PLGA microsphere/P(NIPAAm-*co*-AAm) hydrogel combination systems for drug delivery

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PLGA microsphere/P(NIPAAm-*co*-AAm) hydrogel combination systems for drug delivery

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Abstract. A combination drug delivery system was successfully prepared by dispersing drug-loaded-poly (lactic-co-glycolic acid) (PLGA) microspheres into porous poly (N-isopropylamide- co-acrylamide) P(NIPAAm- co-AAm) hydrogel. Scanning electronic microscope (SEM) studies revealed that PLGA microspheres were encased into the interconnected porous structure of P(NIPAAm-co-AAm) hydrogel. In vitro release of Bovine serum albumin (BSA) from combination system exhibited a lower burst release followed by a slower sustained release as compared to BSA release from the PLGA microspheres and P (NIPAAm-co-AAm) hydrogels alone. These results demonstrated that the combination systems have the potential application in drug delivery.

1. Introduction

With the fast growth of biomedical and biotechnology, protein drugs have been being extensively investigated because of its high bioactivity, incredible selectivity, fewer side effects, and great potential to clinical application [1-2]. Most of these drugs cannot be orally administered due to gastro- and/or intestinal degradation. Intravenous injection is the most common method of administration, but frequent injection leads to patients' low compliance [3]. Therefore, controlled-release drug delivery was developed by encapsulating proteins into polymeric materials for sustained release of protein drugs in expected time.

Controlled-release drug delivery systems are becoming increasingly important in biomedicine, microspheres and hydrogels using for drug delivery systems have been attracted wide attention [4-6]. These drug delivery systems protect protein and peptide structures from rapid degradation in the bloodstream, and to localized or target drug delivery in a sustained manner. Poly(N-isopropylacrylamide) (PNIPAAm) hydrogel have been widely applied in drug delivery systems, the release rate of loaded drugs in PNIPAAm hydrogels could be activated and self-controlled by responding to a temperature trigger provided at a target site [7-9]. However, the conventional PNIPAAm hydrogels for protein delivery have several limitations. Firstly, protein-based drugs cannot completely be released from the gel networks because of its limited pore size. Additionally, the drugs loaded directly into the swollen PNIPAAm hydrogels were found to release too quickly at the initial stage [10-11], the rapid burst release reduces the drug efficacy and the release period. Microspheres

prepared with biodegradable polymers were developed as drug delivery show excellent sustained release period over weeks [12-14]. However, microspheres were cleared by the phagocytes after administration and also exhibited a large burst release in the first few hours or days after administration [15-16].

In order to overcome the above limitations, we incorporated poly (D,L-Lactide-co-glycolide) (PLGA) microspheres into poly (N-isopropylamide-co-acryla-mide) [P(NIPAAm-co-AAm)] hydrogels to produce a combination delivery system. The combination system would reciprocally compensate for the disadvantages of individual microspheres and hydrogels delivery system, produced an auto adjustable function to external temperature changes, and localized delivery system with a sustained protein release behavior. Firstly, PLGA microspheres were prepared by emulsion-solvent evaporation, and then drug-loaded microspheres were dispersed in the solution based on N-isopropylacrylamide (NIPAAm), and the mixture was performed copolymerization/ crosslinking reaction to form a gel which could act as drug reservoir. In this case, aggregation of microspheres would be prevented because they were dispersed in hydrogels. Thus, by combining microspheres and hydrogels, protein drug could be released in a controlled manner for a long period of time.

In this article, Bovine serum albumin (BSA) was chosen as a model protein drug to investigate the in vitro release properties of P (NIPAAm-*co*-AAm) hydrogels, PLGA microspheres and microsphere-loaded hydrogel combination systems. Distribution of PLGA microspheres in the hydrogels was determined by Scanning electronic microscope (SEM).

2. Experimental

2.1. Preparation of Combination systems.

PLGA microspheres with BSA were dispersed in distilled water first. Then, various amounts of NIPAAm, AAm monomer and MBAAm crosslinker were dissolved well in the above aqueous solution with dispersed PLGA microspheres. The feed mixture of monomer, crosslinker, microspheres, initiator and solvent was stirred for 10 min at room temperature. Subsequently, APS solution and TEMED were added into the mixture to initiate the copolymerization/ crosslinking reaction at 18 oC for 3 min, and then was performed at -18 oC for polymerization. Twenty hours later, PLGA microsphere/ P(NIPAAm-co-AAm) hydrogel combination systems were prepared.

The PLGA microspheres/P (NIPAAm co-AAm) hydrogels combination systems prepared in this paper were labeled PLGA-0, PLGA-5, PLGA-10, PLGA-15 and PLGA-20， respectively. The notation indicates the amount of PLGA microspheres incorparated into hydrogels. For example, PLGA-0 is the sample of P (NIPAAm-co-AAm) hydrogels without incorporating PLGA microspheres, PLGA-5 is the sample for which the amount of PLGA microspheres incorparated into P(NIPAAm-co-AAm) hydrogels was 5 mg.

2.2. Scanning electronic microscope examination.

The internal structure of the hydrogels (not or encapsulated with microspheres) was observed by a scanning electron microscope (SEM; S-3400N, Hitachi, Japan) at an accelerated voltage of 15 kV. The hydrogel combination systems were first equilibrated in distilled water at room temperature to reach the equilibrium states and swollen. Swollen hydrogel combination systems were quickly frozen in liquid nitrogen and then lyophilized for 72 h. The dried samples were cut to the appropriate size with sharp blade, sputtered with gold, and observed at ambient temperature.

2.3. Release study

BSA was selected as the model protein to investigate the release behavior from the porous hydrogels, PLGA microspheres and microsphere/hydrogel combination systems. The hydrogel and combination systems were loaded with BSA by immersing them in 5 mg/ml of BSA solutions at 4 °C for 48 h, respectively. The in vitro release studies were carried out in PBS buffer (pH 7.4) at 37°C. The BSA-loaded samples were transferred into a beaker containing 10 ml PBS, which was shaken with a speed

of 100 rpm. At specific time intervals, 0.5 ml of solution was withdrawn from the release medium and replaced with 0.5 ml of fresh PBS solution. The amount of BSA released from the samples was measured by a UV-Vis spectrophotometer (LAMBDA 500, PerkinElmer Instruments) at 595 nm. The percentage of BSA released was calculated as: $Mi/M\infty$, where Mi is the amount of proteins in the release medium at a given time and $M\infty$ is the total loaded BSA in the samples.

3. Results and discussion

3.1. Morphology.

The interior morphology of the hydrogel was shown in Figure 1. PLGA microspheres were dispersed in porous P (NIPAAm-*co*-AAm) hydrogels (as shown in Figure 1a and 1b). The microspheres based hydrogel composition scaffolds were successfully obtained in presented study. When the microparticle size was smaller than the pore size of hydrogels, microspheres were encased into the pore structure of P (NIPAAm-*co*-AAm) hydrogel. The bigger microspheres were filled the pores of combination systems, which reduced the interconnected porous structure of combination systems. PLGA microspheres dramatically affected the pore structure of P (NIPAAm-*co*-AAm) hydrogels. When PLGA microspheres were not incorporated in P (NIPAAm-*co*-AAm) hydrogels, a loose structure was observed in the hydrogel with interconnecting pores which could be the main drug penetration channels (Figure 1d). With PLGA microspheres incorporating in the hydrogels, the interconnecting and the pore size of the porous structure decreased (Figure 1c). Because the incorporated PLGA microspheres occupied the spaces of the mixture, which hindered the ice crystals free growth in freezing polymerization[17,18]. So the continuous ice phase cannot be formed in the freezing polymerization stage, in turn reduced the inter-connectivity of pore in PLGA microspheres/ P (NIPAAm-*co*-AAm) hydrogels scaffolds. Moreover, with PLGA microspheres dispersing into the reaction mixture, it affected the crosslinking of hydrogels and the forming of the ice crystal in the second polymerization, which in turn affected the interconnecting and pore size of the resulting swollen network.

3.2. In vitro BSA release from different delivery system

The cumulative release amounts of BSA from the porous hydrogels, PLGA microspheres and microsphere/ hydrogel combination systems at 37°C were presented in Figure 2. As shown in Figure 2, BSA was released quickly from PLGA microspheres and porous P (NIPAAm-co-AAm) hydrogel delivery systems. The PLGA microspheres had an initial burst release of approximately 43% (w/w) (24 h). The porous P (NIPAAm-co-AAm) hydrogels showed a burst release at the initial stage (ca. 60% in 24 h) and then the release stopped, but not all the loaded BSA was released from the hydrogel networks. The combination drug delivery system had an initial release (ca. 13% in 24 h) followed by a slow sustained release for 30 days. A lower initial burst release and the slower release after the initial burst release were observed from the combination systems as compared to BSA release from the microspheres and hydrogels alone. The decrease in the burst release might be due to incorporation of the microspheres into the P (NIPAAm-co-AAm) matrix, which created another barrier for diffusion of BSA into release medium. Moreover, since burst release is due to release of drug that is close to microsphere surface, incorporating the microspheres into hydrogels is likely to decrease burst release. The slower release rate after the initial burst release is the reason that the incorporated PLGA microspheres dramatically reduced the interconnected pore structure of P (NIPAAm-co-AAm) hydrogels. Interconnected pore structure is the main drug penetration channels, thus it was resulted the low permeability of BSA from combination systems. As shown in Figure 2, the release profiles of BSA from combination delivery systems showed suppression of the initial burst and sustained release thereafter. Apparently, more BSA loaded PLGA microspheres incorporated into hydrogels led to more higher initial burst release and faster release rate. This finding indicates that drug release from this combination delivery system can be controlled for prolonged time periods by simply varying the proportion of PLGA microspheres in the hydrogels.

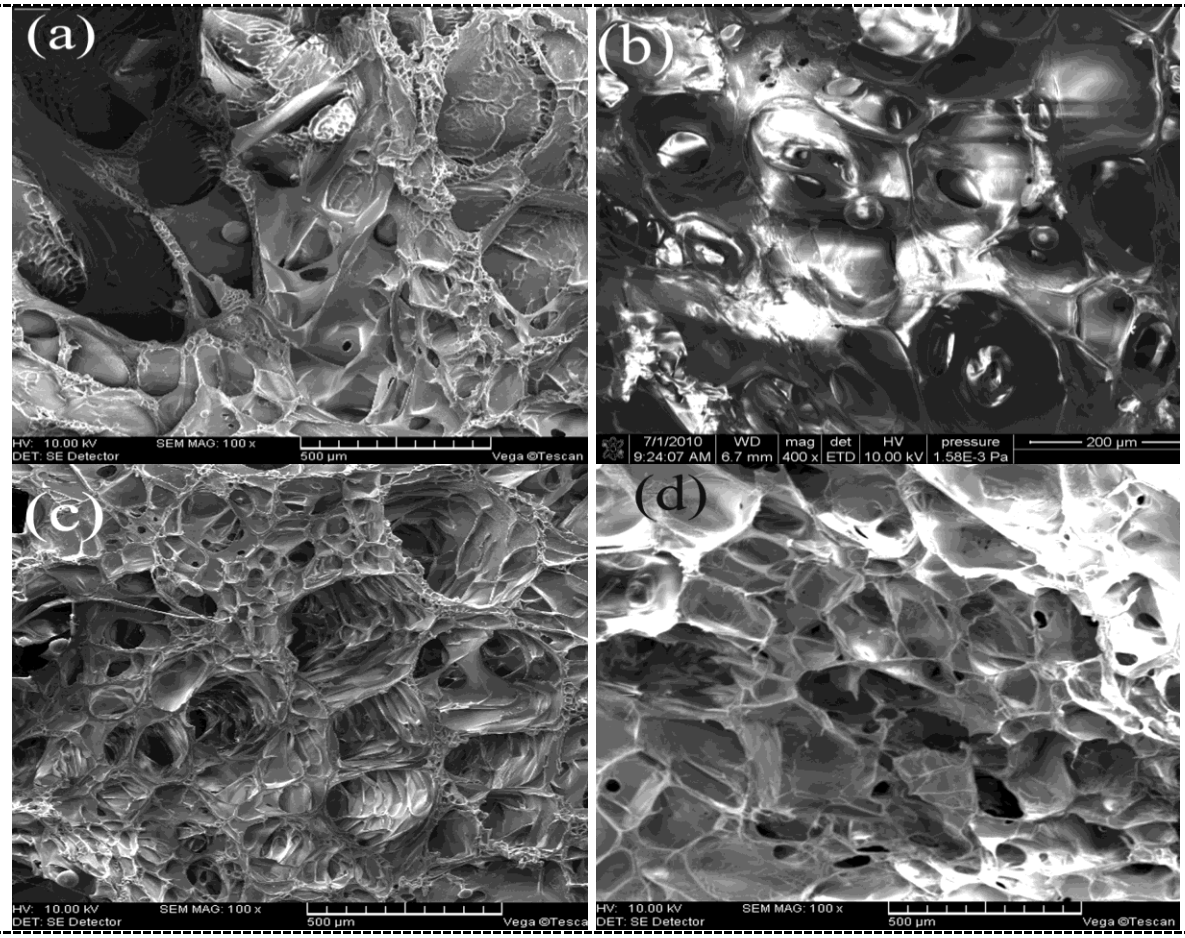

Figure 1. SEM images of PLGA microspheres /P(NIPAAm- co-AAm) hydrogel combination systems:(a), (b), (c) microsphere-loaded hydrogel combination systems; (d) porous P(NIPAAm-co-AAm) hydrogel.

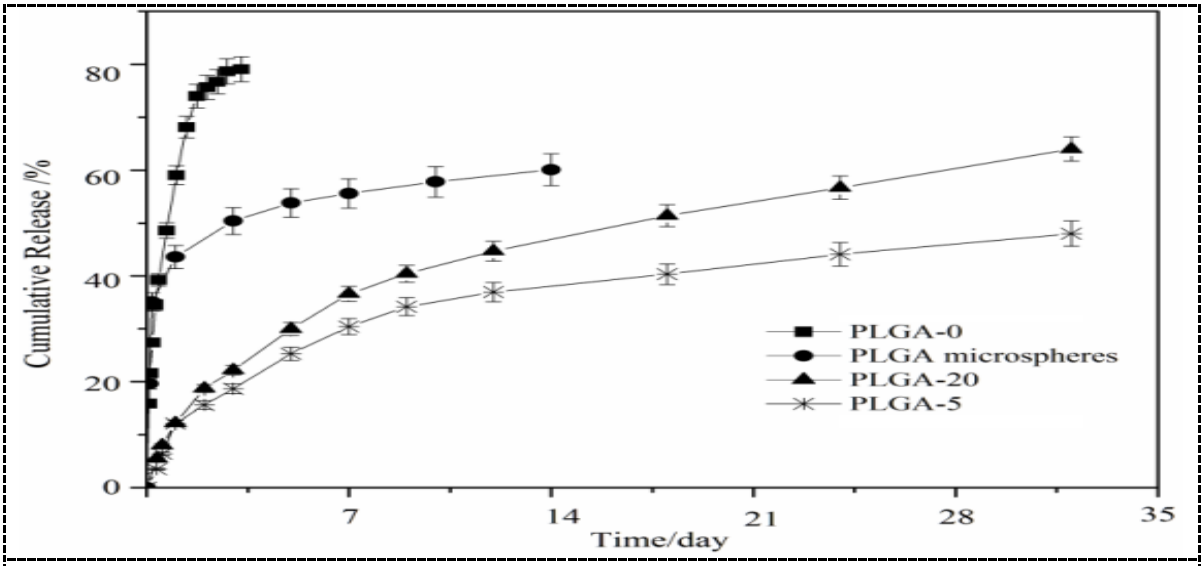

Figure 2. In vitro release of BSA from PLGA microspheres, P (NIP AAm-coAAm) hydrogel and combination systems at 37 ℃.

4. Conclusions

A combination drug delivery system was successfully prepared by dispersing drug-loaded- PLGA microspheres into porous P (NIPAAm-co-AAm) hydrogel. The drug delivery system can sustain release of protein for an extended period of time. SEM results showed that PLGA microspheres were encased into the interconnected porous structure of P (NIPAAm-co-AAm) hydrogel. With PLGA microspheres dispersing into the reaction mixture, the interconnecting and the pore size of the porous structure decreased. In comparison with the release behavior of BSA from porous P (NIPAAm-*co*-AAm) hydrogel and the PLGA microspheres/hydrogels combination systems, it exhibited a prolonged release of BSA from the combination systems. Thus, the PLGA microspheres/ hydrogels combination systems have remarkable potential for application in protein or other bioactive macromolecule drug delivery.

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