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Thermosensitive Chitosan as an Injectable Carrier for Local Drug Delivery

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Abstract: Two types of injectable system using thermosensitive chitosan (chitosan-g-NIPAAm), hydrogel and microparticles (MPs)-embedded hydrogel were developed as drug carriers for controlled release and their pharmaceutical potentials were investigated. 5-Fluorouracil (5-FU)-loaded, biodegradable PLGA MPs were prepared by a double emulsion method and then simply mixed with an aqueous solution of thermosensitive chitosan at room temperature. All 5-FU release rates from the hydrogel matrix were faster than bovine serum albumin (BSA), possibly due to the difference in the molecular weight of the drugs. The 5-FU release profile from MPs-embedded hydrogel was shown to reduce the burst effect and exhibit nearly zero-order release behavior from the beginning of each initial stage. Thus, these MPs-embedded hydrogels, as well as thermosensitive chitosan hydrogel, have promising potential as an injectable drug carrier for pharmaceutical applications.

Keywords: thermosensitive hydrogel, chitosan, microparticle, injectable, drug delivery.

Introduction

In recent years, significant advances have been made in the controlled drug delivery system using injectable polymeric materials. These systems have been attempted and developed to apply for therapeutic applications. Among these systems, the development of new injectable drug delivery systems has received considerable attention over the past years.[1-3] This interest has been sparked by various advantages of injectable drug delivery system, which include ease of application, localized delivery for a site-specific action, prolonged delivery period, decreased body drug dosage with concurrent reduction in undesirable side effects common to most forms of systemic delivery, and improved patient compliance and comfort.[4]

Most of approaches using injectable polymers have been mainly studied with hydrogels, emulsions, liposomes, micelles, biodegradable micro- and nanoparticles.[4] These injectable polymeric systems for a promising drug delivery application typically consist of hydrogels (based on stimuli-sensitivity) and particulates. Thermosensitivity-based approach in the stimuli-sensitive hydrogels can be more advantageous for biomedical applications, as it does not require organic solvent, copolymerization agent, or an externally applied trigger for gelation. Some polymers undergo abrupt changes in solubility in response to increases in environmental temperature (lower critical solution temperature, LCST). This phase separation is generally viewed as a phenomenon governed by the balance of hydrophilic and hydrophobic moieties on the polymer chain. Various kinds of thermosensitive polymers have shown potentials for the local and injectable drug delivery systems. Especially, poly(*N*-isopropylacrylamide) (PNIPAAm) as a representative of thermosensitive polymers has been used for the drug localization through sol-gel transition around 32 °C. There are a lot of attempts for the design of NIPAAm-based drug carriers and their improvements.[5-8]

Chitosan, poly[(1,4)-2-amino-2-deoxy-*D*-glucopyranose], can be obtained by the *N*-deacetylation of chitin. Chitosan and its derivatives are considerable as drug carrier due to their unique properties in biomedical applications. The availability of chitosan, its biocompatibility and its chemical and biological properties make it attractive biomaterials for variety of pharmaceutical applications, especially in the areas of drug delivery and wound dressing. It has been used or tested in different forms, such as tablets, matrix and microparticles for the purpose of sustained release, controlled drug delivery, and mucosal formulation, more recently, drug absorption enhancement for protein drug delivery and vaccine development.[9-11]

Also, particulate systems using biodegradable polymer

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such as poly(lactide-*co*-glycolide) (PLGA) and polylactide (PLA) have been constantly studied as another injectable drug carriers, which may be incorporation of various drugs, improvement of drug stability and controlled release property.[12-14] However, the particulate system has a limit that can be moved by body fluids when it was injected into a target site as a suspension type. So they should be required to localize therapeutic agents at the specific site. The double emulsion process is a water-in-oil-in-water (w/o/w) method and suitable for encapsulating water-soluble drugs like peptides, proteins, and vaccines, unlike the o/w method which is ideal for water-insoluble drugs like steroids.[15]

In our previous study, chitosan-*g*-NIPAAm as thermosensitive chitosan was physico-chemically characterized and investigated their biological responses *in vitro* and *in vivo*.[16,17] The aim of this study is to investigate the potentials of thermosensitive chitosan hydrogel as a new injectable drug delivery system by *in-situ* gelation. Drug release behaviors from hydrogel with or without PLGA MPs have been investigated *in vitro*.

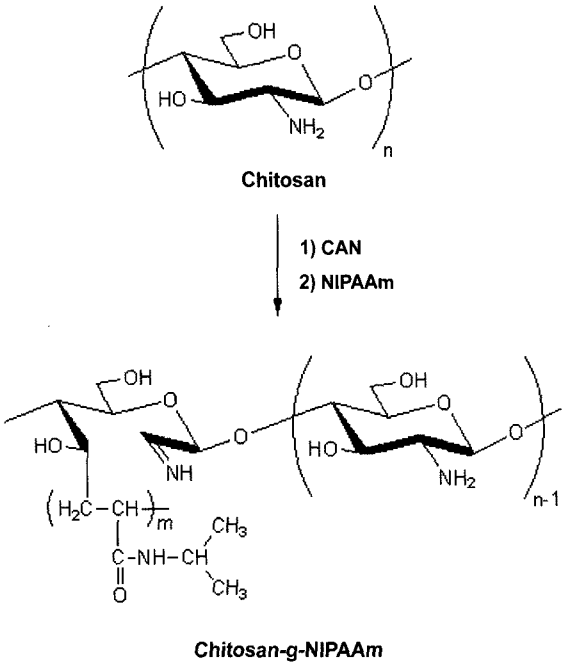

Figure 1. Synthetic procedure of thermosensitive chitosan.

Experimental

Materials. Chitosan (Deacetylation degree 85%, Mw. 2×10^5) was kindly supplied by Jakwang Co., Korea. Ceric ammonium nitrate (CAN), *N*-isopropylacrylamide (NIPAAm), heavy mineral oil and decane were purchased from Aldrich. 5-fluorouracil (5-FU) and bovine serum albumin (BSA) as a model drug were purchased from Sigma. L-Lactide and glycolide were purchased from PURAC (USA). Bicinchoninic acid (BCA) kit was purchased from Pierce (USA). NIPAAm was recrystallized from *n*-hexane and all other reagents and solvent were used without further purification.

Preparation of Thermosensitive Chitosan. Thermosensitive chitosan was synthesized by the graft polymerization of NIPAAm onto chitosan using CAN as an initiator under N_2 at 30 °C for 15 h as shown in Figure 1. CAN solution dissolved in 0.1 N HNO_3 was used for the reaction.

After the reactions, the resultant was precipitated in excess acetone and filtered to separate products. To remove the homopolymer of NIPAAm, the product was followed by soxhlet extraction with methanol for 48 h. The resulting product was dried under reduced pressure at 30 °C for 24 h.[16]

Preparation and Characterization of 5-FU Loaded PLGA MPs. PLGA copolymer for the preparation of microparticles (MPs) was synthesized by a conventional method.[18] A modified double emulsion method was employed to fabricate 5-FU loaded MPs. Briefly, 1 mL of an aqueous solution of 1% (w/w) 5-fluorouracil was emulsified in a solution of PLGA in methylene chloride (25 mg/mL) by sonication. Then, 40 mL of a 3% (w/v) poly(vinyl alcohol) (PVA) was added to this primary emulsion and sonicated under the above conditions to obtain the double emulsion. The double emulsion was then diluted into 8% (v/v) isopropyl-alcohol solution under magnetic stirring for removal of organic solvent. Finally, MPs were isolated by centrifugation at 20,000 × g for 15 min at 10 °C, washed three times. The shapes and surface characteristics of MPs were examined by scanning electron microscopy (SEM) (S-2300; Hitachi Instrument, Japan) at 20 kV. The average size of MPs was determined by Zetasizer S (Instrument Ltd, Malvern, UK).

Quantitative Analysis of 5-FU and BSA. The 5-FU contents in thermosensitive chitosan hydrogels and PLGA MPs were analyzed by high performance liquid chromatography (HPLC). The HPLC system consists of a pump (JASCO PU-1580), a loop injector (Rheodyne, 7725i) with 20 μL loop, a fixed wavelength UV absorption detector (JASCO UV-1575) set at 266 nm, and a reversed phase C18 column (Kromasil). The mobile phase was a mixture (V/V) of 5% methanol and 95% sodium acetate buffer (0.01 M) at pH 4. The flow rate was 0.5 mL/min and the concentration of 5-FU was calculated from the calibration curve using the peak area ratio. The BSA content in hydrogel was analyzed using enzyme-linked immunosorbent assay (ELISA) and BCA kit.[19]

Drug Loading Efficiencies. The drug loading efficiency of thermosensitive chitosan hydrogels was determined after complete dissolution of drug loaded polymer matrix at 4 °C. Then, the solution was heated to 50 °C for 10 min. After polymer precipitation, the solution was collected and centrifuged at 7,500 rpm, and then 0.5 mL of supernatant was removed for measurement.[7]

The weighed MPs after drying under reduced pressure were dissolved in acetonitrile and then methanol was added to preferentially precipitate the polymer. The drug in the fil-

trate passed through a membrane filter (pore diameter, 0.45 μm) was properly diluted and analyzed.[13]

***In vitro* Release Test.** Two different methods for the preparation of drug loaded hydrogel using thermosensitive chitosan were carried out. BSA or 5-FU loaded polymer matrix were prepared from aqueous solution containing 7% (w/v) chitosan-g-NIPAAm with 0.2% (w/v) BSA or 1% (w/v) 5-FU. The solution was kept at 4 °C overnight to allow the solubilization of the polymer. The 0.6 mL of drugs/polymer solution was added dropwise using a syringe into 50 mL mineral oil kept at 37 °C, which is above the LCST of the polymer. Then, the mineral oil was covered with 5 mL decane to reduce surface tension and to aid the penetration of the solution drop at the air/oil interface. The formed matrix was washed with *n*-hexane, dried at 37 °C for 1 h.[7] As direct-gelling method, the drug/polymer solution with same concentration was dropped into teflon mold (1×1 cm^2) and kept at 37 °C oven. After a phase separation, drug loaded hydrogels drawn out of a mold were used. Drug loaded hydrogels were placed into a vial with 10 mL PBS (poly(butylene succinate), 0.01 M, pH 7.4) solution at 37 °C. At predetermined time intervals, 1 mL of the release medium was collected from each sample in shaking water bath (37 °C, 50 rpm) and replaced by fresh medium.

For the release test of MPs-embedded hydrogel, 0.6 mL of sample prepared by mixing 7% (w/w) thermosensitive chitosan solution and 50 mg of 5-FU loaded PLGA MPs at room temperature was injected into dialysis bag (MWCO 12,000), and 50 mg of 5-FU loaded PLGA MPs was used as a control. Then, dialysis bags were placed into vials with 50 mL of PBS solution and the 5 mL of medium were taken as predetermined time intervals. Finally, the release medium was analyzed by HPLC.

Results and Discussion

Characterization of chitosan-*g*-NIPAAm and PLGA MPs. In our previous report, thermosensitive chitosan was prepared by the graft polymerization of NIPAAm into chitosan using CAN as an initiator. Chitosan-*g*-NIPAAm has shown thermosensitive behavior. This copolymer can form thermally reversible hydrogel, which exhibits a LCST around 32°C in aqueous solutions. The phase transition behavior of the aqueous solution was similar to its of PNIPAAm, which supports that thermosensitivity of the copolymer depends on the grafting percentage of PNIPAAm onto chitosan backbone. In fibroblast culture, they showed nontoxic and biocompatible properties in comparison with alginate gel as a control. Herein, two types of approach based on thermosensitivity of chitosan-*g*-NIPAAm, the hydrogel and MPs-embedded hydrogel have studied as an injectable drug carrier.

The molecular weight of chitosan-*g*-NIPAAm and PLGA (75:25) used for this study were measured to be 450,000 (PDI, 1.08) and 120,000 (PDI, 1.33), respectively. The grafting percentage of chitosan-*g*-NIPAAm was measured to be 700%. SEM images of the MPs revealed the regular shapes (Figure 2) and the obtained MPs were spherical, smooth and separated each other. The particle size of BSA or 5-FU loaded PLGA MPs were observed in the range of 20 to 30 μm, although it varied depending on the preparation condition and particular batch. The recovery ratio of obtained MPs revealed to be about 70%.

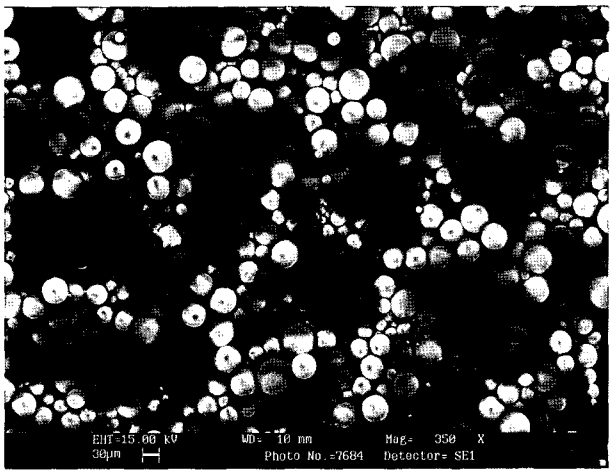

Figure 2. SEM image of 5-FU loaded PLGA MPs.

Loading Efficiencies. For investigating the drug loading efficiency of thermosensitive chitosan hydrogel, two types of drug loaded matrix were prepared by using the phase transition property under the contrary environment and the results was summarized in Table I. When the polymer solution was dropped into the warm oil passing through a decane layer, the first event to occur was a thin skin formation by polymer precipitation at the interface, followed by the droplet shrinking. This shrinkage expelled water out of the droplets, creating an aqueous layer between the solidified skin of the droplet and the oil phase, preventing further contact. The solidified matrix was isolated immediately by washing with hexane to remove any residual surface oil. In the oil/decane system, the drug diffusion from hydrogel matrix was prevented by the low solubility of drug at an external environment. Therefore, the drug loading efficiency of hydrogel by this method is higher than direct-gelling method with aqueous environment.

Table I. Loading Efficiencies of 5-FU and BSA Loaded Thermosensitive Chitosan Hydrogel (Mean±Standard Error of the Mean, n=3)

	5-FU	BSA
Oil/decane system	47±2.1	74±5.6
Aqueous system	44±3.2	63±6.2

In the preparation of PLGA MPs, it is notable that the loss of 5-FU during the encapsulation process by w/o/w double emulsion method because 5-FU is a water-soluble drug. The solvent extraction was adopted because isopropyl alcohol could prevent 5-FU from diffusing out of secondary water phase. The loading efficiency of 5-FU loaded PLGA MPs showed 22.6%, approximately.

***In vitro* Release Profiles.** *In vitro* release profiles of 5-FU and BSA from thermosensitive chitosan hydrogel shown in Figures 3-4 demonstrated different behaviors with the initial burst. The release rates of 5-FU were faster than BSA, resulting from the difference in drug molecular weight. Additionally, there were no significant differences in release profiles according to 5-FU loading method and 5-FU release from hydrogels was extended over 24 h. The initial burst during the first 6 h represented about 70% of the total 5-FU content, which was poorly entrapped into the hydrogel matrix. Almost of them were placed on the polymer surface. BSA release from hydrogel showed different profiles, depending on the preparation method as shown in Figure 4. The initial burst of BSA in oil/decane system was about 35%. On the other hand, the initial burst in aqueous system revealed 55%, approximately. This difference came from the first stage of phase transition when drugs were entrapped into hydrogel matrix. Because drug loaded matrix in the former was prepared by nonaqueous condition, drugs that were not diffused out would be incorporated more strongly into the matrix. However, drugs in the latter were weakly incorporated into the matrix due to free aqueous conditions. BSA release of each system showed sustained release over 60 h and their behaviors support that the different gelation condition do not affects the release profile but only the amount of incorporated drugs.

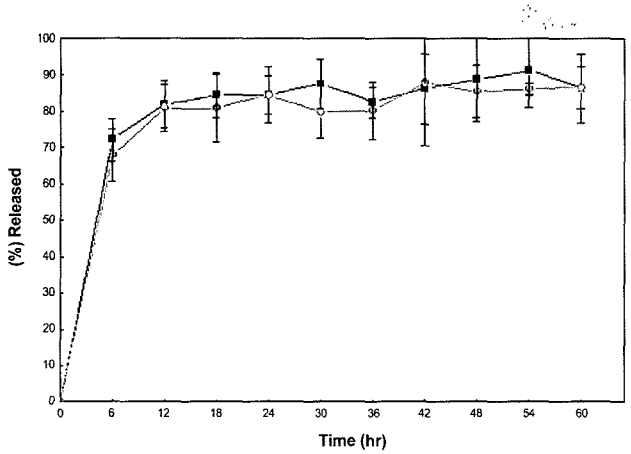

Figure 3. *In vitro* release of 5-FU from thermosensitive chitosan hydrogel (Mean±standard error of the mean, n=3). ●: Oil/decane system, ■: Aqueous system.

The drug release rate from PLGA MPs is determined in general by the polymer degradation time depending on the molecular weight of PLGA and the ratio of monomers. Due to very fast release of 5-FU from hydrogel, 5-FU loaded PLGA MPs prepared by double emulsion method were incorporated into hydrogel matrix. Figure 5 represents *in vitro* release profiles of 5-FU from each polymer carriers. The burst effects are attributed to the presence of drug crystals spread over the periphery of the MPs. 5-FU release from MPs showed an initial burst effect above 40% in the initial 1 day and lasted over 9 days. However, the result from MPs-embedded hydrogel matrix showed the release profile to a zero-order pattern with a decreased initial burst.

The main release mechanism of PLGA MPs is a hydrolysis of ester groups under aqueous condition. When PLGA MPs were incorporated into thermosensitive chitosan solution, their free contacts with water were hindered by ther-

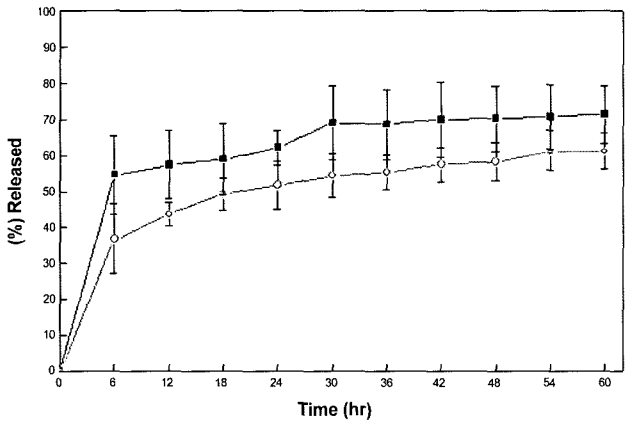

Figure 4. *In vitro* release of BSA from thermosensitive chitosan hydrogel (Mean±standard error of the mean, n=3). ●: Oil/decane system, ■: Aqueous system.

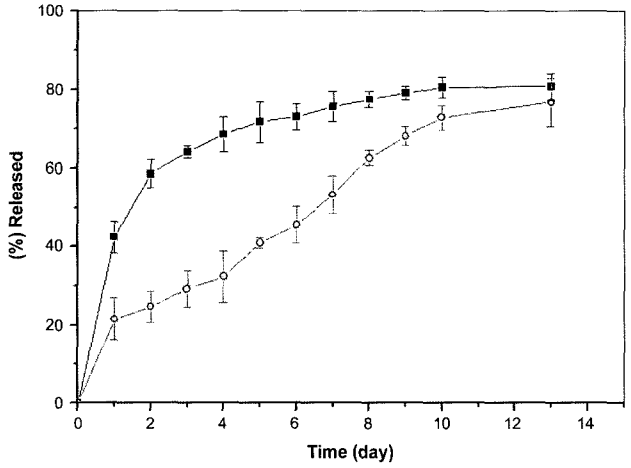

Figure 5. *In vitro* release of 5-FU from PLGA MPs and PLGA MPs-embedded hydrogel (Mean±standard error of the mean, n=3). ■: PLGA MPs, ●: PLGA MPs-embedded hydrogel.

mosensitive chitosan hydrogels. As the degradation of PLGA from the inside of hydrogel was slowly progressed, the drugs released from MPs diffused out through hydrogel network but the release period of 5-FU was not extended as compared with PLGA MPs. This result demonstrates that hydrogel itself may not affect the release rate of small molecules like 5-FU.

Conclusions

Chitosan-g-NIPAAm was developed as a drug carrier for an injectable and local delivery system. An aqueous solution of newly developed injectable thermosensitive chitosan copolymer showed a free flowing sol at room temperature but became a gel at body temperature above LCST. Therefore, drug/copolymer mixtures could be simply prepared by mixing a drug solution and an aqueous solution of thermosensitive chitosan copolymer at room temperature. These mixtures could be conveniently injected by using a syringe to the specific site and solidified at body temperature. Two kinds of release profiles from thermosensitive chitosan hydrogel showed a potential as a drug delivery carrier on the basis of these supporting results. MPs-embedded hydrogel was designed as another injectable carrier, which would be effective to the drug localization by trapping the particles around the injection site. They have shown a zero-order release behavior with a reduced burst effect.

In summary, thermosensitive chitosan can be utilized as an injectable drug carrier using sol-gel transition and more suitable for protein drugs with high molecular weight. They also can control the release kinetic from drug loaded MPs and make them possible to localize on the disease site. Therefore, thermosensitive chitosan has a potential as an injectable drug carrier for the local drug delivery and also can be useful for other biomedical applications.

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