received 11 December 2013
Received in revised form 20 February 2014
Accepted 2 March 2014
Available online 21 March 2014

Keywords:
Hydrogel
Poly(ε-caprolactone)/poly(ethylene glycol) block copolymer
Hyaluronic acid
Hybrid
Degradation
Drug release

ABSTRACT

In this work, a new hydrogel was constructed using poly(ε-caprolactone-co-1,4,8-trioxa[4.6]spiro-9-undecanone)–poly(ethylene glycol)–poly(ε-caprolactone-co-1,4,8-trioxa[4.6]spiro-9-undecanone) triblock copolymers (PECT) with hyaluronic acid (HA) in order to expand application scopes of PECT hydrogel. The rheological and sol–gel phase transition behaviors were investigated by rheometer and test tube inversion method, and the interior morphologies of hydrogel systems were observed by scanning electron microscope (SEM). With the introduction of HA, certain properties of PECT hydrogel, such as viscosity and morphology, have present trends with regularity. Furthermore, with the participation of HA, the degradation and release of acetylsalicylic acid was slightly affected, however, the drug release mechanism of hydrogel has not been changed. PECT/HA hydrogel is confirmed to be non-toxic through a test to NIH3T3 cells. In conclusion, blending with HA is a feasible and safe method to tune properties of PECT hydrogel.

1. Introduction

Thermo-sensitive injectable *in situ* hydrogel systems are fluids in aqueous solutions at room temperature, and gel under physiological conditions (Brandl et al., 2010; Bruggeman, de Bruin, Bettinger, & Langer, 2008). Injectable hydrogels allow good physical integrations into the defect, potentially avoiding an open surgery procedure and facilitating the use of minimally invasive approaches for drug delivery (Bajpai, Shukla, Bhanu, & Kankane, 2008; Chang, Ci, Yu, & Ding, 2011; Ehrbar, Schoenmakers, Christen, Fussenegger, & Weber, 2008).

Thermosensitive hydrogels based on tri-block copolymer poly(ε-caprolactone)–poly(ethylene glycol)–poly(ε-caprolactone) (PCL–PEG–PCL, PCEC) hydrogel were developed previously and used widely. This biocompatible hydrogels can degrade *in vivo* for a few months (Gong, Shi, Wu, et al., 2009; Gong, Shi, Dong, et al., 2009; Ma, Miao, & Song, 2010). However, due to the strong crystalline of PCL, for preparing the hydrogels, PCL–PEG–PCL polymer should be dissolved in water at a temperature above the melting point of PCL initially and then incubated the mixture in the environment of 4 °C for 24 h, which undoubtedly complicates the process and limits its applications clinically (Gou et al., 2009; Liu et al., 2008; Ruel-Gariépy & Leroux, 2004).

To solve this problem, a new tri-block copolymer, poly(ε-caprolactone-co-1,4,8-trioxa[4.6]spiro-9-undecanone)–poly(ethylene glycol)–poly(ε-caprolactone-co-1,4,8-trioxa[4.6]spiro-9-undecanone) (PECT), which is a modified PEG/PCL polymer by pendant cyclic ether, was developed in our previous work(Wang, Chang, et al., 2012). The introduction of pendant cyclic ether destroyed the isotacticity of PCL, and decreased the crystallinity. Therefore, to get the hydrogel, the process of sol–gel phase transition can avoid the pre-quenching treatment which is needed for PCL–PEG–PCL gelation. The polymer can be made into freeze-dried powder which ensures a convenient storage for the polymer. And the freeze-dried powder of drug-loaded PECT nanoparticles can disperse in water to form a stable self-assembling nanoparticle aqueous dispersion rapidly at ambient temperature, furthermore, the hydrogel formed from PECT nanoparticles has an outstanding controllable thermal gelation behavior and excellent

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http://dx.doi.org/10.1016/j.carbpol.2014.03.016
0144-8617/

biocompatibility and biodegradability (Wang, Deng, et al., 2012). However, the hydrogel has a shortage of strength. To expand its application, the viscosity of PECT hydrogel must be enhanced to adapt to different injection situations, for example, when applied in some active parts of body, the hydrogel needs a high viscosity. For this case, necessary changes were needed to be introduced to our system.

Hyaluronic acid (HA) is a naturally linear ionic polysaccharide made of repeating disaccharide units of D-glucuronic acid and N-acetyl glucosamine linked by β(1,4) and β(1,3) glucosidic bonds (Bae, Yoon, & Park, 2006; Burdick, Chung, Jia, Randolph, & Langer, 2005). The physical and chemical properties of HA have been investigated and reviewed thoroughly (Jeon et al., 2007; Jia et al., 2006; Park, Tirelli, & Hubbell, 2003). Owing to a high level of molecular weight value and strong intermolecular interactions, HA aqueous solutions are highly viscous and shear-thinning, and crosslinked HA has been widely used (Sannino et al., 2004; Segura et al., 2005; Xu, Jha, Harrington, Farach-Carson, & Jia, 2012). Furthermore, HA is a kind of proteoglycans (PGAs) which are a major components of the extracellular matrix. Thus, with a standout biocompatibility, HA has been widely utilized in the field of cancer metastasis, wound healing, cell differentiation, and motility (Palumbo et al., 2013; Zhou et al., 2012). However, as a hydrophilic material, HA will potentially result in the crystallization of hydrophobic drugs. Therefore, if HA is employed as drug carrier alone, the effect of drug delivery can be impacted (Gong, Shi, Dong, et al., 2009; Xu et al., 2013). Therefore, HA is not suitable for drug delivery separately.

HA has been introduced to drug delivery hydrogel systems frequently and has achieved a desirable result (Lin, Chen, & Kuan, 2013). In our study, a novel hydrogel in which HA and PECT crosslinked as hydrogel network has been structurally enhanced. The network generated through intermolecular forces for HA's remarkable strength and excellent viscosity (Lee, Chung, & Kurisawa, 2008; Mao, Yin, & Yao, 2003; Yamane et al., 2005), HA's physical property has a great influence on the properties of PECT hydrogel.

2. Materials and methods

2.1. Materials

PECT was synthesized by ring opening polymerization of ε-caprolactone using PEG as the initiator and $Sn(Oct)_2$ as the catalyst following our previous method (Wang, Chang, et al., 2012). Sodium hyaluronate (M_w: 64 kDa; 210 kDa; 1000 kDa) was purchased from Shandong Freda™ Biopharm Co., Ltd. (Jinan, China). ASA was purchased from Aladdin™ Industrial Co., Ltd. Strips of porcine small intestinal submucosa (SIS) were cut from sheets obtained from Cook Biotech™ Inc., West Lafayette, IN. All other reagents were from the Jiangtian™ Co., and they were of analytical grade and were used as received without further purification.

2.2. Preparation of aqueous liquid of PECT/HA and gel formation

In order to improve the dispersibility of PECT, the PECT polymer was made into freeze-dried powder according to the following methods: Firstly, 5 g PECT polymer was dissolved in 15 ml tetrahydrofuran, and then the mixture was dropped into stirring water gradually with a constant speed. Leave it overnight in order to evaporate the THF thoroughly. Having been frozen and lyophilized, the freeze-dried powder of PECT was obtained.

In order to get HA, sodium hyaluronate need to be deprotonated. 1 g sodium hyaluronate was dissolved in 100 ml deionized water and dialyzed (MWCO: 8–14 kDa) for 12 h in dilute acid followed by lyophilized.

Table 1
Formulations prepared and tested.

Acronyms	PECT nanoparticles (%, w/w)	HA (%, w/w)	HA MW (kDa)
PECT	25	0	–
PECT/1% HA_{64}	25	1	64
PECT/1% HA_{210}	25	1	210
PECT/1% HA_{1000}	25	1	1000
PECT/2% HA_{64}	25	2	64
PECT/2% HA_{210}	25	2	210
PECT/2% HA_{1000}	25	2	1000

The aqueous HA liquids were prepared by dissolving HA with discrete amounts (0.01 g, 0.02 g) and molecular weights (64 kDa, 210 kDa, 1000 kDa) in 1 ml water under continuous stirring at 20 °C until clear solutions were obtained. PECT/HA mixture aqueous liquids were prepared by adding 0.25 g PECT to 750 mg HA liquids. Besides, a PECT solution (25%, w/w) which was HA free was also prepared. The compositions and the acronyms of the formulations tested are summarized in Table 1.

The mixtures were injected into test tubes separately and then the test tubes were placed in Vapor-bathing Constant Temperature Vibrator at 37 °C under shaking at 70 rpm, and their states were observed every 10 s.

2.3. Rheology analysis

The phase transition behaviors of PECT/HA hydrogels were investigated by a Fluids Rheometer (Stress Tech, Reologica Instruments AB). 1 ml polymer aqueous solutions were placed between parallel plates of 25 mm diameter and a gap of 0.5 mm at 20 °C for 20 min. The data were collected under a controlled stress (0.01 Pa) at a frequency of 1.0 Hz. The heating rate was 0.5 °C min^{-1}.

2.4. Interior morphology of PECT/HA hydrogel

SEM (S-4800, Hitachi) was employed to investigate the interior morphologies of PECT/HA hydrogel samples. The PECT/HA hydrogel samples were quickly frozen in liquid nitrogen and lyophilized for 72 h. Freeze-dried hydrogels were then fractured with care and the interior morphologies were visualized.

2.5. In vitro degradation of PECT/HA hydrogels

1 g aqueous solutions of PECT/HA were placed in test tubes and placed at 37 °C for 12 h to produce stable gels. Then 10.0 ml of 0.01 M phosphate-buffered saline (PBS, pH 7.4) was added to the test tubes. 3 parallel samples should be set. *In vitro* degradation was conducted under shaking (70 rpm) at 37 °C.

The mediums were replaced by a 10 ml aliquot of fresh PBS every day. At preset time intervals the residual samples were separated from the medium and lyophilized, and the weight loss examined gravimetrically.

2.6. In vitro release study

The ASA-loaded PECT/HA aqueous liquids were prepared by the following methods. 4.5 mg ASA, mixed with 100 mg PECT polymer, was dissolved in 2 ml tetrahydrofuran, and then the mixture was dropped in 10 ml stirring water dropwise. The fresh mixture was left overnight, in order to make sure that the tetrahydrofuran was evaporated thoroughly, with following steps of freeze and lyophilization. And the ASA-loaded freeze-dried powder was prepared. 750 mg aqueous HA solutions in which the mass of HA is 10 mg and 20 mg, respectively, should be prepared at the same time. And then add 250 mg ASA-loaded freeze-dried powder to 750 mg

stirring aqueous HA solutions in test tubes until the solutions were clear and transparent. Keep the solutions in water bath at 37 °C for 12 h to form stable hydrogel systems. *In vitro* release was observed under shaking at 70 rpm at 37 °C. At definite time intervals, 8 ml of PBS was collected to determine the amount of released drug and then another 8 ml of prewarmed fresh PBS replenisher was added to substitute. The collected solution was lyophilized and then dissolved in acetonitrile to determine the total amount of ASA in the released liquid. It should be noted that the collected solution of ASA-loaded PECT/1%HA_{210} was separated into two equal parts: one part was freeze-dried and then dissolved in acetonitrile to determine the total amount of ASA in the release liquid; the other part was filtered by ultrafiltration (cut-off 10,000 g mol^{-1}, Stirred Cells, Millipore Co., USA) to obtain released ASA soluble in PBS.

The amount of ASA in the release liquid was determined by ultraviolet and visible spectrophotometer. The detection wavelength was set at 296 nm. The injection volume was 3 ml. The accumulated release was calculated according to Eq. (1).

$$E = \left(\frac{V_E \sum_{1}^{n-1} C_i + V_0 C_n}{m_0} \right) \times 100\% \quad (1)$$

where E is the accumulated release (%), V_E is the sampling volume (5 ml), V_0 is the initial volume (10 ml), C_i and C_n are the drug concentrations (μg ml^{-1}), i and n are the sampling times, and m_0 is the mass of drug in the gel (mg).

2.7. Analysis of cytotoxicity

All the studied cells were maintained in Institute of Radiation Medicine Chinese Academy of Medical Sciences, Tianjin, China. The cytotoxicity of PECT/HA_{210} mixtures was evaluated by cell viability assay on NIH3T3 cells. Briefly, NIH3T3 cells were plated at a density of 5×10^3 cells per well in 100 μl of DMEM medium in 96-well plates and grown for 24 h. The cells were then exposed to PECT/HA mixtures at different concentration for 48 h, and the cells viability were measured using the methylthiazoletetrazolium (MTT) method. These assays were repeated 6 times, and results were expressed as mean value ± S.D.

3. Results and discussion

3.1. Structure and physico-chemical properties of hydrogels

On the basis of our previous study, aqueous solutions of PECT/HA were chosen as precursors of *in situ*-forming hydrogels. An aqueous solution of HA in double distilled water was mixed with PECT freeze-dried powder. The resulting solution existed in a translucent emulsion–sol state that flowed when tilted at room temperature, and gel at 37 °C. The phase transition time from solution to gel at 37 °C was approximately 10 s.

The impact of a series of factors such as the concentration and molecular weight of HA was studied. The state of six mixtures at room temperature and 37 °C were shown in Fig. 1. The results showed that all the solutions with a precise ratio of PECT nanoparticles and HA could transfer into hydrogels. In addition, the state of hydrogels was still dependable within 10 min when placed in the condition of room temperature.

The morphologies of hydrogels were observed by SEM. The micro appearances of hydrogels were displayed in Fig. 2. Analogous to the PECT hydrogel (Fig. 2G), all the PECT/HA hydrogels were porous. What is more, the size of the holes varies with the content and molecular weight of HA. In particular, the diameters of the holes

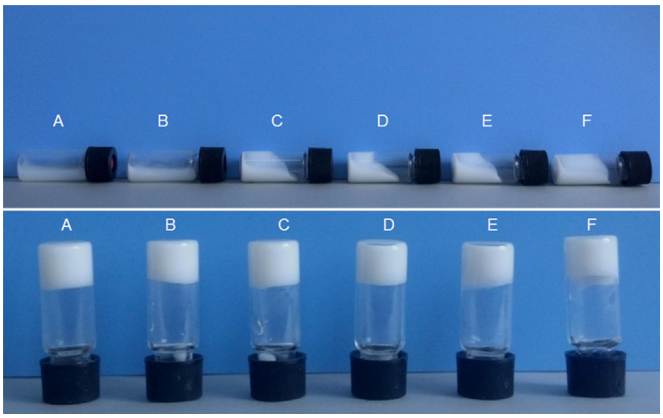

Fig. 1. Photographs of mixtures at room temperature (above) and *in situ*-formed hydrogels at 37 °C (below). From left to right: (A) PECT/1% HA_{64}; (B) PECT/1% HA_{210}; (C) PECT/1% HA_{1000}; (D) PECT/2% HA_{64}; (E) PECT/2% HA_{210}; and (F) PECT/2% HA_{1000}.

decrease with the increase of HA in the hydrogel when the molecular weight of HA is fixed (Fig. 2A, D, G or Fig. 2B, E, G). Likewise, when the amount of HA in the hydrogel is fixed, the diameters similarly decrease with the molecular weight of HA (Fig. A–C/Fig. 2D–F). For the former case, it is no doubt that when the HA molecular weight is fixed, the more of the HA molecules, the denser of the HA chains in the hydrogel, PECT nanoparticles were united with the HA chains, so the increase of HA molecular will necessarily lead to the shrink of holes. Obviously, when it comes to the latter case, the amount of HA is fixed, which means that the number of molecules is certain. While to the single molecule, when the molecular weight is larger, the molecular chain is longer, along with a better strength and stickiness. It can be argued that with a fixed amount of HA, a larger molecular weight will result in less molecular chains, and larger holes will be shown. Although the number of molecular chains is reduced, the stickiness between molecules will diminish the gap, hence, the hole size will decrease. However, PECT/2%HA_{1000} hydrogel was not homogeneous as shown in Fig. 2F, which means the aqueous solution of PECT/2%HA_{1000} is not in a good dispersion for the molecular weight of HA_{1000} is too high to fully dissolve in water with a concentration of 2%, so that PECT/2%HA_{1000} hydrogel is not qualified as drug carrier.

3.2. Rheology tests of PECT/HA

To gain a better understanding of the phase transition of PECT/HA, the change in the storage/loss moduli of the PECT/HA aqueous solution as a function of temperature from 20 to 60 °C was examined. For rheology test determination, the formation and destruction of hydrogel networks are involved in sol–gel and gel–sol phase transitions, respectively. Hence, it will be accompanied by an abrupt change in viscosity. This can be illustrated by the variation in storage modulus G′ and loss modulus G″ of hydrogels. The material stiffness can be estimated from the storage modulus G′ which reflects the strength of hydrogel. In other words, larger value of G′ indicated a higher strength. The gelation process can be confirmed when G′ is equal to G″ which can evaluate the thermo responsive property (Islam, Riaz, & Yasin, 2013; Meena, Lehnen, Schmitt, & Saake, 2013). The mechanism of the thermosensitive sol–gel phase transition can be attributed to the hydrogen bond. As is well-known, hydroxy polymer can stabilize some compounds, and contribute to the formation of a water shield around some macromolecules in solutions. Thus, at low temperatures, hydrogen bonds exist both between the OH groups of HA and PECT and between HA and water owing to the hydrophilicity of HA. Meanwhile, low temperature reduces the mobility of HA

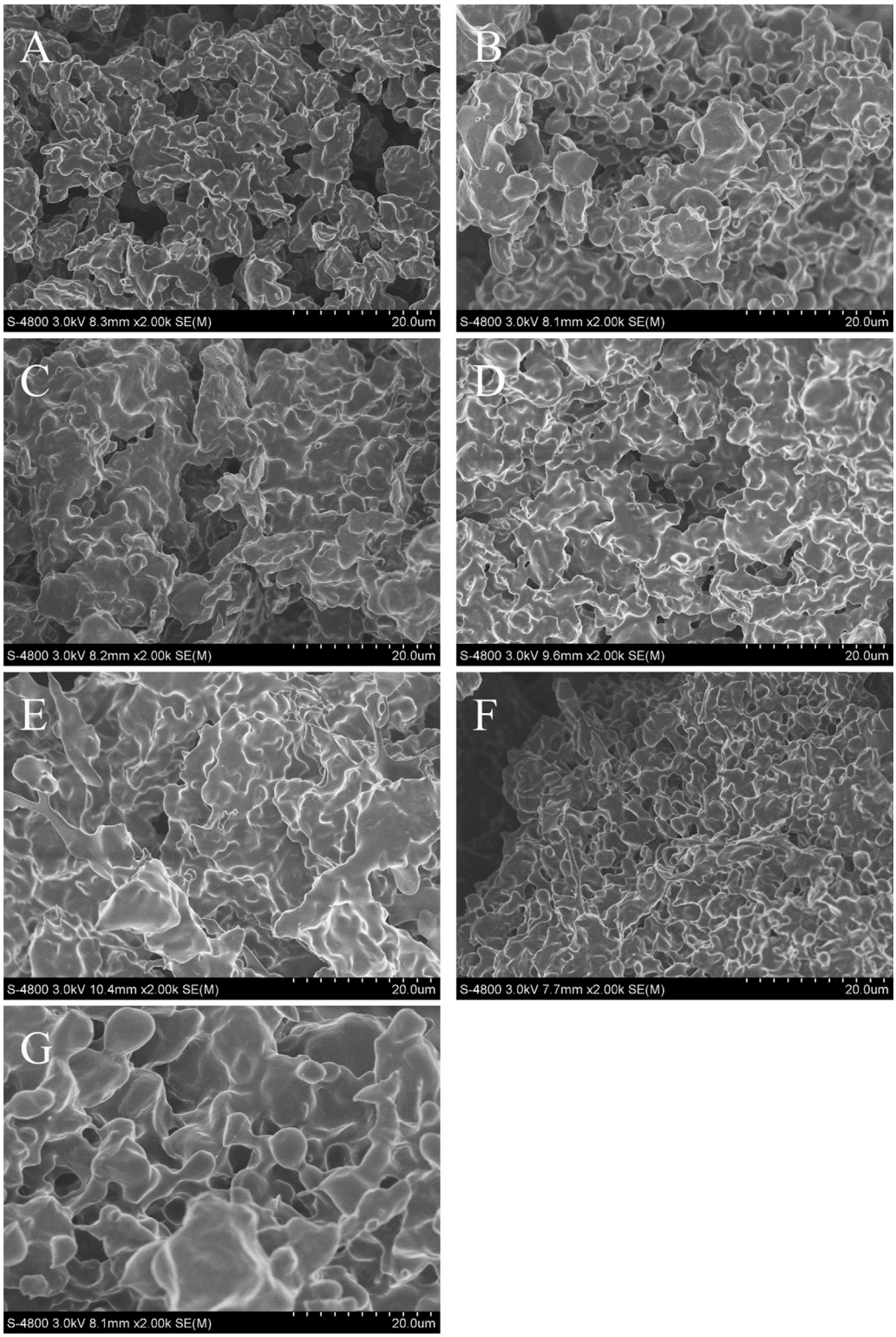

Fig. 2. SEM micrographs of the internal structures of (A) hydrogel of PECT/1% HA_{64}; (B) hydrogel of PECT/1% HA_{210}; (C) hydrogel of PECT/1% HA_{1000}; (D) hydrogel of PECT/2% HA_{64}; (E) hydrogel of PECT/2% HA_{210}; (F) hydrogel of PECT/2% HA_{1000}; and (G) hydrogel of PECT.

molecules, which obstruct the association of HA chains. Because of the difficulty to create contacts between junction chains, it is hard to form a network. When the temperature is elevated, hydrogen bonds are weakened, and the water molecules which were surrounding the polymer are removed. Then the dewatered chains associate with each other due to hydrophobic interactions. As a result, the hydrogel is formed (Tang, Du, Hu, Shi, & Kennedy, 2007).

As shown in Fig. 3, the results showed that at low temperatures the viscosity of the mixtures of PECT/HA is very despondent and $G' < G''$, indicating that they are in the sol state in which the PECT polymer formed single nanoparticles. As the temperature increases, the viscosity increases sharply to a maximum value and then gradually drops, which suggest the formation and disruption of the gel state. When the concentration of HA in the hydrogel is 1%, as the

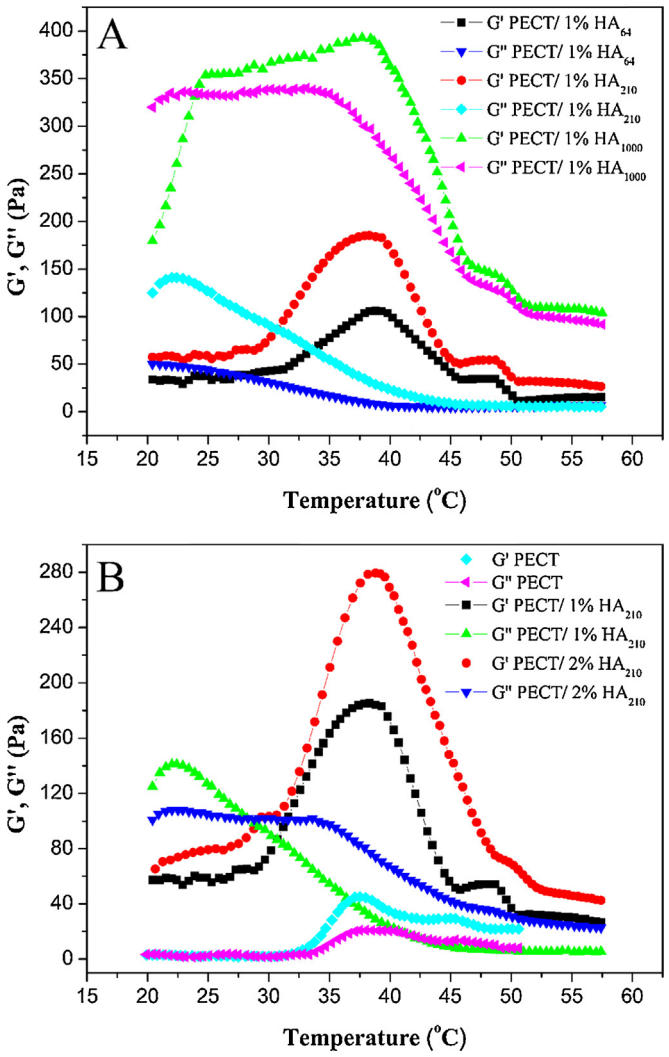

Fig. 3. Comparison of temperature dependence of storage modulus G′ and loss modulus G″ of (A) PECT nanoparticles (25%) with HA (M_w: 64 kDa, 210 kDa, 1000 kDa; concentration: 1%) aqueous dispersions; (B) PECT nanoparticles (25%) with HA (M_w: 210 kDa; concentration: 0%, 1%, 2%) aqueous dispersions, at a healing rate of 0.5 °C min^{-1} and at a frequency of 1 rad s^{-1}.

molecular weight varies from 64 kDa to 1000 kDa, the maximum G′ of the blend polymers increased from 106 Pa to 400 Pa (as shown in Fig. 3A), which implies that the bulk of molecules may affect the gel stiffness. As we all know, the bulk of molecules of HA will influence the mobility of HA polymers, the larger of the molecular weight, the less flexibility of the polymer chain. Since HA polymer contributes to the strength of the hydrogel, long-chain molecules will probably result in a sturdy structure. One more factor contribute to the phenomenon may be HA with larger molecular weight impeding HA/HA or PECT/HA chain to chain interactions. Upon heating, at the same time, from 20 to 60 °C, a rapid increase of G′ indicated that the temperature of incipient gelation is near 27 °C. After incubation above 40 °C, a gradually decreased G′ will be encountered, this trend is in accordance with the viscosity.

When the molecular weight of HA is fixed, as the concentration of HA varies from 0% to 2%, the maximum value of G′ varies from 42 Pa to 280 Pa (Fig. 3B). The probable reason is that as HA chains increases, the interchain force will rise which result in a hydrogel with a stronger structure. It seems that the combination of the inclusion complexation between HA and the PECT block copolymer and the hydrophobic interaction of the PECT block copolymer provides the driving force which contributes to the rapid formation of the supramolecular hydrogel.

3.3. In vitro degradation

As delivery carriers, hydrogels participate in *in vivo* metabolic processes involving hydrogels and the mechanism of release of the encapsulated drug, thereof, the degradation properties are significant predictors.

Two factors were investigated in this work including the concentration and molecular weight of HA, as shown in Fig. 4. Hydrogel was formulated with discrete concentrations, i.e., 0%, 1%, and 2% and molecular weights, i.e., 64 kDa, 210 kDa, 1000 kDa of HA as a model formulation cultured in PBS solutions. Details of the influence of the factors on the degradation process are discussed below.

3.3.1. Effect of the concentration of HA

Fig. 4A shows the mass-change of hydrogel, in which HA's molecular weight is 210 kDa, during a degradation process of 40 days, we found that the degradation ratio decrease 4.6% with the increase of the concentration of HA from 0% to 2%, the result showed that the degradation ratios increase with the increase of the concentration of HA.

Therefore a conclusion can be reached that HA can slow down the degradation of hydrogel. The reason is that HA molecular chains can reinforce PECT hydrogel.

3.3.2. Effect of the molecular weight of HA

As shown in Fig. 4B, the influence of molecular weight of HA was investigated. It showed that with the increase of molecular weight of HA from 64 kDa to 1000 kDa, the degradation rate was slowed down from 22% to 14%, which suggested that HA with a larger molecular weight is harder to degrade during a given period of time, ceteris paribus This can ascribe to HA with a larger molecular weight has a longer molecular chain.

However, the curve in Fig. 4B also showed that the weight losses of hydrogels with HA, whose molecular weight were 64 kDa and 210 kDa, were very close. Then the conclusion can be reached that when the gap between the molecular weights of HA was not very huge, the weight losses of the hydrogels in the degradation process will be very limited.

3.4. In vitro drug release profile of PECT/HA hydrogel

The effect of the factors such as concentration and molecular weight of HA in the hydrogel on the release of drug was studied for hydrogel formulations containing 10 ± 0.3 mg of ASA and different concentrations, i.e., 0%, 1%, and 2% and molecular weights, i.e., 64 kDa, 210 kDa, and 1000 kDa of HA as a model formulation cultured in PBS solutions. Details are discussed below.

3.4.1. Effect of the concentration of HA

The effect of concentration of HA was shown in Fig. 5A. The 24 day's release ratio of ASA from hydrogel was slightly decreased from 73% to 67% with the percentage composition of HA in the hydrogel increased from 0% to 2%. Despite this trend, the difference in data is not very notable.

3.4.2. Effect of the molecular weight of HA

Fig. 5B showed that with the increase of molecular weight of HA from 64 kDa to 1000 kDa, the 24 day's release ratio of ASA was dropped from 70% to 52%, which suggested that HA with a larger molecular weight may be a barrier for ASA to release. However, if the gap of HA's molecular weight, such as 64 kDa and 210 kDa, is not huge, then the difference of the 24 day's release ratio is not notable.

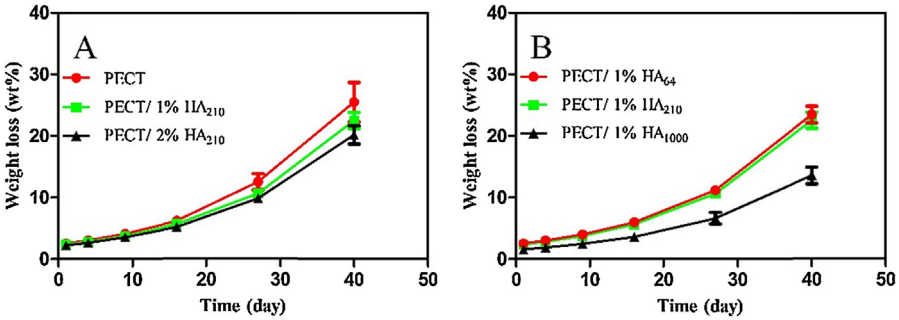

Fig. 4. Weight loss of (A) PECT nanoparticle (25%) with HA (M_w: 210 kDa; concentration: 0%, 1%, 2%) hydrogel in 0.01 M PBS (pH = 7.4); (B) PECT nanoparticle (25%) with HA (M_w: 64 kDa, 210 kDa, 1000 kDa) hydrogel in 0.01 M PBS (pH = 7.4).

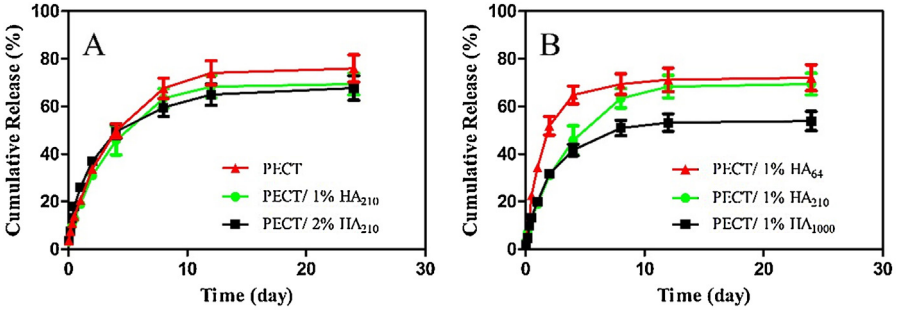

Fig. 5. *In vitro* release of ASA from (A) PECT nanoparticle (25%) with HA (M_w: 210 kDa; concentrations: 0%, 1%, 2%) hydrogel in 0.01 M PBS (pH = 7.4); (B) PECT nanoparticle (25%) with HA (M_w: 64 kDa, 210 kDa, 1000 kDa) hydrogel in 0.01 M PBS (pH = 7.4).

3.4.3. *Effect of HA on the mechanism of release behavior*

As for the ASA-loaded PECT/1% HA_{210} hydrogel, nanoparticles were produced during degradation of the PECT hydrogels, and ASA-loaded nanoparticles were released in the process. These nanoparticles were separated from the release liquid and the residual release liquid was analyzed by UV to indirectly confirm the amount of ASA that was encapsulated in nanoparticles (Fig. 6A). The cumulative release of free ASA soluble in PBS was approximately 15%. Hence, it could be concluded that 55% of the loaded ASA might be incorporated in PECT nanoparticles. The TEM (Fig. 6B) results confirmed that it was the drug-loaded nanoparticles which were released from hydrogel. The consequence indicated that the introduction of HA has not changed the mechanism of release behavior of hydrogel.

It is well accepted that release mechanism of hydrophobic drugs from a hydrogel was attributable to the combination of diffusion and degradation of hydrogel carrier. The results meet well with the diffusion mechanism of hydrophobic drugs from a hydrogel. The hydrophobic drugs which were wrapped within the nanoparticles need a process of diffusion from the nanoparticle to the hydrogel, and then released from hydrogel to the surrounding liquid. The driving force of the release is believed to be the concentration gradient between the inner and outer of the hydrogel. With the shrink of the concentration gradient, the speed of release is decreased. Therefore, an equilibrium can be achieved before the complete degradation of hydrogel. The degradation of hydrogel also contributes. Since the hydrogel is formed by PECT nanoparticles with HA who contributes a lot to the intensity of the hydrogel, with the degradation of the hydrogel. HA chains break down and nanoparticles are released which leads to the release of ASA for it is carried in nanoparticles. Furthermore, the introduction of HA has little effect on the degradation and drug release behavior of

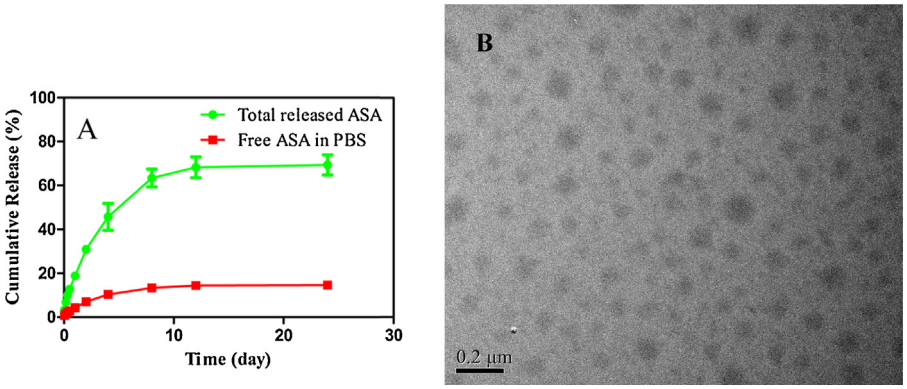

Fig. 6. (A) *In vitro* release of ASA from PECT/1% HA_{210} hydrogel. The drug loading was 1 wt.%. Each point represents the average result of three parallel experiments. The unencapsulated ASA in PECT NPs was filtered to indirectly determine how much ASA was encapsulated. (B) A TEM image of nanoparticles in the released liquid.

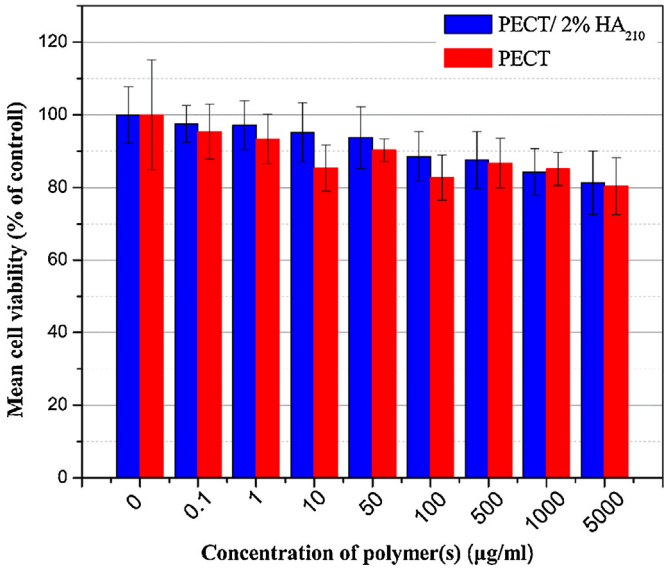

Fig. 7. Cytotoxicity of PECT and PECT/2%HA_{210} on NIH3T3 cells after cultured for 48 h.

hydrogel if the molecular weight of HA is not more than 21 kDa and concentration not more than 2%. Yet during the release period, the degradation rate of hydrogel is ca. 10%, which indicate that hydrogel degradation plays a less important role in this release process.

3.5. *Safety evaluation of PECT/HA mixtures in vitro*

Toxicity test was performed on PECT/HA mixtures. As shown in Fig. 7, PECT/HA mixtures at the concentration of 5000 μg ml^{-1} did not cause any harmful effect on NIH3T3 cells with the negative control (normal saline). Meanwhile, the cytotoxicity of blank PECT was evaluated by cell viability assay on NIH3T3 cells as normal cells. The result suggested that PECT/HA hydrogel was very lower toxic even than the blank PECT hydrogel, exactly non-toxic. So, the prepared mixtures might be safe vector for intravenous injection.

4. Conclusion

A series of injectable, thermosensitive hydrogels were formed by physical interactions between PECT nanoparticles and HA chains via hydrogen bonds and hydrophobic interactions between HA chains. The hydrogels are liquid in aqueous solutions at room temperature, and gel at 37 °C. Our study also demonstrated that the introduction of HA into PECT hydrogel can be considered an effective method to tune the properties such as mechanical strength, viscosity and morphological appearance. In addition, it has no influence on the mechanism of drug release and a negligible influence on the degradation and drug release of hydrogels to be applied to various situations. Since the hydrogels have been proved to be non-toxic and biocompatible, the copolymer hydrogels are expected to be considered for a wide range of applications in drug delivery.

Acknowledgement

This project was supported by the National Natural Science Foundation of China (81371667).

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