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Poly (L-lysine) Based Semi-interpenetrating Polymer Network as pH-responsive Hydrogel for Controlled Release of a Model Protein Drug Streptokinase

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Abstract With the aim of developing a pH-sensitive controlled drug release system, a poly (L-lysine) (PLL) based cationic semi-interpenetrating polymer network (semi-IPN) has been synthesized. This cationic hydrogel was designed to swell at lower pH and de-swell at higher pH and therefore be applicable for achieving regulated drug release at a specific pH range. In addition to the pH sensitivity, this hydrogel was anticipated to interact with an ionic drug, providing another means to regulate the release rate of ionic drugs. This semi-IPN hydrogel was prepared using a free-radical polymerization method and by crosslinking of the polyethylene glycol (PEG)-methacrylate polymer through the PLL network. The two polymers were penetrated with each other via interpolymer complexation to yield the semi-IPN structures. The PLL hydrogel thus prepared showed dynamic swelling/de-swelling behavior in response to pH change, and such a behavior was influenced by both the concentrations of PLL and PEG-methacrylate. Drug release from this semi-IPN hydrogel was also investigated using a model protein drug, streptokinase. Streptokinase release was found to be dependent on its ionic interaction with the PLL backbones as well as on the swelling of the semi-IPN hydrogel. These results suggest that a PLL semi-IPN hydrogel could potentially be used as a drug delivery platform to modulate drug release by pH-sensitivity and ionic interaction.

Keywords: semi-interpenetrating polymer network, poly (L-lysine), pH sensitivity, controlled protein drug release, streptokinase

INTRODUCTION

Recently, a significant interest has been focused on the development of the pH-responsive drug delivery systems, since this pH-dependent behavior offers specific advantages for the treatment of certain diseases [1-3]. For instance, some disease sites have different pHs than the normal sites [4,5]; such as that the tumor site is normally presented with an acidic environment (pH ~6.0) due to the production of acidic metabolites, whereas many inflammation sites produce an alkaline pH surrounding. Aside from these disease sites, the normal gastrointestinal (GI) system also composes of an acidic environment [6], and the endosomes within the cells have a pH of 5.5 [7]. Such a variety in pH allows specific targeting and delivery of active drugs or genes to these areas by pH-responsive polymeric delivery systems.

Among pH-sensitive polymers, cationic and anionic polymers have been utilized for the controlled drug release based on their dynamic swelling/de-swelling behavior against pH changes [8-10]. Functionalized methacrylate polymers are also well noted for their pH-sensitivity due to their modified side chains as well as the presence of the carboxylic acid moieties [10]. Hydrogels made of those pH-sensitive polymers have been demonstrated to display sharp responses towards external pH changes and also the ability to control the permeation of entrapped drugs in correspondence to the swelling/de-swelling behavior. Thus, these pH-sensitive hydrogels have been found to possess many potential medical applications such as drug delivery devices or on/off switches [9,10]; by virtue of their rapid response to small pH changes under physiological conditions. In addition, such pH-sensitive hydrogel can facilitate the release of protein drugs which, due to the high molecular weights, often can hardly be released from the carrier matrices. In general, to enhance the release rate of a protein drug, water-soluble additives such as porogen is usually incorporated into the carrier matrices to provide a channel for the release [11]. The use of a pH-responsive hydrogel system, however, can directly facilitate such a protein release without the release enhancer, simply by regulating the swelling/de-swelling behavior of the hydrogel. In addition, a pH-sensitive polymer hydrogel can also regulate protein release via an ionic interaction with the protein drug.

Herein, we report the development of a semi-

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interpenetrating polymer network (semi-IPN) hydrogel delivery system based on free radical polymerization and interpolymer crosslinking of poly (L-lysine) (PLL) and polyethylene glycol (PEG) methacrylate for a regulated release of a model protein drug, streptokinase, in response to an external pH-change. In contrast to an acrylate-based ionic hydrogel system which manifests limited biodegradability, this PLL-based hydrogel represents a biocompatible polypeptide system with a biodegradability that can be regulated due to the presence of amenable crosslinking and conjugation chemistries. Aside from its use in protein delivery, as a cationic polymer, this PLL-based hydrogel can presumably also be employed in gene delivery due to its high capability in condensing anionic DNA products [12,13].

MATERIALS AND METHODS

Materials

Poly (L-lysine) (MW: 80 kDa) and polyethylene glycol (PEG, MW: 10 kDa) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). PEG (MW: 360 Da)-methacrylate and PEG (MW: 560 Da)-dimethacrylate were obtained from Aldrich Chemical Co. (Milwaukee, WI, USA). Ammonium persulfate (APS), tetramethylethylene diamine (TEMED) were obtained from ICN Bio-medicals, Inc. (Aurora, OH, USA) and Bio-Rad Laboratories (Richmond, CA, USA), respectively. PEG dialdehyde was from Shearwater Inc. (Huntsvill, OH, USA). Streptokinase was purchased from Sigma Chemical Co. (St. Lous, MO, USA). The chromogenic substrate D-Val-Leu-Lys-*p*-nitroanilide (S-2251) was obtained from Pharmacia Hepar Inc. (Franklin, OH, USA).

pH Mesurement of the Mixture of PLL and PEG-Methacrylate Polymers

In order to examine the interpolymer interaction between PLL and PEG, the pH change in the mixture containing PLL and PEG (MW : 10 kDa) polymers were measured in accordance with the weight ratio of PEG versus PLL. In addition, the pH changes in mixtures containing PLL and PEG-methacrylate polymers as well as PLL polymer and PEG-methacrylate monomers were also measured accordingly.

Fabrication of the Interpenetrating Polymer Network

To make the semi-IPN hydrogel, 100 mg PEG methacrylate monomer, 50 mg PEG-dimethacrylate, 100 mg PLL, 50 mg PEG dialdehyde and 0.5 mL distilled water were added sequentially to a test tube (20 mm outer diameter × 150 mm in length). Following mixing, 10 mg of the polymerization initiator, APS, and 10 μL of the polymerization catalyst, TEMED, were added. Polymerization of the hydrogel was carried out at 4°C for overnight. Fig. 1 presents an illustration of the synthesis of the PLL-based semi-IPN hydrogel. The synthesized hydrogel was removed from the tube, washed with distilled water to remove APS and TEMED, and then dried under a vacuum. To prepare the protein-loaded hydrogel, streptokinase was added to the above polymer mixture, and polymerization and crosslinking of the hydrogel were then carried out. As a control, crosslinked PLL hydrogel was prepared by reacting 100 mg PLL with 100 μL glutaraldehyde (25%) at 4°C for 24 h. All hydrogel products were stored at 4°C under a vacuum.

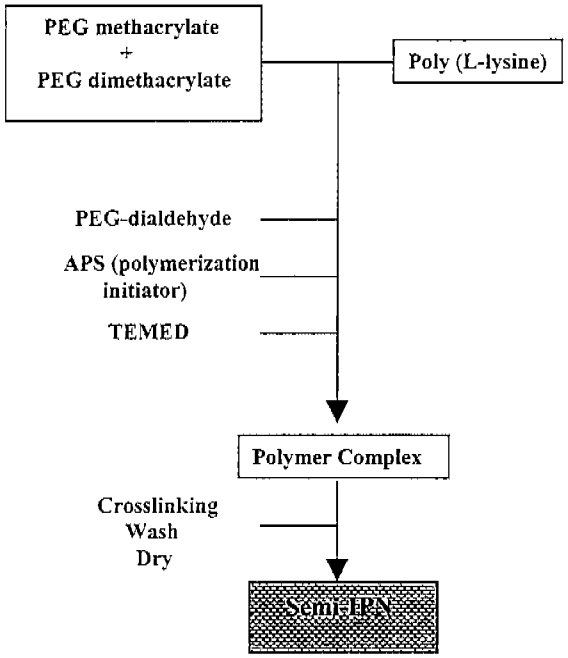

Fig. 1. Schematic diagram of the synthesis of PLL/PEG-dimethacrylate semi-IPN hydrogel.

Swelling Experiments

Swelling experiments were conducted by equilibrating the selected dry hydrogel sample in buffer until a constant weight was obtained. Acetate buffer was used for pH values from 2.0 to 5.0, phosphate buffer for pH values from 5.0 to 7.4, and borate buffer for pH from 7.4 to 11.0. The total buffer concentration was maintained at 0.01 M, and the total ionic strength (μ) was adjusted by the addition of sodium chloride. The buffer solution was replaced frequently throughout the swelling process to insure complete equilibration of the hydrogel at the desired pH. The equilibrium swelling ratio was determined as the mass ratio of the wet versus the dry hydrogel. To examine the reversibility of the hydrogel response towards external pH changes, swelling studies were conducted by using short-time pulsatile pH change from either 5.0 to 7.4 or 5.0 to 11.0. The weight of the hydrogel was measured periodically during the experiment to monitor the swelling behavior.

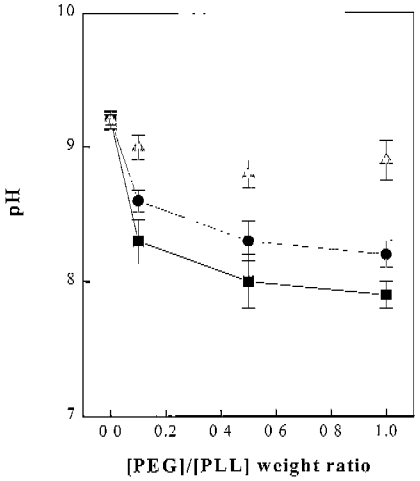

Fig. 2. The pH changes in solutions containing the PLL and PEG-methacrylate monomer/polymer in relation to the PEG/PLL weight ratios. The pH change in solutions containing: (▲) PLL and PEG-methacrylate monomers; (●) PLL and PEG-methacrylate polymer ; and (■) PLL and PEG polymer (MW: 10 KDa). The molecular weight of PEG in the PEG-methacrylate monomer was 350 Da.

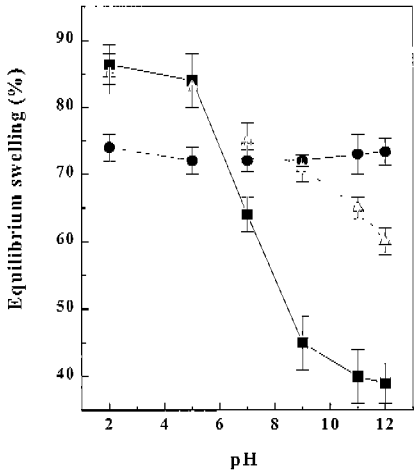

Fig. 3. Equilibrium swelling of (■) PLL-PEG methacrylate polymer IPN hydrogel; (▲) crosslinked PLL hydrogel; and (●) crosslinked PEG-methacrylate polymer hydrogel in accordance to pH changes.

Drug Release Experiments

Drug release experiments were conducted by placing the swollen, drug-loaded hydrogel samples into vials containing 5.0 mL buffer solution. The vials were placed onto a shaking water bath maintained at 37°C and gently agitated. At selected time intervals, individual hydrogel samples were removed from the solution, gently wiped to dry the excess buffer on the surface, weighed, and then placed into vials containing a freshly prepared buffer solution. To examine the effect of volume change of the hydrogel on drug release, swollen hydrogel samples were transferred into a high pH buffer solution (pH 9.0) to allow for de-swelling, whereas dried hydrogel samples were transferred to a lower pH buffer solution (pH 5.0) to allow for swelling. The release of streptokinase from the hydrogel was quantified by using the chromogenic S-2251 assay, which was designed to measure the produced plasmin activity. Absorbance was measured at 405 nm and then converted into the quantity of released streptokinase.

RESULTS AND DISCUSSION

Fabrication of Semi-IPN Hydrogel

To fabricate a semi-IPN hydrogel, interpolymer complexation between polymer pairs through secondary binding forces (*e.g.* electrostatic interaction, hydrogen bond, etc.) is required [14,15]. In our hydrogel system, the hydrogen bonds formed between the amino groups on PLL and the ether groups on PEG methacrylate polymer induced interpolymer complexation, causing the formation of the IPN structures. This complexation occurred only at a pH that was high enough to retain substantial deprotonation of the amine groups. To this regard, complexation would affect the acid-base equilibrium in the system, causing a reduction of the pH in the solution. This phenomenon has already been reported by other investigatiors [14,15] on the formation of a similar semi-IPN hydrogel between polyacrylic acid and PEG. Therefore, in our study, the same methodology was adopted to corroborate the complexation between PLL and PEG, by measuring the change in pH in the solution containing these two polymers. As shown in Fig. 2, comparing to the mixture of PLL and PEG-methacrylate monomer, the mixture of PLL and PEG-methacrylate polymer showed a more evident decrease in pH when increasing the PEG-methacrylate polymer/PLL weight ratio. It should be noted that interpolymer complexation, which requires the entanglement of polymer chains, should be more effective if the two polymers possess comparable molecular weights. To this regard, the presence of a less significant pH decrease in the mixture containing PLL and PEG-methacrylate monomer simply suggested the inability to produce a stable interpolymer complexation in this solution. On the other hand, the pH change in the mixture of PLL and PEG-methacrylate polymer was comparable to that of PLL and PEG-10 kDa polymer, indicating the occurrence of successful interpolymer complexation in both systems. Besides the more obvious decrease in pH, phase separation was also observed in these two systems.

To confirm the presence of interpolymer interaction and IPN network formation, the glass transition temperature (Tg) of the semi-IPN hydrogel was measured by using a differential scanning calorimeter (DSC). Unlike the crosslinked PEG-methacrylate polymer, the PLL/PEG-methacrylate IPN hydrogel did not show a

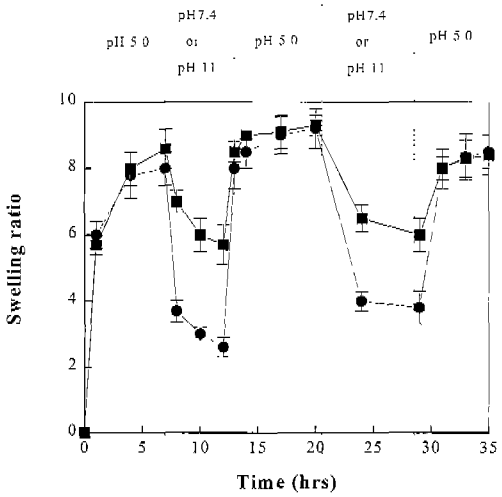

Fig. 4. The change in swelling ratios of the PLL-PEG methacrylate IPN hydrogels placed successively in buffered solutions at (■) pH 5.0 and pH 7.4; (●) pH 5.0 and pH 11.

detectable Tg (data not shown); presumably due to the significant lack of chain mobility within this type of hydrogel. Such a hindrance in Tg in the polymer blends implicitly suggested the miscibility and interaction between the two polymer chains. To explain this event, a likely mechanism would be that physical entanglement of polymer chains occurred first, followed by an interpolymer complexation produced by the formation of hydrogen bonds. This inter-polymer complexation could affect both the swelling properties and the drug release patterns of the hydrogel.

Equilibrium Swelling of Semi-IPN Hydrogel

Fig. 3 shows the equilibrium swelling of the studied hydrogel systems at different pH. While the nonionic PEG-methacrylate hydrogel displayed a low and pH-independent swelling behavior, the crosslinked PLL and PLL-PEG IPN hydrogels exhibited relatively high and pH-dependent characteristics. As seen, high swelling ratios were observed at low pH for these hydrogels due to protonation of the amine groups on PLL. The ionic repulsion between the protonated amino groups led to swelling of the PLL-containing hydrogel. Contrarily, low swelling ratios were observed at high pH due to the lack of interaction between the unprotonated amino groups. Between these two PLL-based hydrogels, the semi-IPN hydrogel yielded a much reduced swelling ratio than the PLL hydrogel did at the same pH, possibly due to the formation of hydrogen bonds in the former thereby reducing the swelling ratio at a high pH.

Pulsatile Swelling Experiments

The swelling behavior of the PLL/PEG-methacrylate semi-IPN hydrogel, when subjected to a pulsatile pH change, was shown in Fig. 4. As seen, the swelling/de-

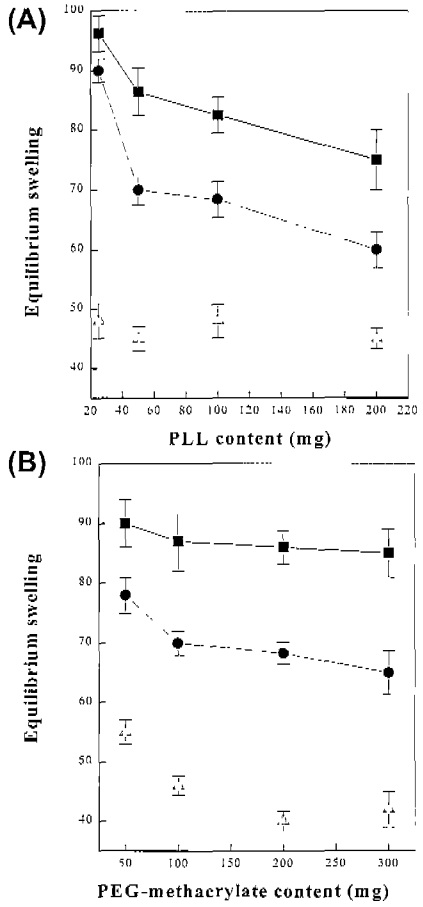

Fig. 5. Effects of (A) PLL content and (B) PEG-methacry-late content on the swelling behavior of the PLL-PEG methacrylate IPN hydrogel. Experiments were conducted at (■) pH 5 ; (●) pH 7.4 ; and (▲) pH 11.

swelling behavior was reversible, as demonstrated after repeatedly cycling of the hydrogel between at pH 5.0 and pH 7.4 (or pH 11) buffers over a 35-hour period. At low pH (pH 5.0), the amino groups on the hydrogel were protonated, rendering the hydrogel to swell due to electrostatic repulsion. On the other hand, the hydrogel collapsed at high pH (7.4 or 11), due to the lack of protonation of these amino groups. When comparing to the crosslinked PLL hydrogel, the PLL-PEG methacrylate semi-IPN demonstrated a lower degree and yet more rapid change in swelling (data not shown); both events were probably attributed to the interpolymer complexation in this IPN hydrogel. The effect of interpolymer complexation was more evident at pH 11, as a rapid collapse was observed when the IPN hydrogel was switched from a pH 5.0 to a pH 11 buffer. Based on this observation, the pH sensitivity of the PLL-PEG methacrylate IPN hydrogel seems to be readily controllable either by altering the extent of protonation of the PLL chains or by changing the degree of interpolymer complexation between the two polymers. This pH-sensitive

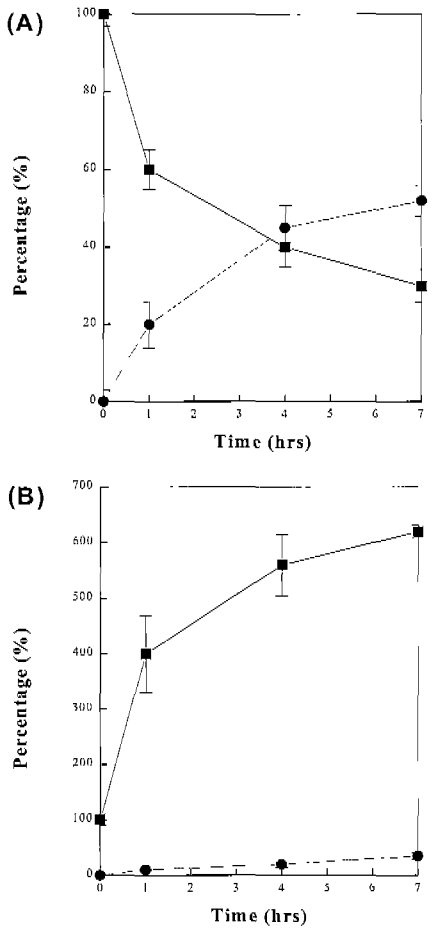

Fig. 6. The effect of (A) de-swelling; and (B) swelling on the release of streptokinase from the IPN hydrogel. Details of experimental design were described in the "Materials and method" section. In brief, experiments in (A) were conducted by placing swollen hydrogel samples in a pH 9.0 buffer whereas, experiments in (B) were conducted by placing dry hydrogel samples in a pH 5.0 buffer. (●) represents the cumulative percentage of streptokinase released from the hydrogel sample, whereas (■) represents the percent change in the swelling ratio of the hydrogel as a function of time.

swelling and de-swelling process appeared to be completely reversible, it could continue for a number of cycles without detectable changes in the swelling/de-swelling ratios should be allowed to reach equilibrium.

The swelling ratio of the hydrogel appeared to be influenced by both the concentration of PLL and PEG-methacrylate. As shown in Fig. 5(A), the semi-IPN hydrogels yielded a reduced degree of swelling at pH 5.0 when increasing the PLL content. This finding was not of surprise, considering that the chain entanglement within the IPN hydrogel was enhanced at a higher PLL concentration. Accordingly, the IPN hydrogel also displayed a similar pattern of reduced swelling at pH 7.4 when the PLL content was increased. In contrast, swelling of the IPN hydrogel at pH 11, when the amino groups on PLL would not be protonated did not reveal any appreciable dependence on the PLL concentration. In this case, the effect on swelling by the increased inter-polymer interaction at higher PLL contents seemed to be quite minimal, simply because of the overall lack of swelling of the IPN hydrogel at high pH due to the absence of repulsion among the unionized amino groups on PLL. On the other hand, the IPN hydrogel yielded almost no reduction in swelling at pH 5.0, when the concentration of PEG-methacrylate was increased (Fig. 5(B)). Obviously, the effect of the PEG-methacrylate concentration on swelling would not be significant at pH 5.0, since all the amino groups on the PLL should be protonated at this pH. Thus, an effective interpolymer complexation could not be achieved at this pH, regardless of the PEG concentration. A more obvious reduction in the swelling ratio was, however, observed at higher pH (*e.g.* pH 11.0), presumably due to the presence of an enhanced degree of interpolymer complexation between the PEG-methacrylate and PLL chains that restricted the water uptake by the IPN hydrogel. Although the actual mechanism that contributes to the swelling behavior of this IPN hydrogel is not yet clear, it seems that the incorporation of PEG-methacrylate and its content are both important factors in regulating the effectiveness of producing the interpolymer complexation during the formation of the IPN network. The results seen in Fig. 5 clearly indicated that swelling of the IPN hydrogel at low pH can be regulated by altering the PLL content whereas at high pH by changing the PEG-methacrylate content. Overall, the conclusion is that by manipulating the content of either PLL or PEG-methacrylate, one could alter the steric shielding of the ionion repulsion or the formation of the intermolecular hydrogen bonds, thereby achieving a tailored swelling pattern for the synthesized IPN hydrogel.

Release of Streptokinase from the Semi-IPN Hydrogel

The pH-dependent swelling/de-swelling properties of an ionic hydrogel have been utilized in the delivery of a variety of protein drugs including insulin [16,17]. To assess if the PLL/PEG semi-IPN hydrogel under development could be employed as a delivery system for protein drugs, the release of streptokinase (MW: 40 kDa), a widely used clinical thrombolytic drug, was examined. Streptokinase was incorporated into the IPN matrices by adding it directly into the reaction mixture during the hydrogel fabrication. To examine the effect of swelling/de-swelling on the release of streptokinase, swollen hydrogel samples were placed into pH 9.0 solution, where the hydrogel would de-swell, and dry hydrogel samples were placed into a pH 5.0 solution, where the hydrogel would swell. As shown in Fig. 6(A), over a 7-hour time span at pH 9.0, more than 40% of the entrapped streptokinase were released from the previously swollen gel samples, accompanied by a reduction in the swelling ratio by approximately 65%. When the dry hydrogel samples were placed into a pH 5.0 buffer to

examine the effect of swelling on streptokinase releae, however, less than 20% of streptokinase were released despite an increase in the gel volume by more than 6-fold (Fig. 6(B)). These findings could be explained by the interaction between the polymer and the protein drug. At the low pH regions where gel swelling occurred, the negative-charged streptokinase molecules might stick to the positive-charged polymer chains via an ionic interaction. It is well understood that drug release from a swollen hydrogel is a diffusion process. Therefore, the ionic interaction between streptokinase and the PLL polymer chain could easily retard streptokinase release, even there was a significant increase in swelling and the hydrogel volumes. Indeed, such an ionic drug and polymer interaction could be wisely used to regulate the drug release rate although our data showed that de-swelling of the hydrogel should be the primary driving force to achieve a complete drug release. Further studies of regulating protein release from this semi-IPN hydrogel by utilizing a self-manipulated pH change in the release environment are currently in progress.

CONCLUSION

A new semi-interpenetrating polymer network (semi-IPN) containing PLL and PEG-methacrylate was synthesized as a pH-responsive controlled drug release carrier. This semi-IPN hydrogel displayed dynamic swelling/de-swelling behavior against pH changes. Variables involved in the synthesis of this semi-IPN hydrogel, such as the PLL and PEG-methacrylate concentrations and the degree of interpolymer complexation were found to significantly influence the swelling properties of the hydrogel. The release of the protein drug from this hydrogel was found to be dependent on both the ionic interaction and the swelling behavior of the hydrogel. Indeed, the de-swelling function of the semi-IPN hydrogel could serve as another effective means to regulate the release of the entrapped protein drug. Overall, our results suggested that the pH-sensitive semi-IPN hydrogel under development could be a useful tool to achieve regulated and desirable drug release in response to environmental pH changes.

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REFERENCES

[1] Vakkalanka, S. K., C. S. Brazel, and N. A. Peppas (1996) Temperature- and pH-sensitive terpolymers for modulated delivery of streptokinase. *J. Biomater. Sci. Polym.* Ed. 8: 119-129.

[2] Ramkissoon-Ganorkar, C., F. Liu, M. Baudys, and S. W. Kim (1999) Modulating insulin-release profile from pH/thermosensitive polymeric beads through polymer molecular weight. *J. Control. Release* 59: 287-298.

[3] Akala, E. O., P. Kopeckova, and J. Kopecek (1998) Novel pH-sensitive hydrogels with adjustable swelling kinetics. *Biomaterials* 19: 1037-1047.

[4] Jain, R. K. (1999) Transport of molecules, particles, and cells in solid tumors. *Annu. Rev. Biomed. Eng.* **1**: 241-263.

[5] Helmlinger F., F. Yuan, M. Dellian, and R. K. Jain (1997) Interstitial pH and pO_2 gradients in solid tumors in vivo: high-resolution measurements reveal a lack of correlation. *Nat. Med.* 3: 177-182.

[6] Rubinstein, A. and D. R. Friend (1994) Specific delivery to the gastrointestinal tract. pp. 267-313 In: A. J. Domb (ed). *Polymeric Site-specific Pharmacotherpay*. Wiley, Chichester, USA.

[7] Behr, J.-P. (1997) The proton sponge : A trick to enter cells the viruses did not exploit. *Chimia* 51: 34-36.

[8] Brazel, C. S. and N. A. Peppas (1996) Pulsatile local delivery of thrombolytic and antithrombotic agents using poly (N-isopropylacrylamide-co-methacrylic acid) hydrogels. *J. Control. Release* 39: 57-64.

[9] Park, T. G. (1999) Temperature modulated protein release from pH/temperature-sensitive hydrogels. *Biomaterials* 20: 517-521.

[10] Markland, P., Y. Zhang, G. L. Amidon, and V. C. Yang (1999) A pH-and ionic strength-responsive polypeptide hydrogel: Synthesis, characterization, and preliminary protein release studies. *J. Biomed. Mater. Res.* 47: 595-602.

[11] Cohen, S., T. Yoshioka, M. Lucarelli, L. H. Hwang, and R. Langer (1991) Controlled delivery systems for proteins based on poly (lactide/glycolic acid) Microspheres. *Pharm. Res.* 8: 713-720.

[12] Choi, Y. H., F. Liu, J. S. Kim, Y. K. Choi, J. S. Park, and S. W. Kim (1998) Poly (ethylene glycol)-grafted poly-L-lysine as polymeric gene carrier. *J. Control. Release* 54: 39-48.

[13] Mislick, K. A., J. D. Baldeschwieler, J. F. Kayyem, and T. J. Meade (1995) Transfection of folate-polylysine DNA complexes: evidence for lysosomal delivery. *Bioconjugate Chem.* 6: 512-515.

[14] Shojaei, A. H. and X. Li, (1997) Mechanisms of buccal mucoadhesion of novel copolymers of acrylic acid and polyethylene glycol monomethylether monomethacrylate. *J. Control. Release* 47: 151-161.

[15] Nishi, S. and T. Kotaka (1985) Complex-forming poly(ethylene glycol): Poly(acrylic acid) interpenetrating polymer networks. 1. Preparation, structure, and viscoelastic properties. *Macromolecules* 18: 1519-1524.

[16] Gombotz, W. R. and A. S. Hoffman (1986), Immobilization of biomolecules and cells within synthetic polymeric hydrogels. pp. 95-126 In: *Hydrogels in Medicine and Pharmacy. I. Fundamentals*. N. A. Pepppas (ed.) CRC Press, Inc., Boca Raton, Florida, USA.

[17] Obaidat, A. A. and K. Park (1997) Characterization of protein release through glucose-sensitive hydrogel membranes. *Biomaterials* 18: 801-806.

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