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Effect of pH on Swelling Property of Hyaluronic Acid Hydrogels for Smart Drug Delivery Systems

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Abstract

Hyaluronic acid(HA) hydrogels were synthesized by immersing HA microbeads in phosphate buffered saline solutions having different pH levels to assess the effect of pH on the swelling ratio of HA hydrogels for smart drug delivery systems. No beads were formed when the HA solution(below pH 9) was crosslinked with divinyl sulfone(DVS) because DVS is a basic solution. The variation regarding the size of the microbead was not significant, suggesting that the bead size is not a function of pH(10 ~ 14). However, the pore size of the microbeads decreased with increasing pH from 10 to 14, leading to the surface smoothness and dense network as a result of higher crosslinking. The swelling ratio of hydrogels increased when the pH rose from 2(acidic) to 6(neutral). Afterwards, it decreased with further increasing pH(basic). The lower swelling ratio may be due to the lack of ionization of the carboxyl groups. On the other hand, a higher swelling ratio is likely due to the increased electrostatic repulsions between negatively charged carboxyl groups on different chains. Experimental results suggested that pH-responsive HA hydrogels can be applicable to the controlled drug delivery systems.

Keywords : pH-Responsive Hydrogel, Hyaluronic Acid, Divinyl Sulfone, Crosslink, Microbead, Swelling Ratio, Drug Delivery System

1. INTRODUCTION

Hydrogels are 3-D high-molecular weight networks composed of a polymer backbone, water and a crosslinking agent. Since the hydrogels have structural similarities to body tissue, they can be used as biomaterial for defected tissue regeneration by replacing water with biological body fluid, cell, and/or medicine[1-11]. Recently, natural polymer hydrogels such as hyaluronic acid(HA), collagen, gelatin, fibrin, alginic acid, and chitosan are gaining tremendous attention in a wide variety of applications in medical, pharmaceutical and related fields[12-16].

Hydrogels are hydrophilic polymer networks, which may absorb from 10 % - 20 % up to thousands of times their dry weight in water[1-3]. They swell dramatically in an aqueous medium; however, they do not dissolve in water at physiological temperature and pH. Hydrogels have been developed as stimuli-responsive materials, which can undergo abrupt volume change in response to compensate changes in environmental parameters such as temperature, pH, ionic strength, solvent composition and pressure[1-11]. These unique characteristics of hydrogels are of great interest in drug delivery, cell encapsulation and tissue engineering[17, 18]. Stimuli-responsive polymers play an important role in the development of novel smart hydrogels. Hydrogels show a minimal tendency to adsorb proteins from body fluids because of their low interfacial tension. Furthermore, the ability for molecules of different sizes to diffuse into(drug loading) to out of(drug release) hydrogels allows for the possible use of dry or swollen polymeric networks as drug delivery systems for oral, nasal, buccal, rectal, vaginal, ocular and parenteral routes of administration[17, 18]. The beauty of the smart hydrogels is that they can perceive the prevailing stimuli and respond by exhibiting changes in their physical or chemical behavior, resulting in the release of the entrapped drug in a controlled manner[17].

Among natural polymers, the HA molecule is stabilized in order to produce a crosslinked gel suitable for soft tissue implantation, resulting in improving its resistance to enzymatic degradation within the dermis without compromising its biocompatibility[13]. Our previous studies revealed that porous polymer networks of the hydrogels are a prerequisite to tissue regeneration as a scaffold because of their good mechanical properties and cell proliferation[4, 12, 13, 16]. HA is a linear

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polysaccharide formed from the disaccharide units of D-glucuronic acid and N-acetyl-glucosamine linked by β(1,4) and β(1,3) glucosidic bonds, as shown in Fig. 1[12, 13, 16, 20, 21]. Water-soluble polymers owe their solubility properties to the presence of functional groups(mainly OH, COOH, NH_2)[7]. The polysaccharide is crosslinked with divinyl sulfone(DVS, $C_4H_6O_2S$) under saline conditions [19-21]. Covalent linkages between polymer chains can be obtained by the reaction of functional groups of a crosslinking agent(vinyl group, CH_2 : CH-) and HA (hydroxyl group, -OH). The network properties can be adjusted by the concentration of the dissolved polymer and the amount of the crosslinking agent. The chemical structure of crosslinked HA with DVS is displayed in Fig. 2[19, 21].

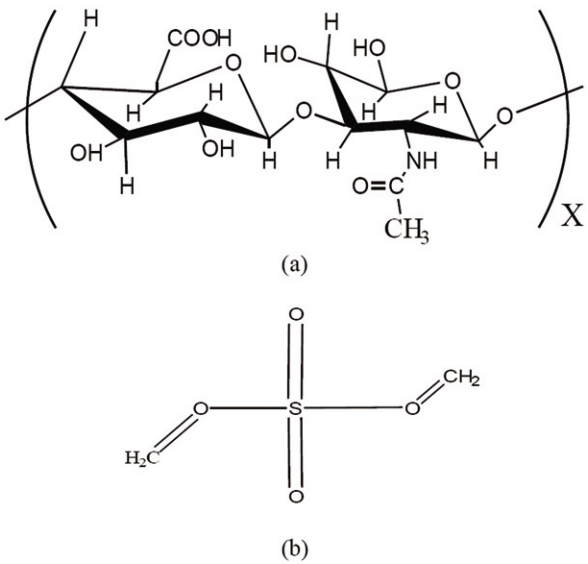

Fig. 1. Chemical structures of (a) hyaluronic acid and (b) DVS.

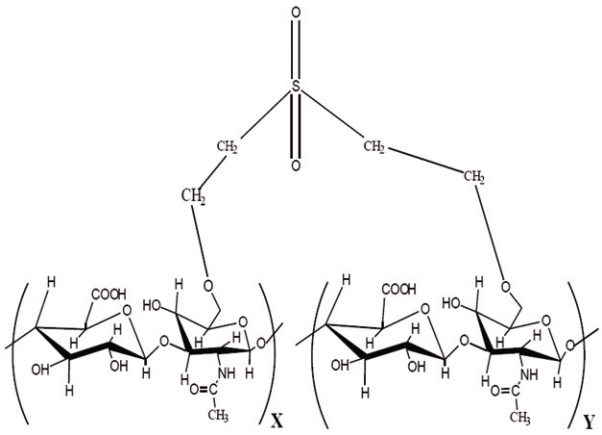

Fig. 2. Chemical crosslinking of HA with DVS.

HA hydrogels crosslinked with DVS are prepared by immersing the microbeads in a phosphate buffered saline solution(NaH_2PO_4). Higher mechanical properties as a result of the dense porous network are found as the degree of crosslinking becomes higher[16].

The pH-sensitive polymers are normally produced by adding pendant acidic or basic functional groups to the polymer backbone[17]. They either accept or release protons in response to appropriate pH and ionic strength changes in aqueous media. Ionic hydrogels containing carboxyl group(-COOH) show either sudden or gradual changes in their dynamic or equilibrium swelling behavior as a result of changing the external pH. The degree of ionization regarding these hydrogels depends on the number of pendant acidic groups in the hydrogel, which results in increased electrostatic repulsions between the negatively charged carboxyl groups on different chains[3, 4, 17, 18]. In the present study, pH-responsive HA hydrogels are synthesized by immersing HA microbeads in phosphate buffered saline solutions in the pH range of 2 to 12 in order to evaluate the effect of pH on the swelling properties of the smart HA hydrogels.

2. EXPERIMENTAL

2.1 Preparation of HA hydrogels

HA solutions of 0.5 wt% concentration were prepared by dissolving sodium hyaluronate(Mw = 1×10^6 Da, Shiseido Co., Japan) in 0.05 mol/L NaOH at room temperature. A pH in the range of 11 to 12 was achieved by adding 10 mol/L NaOH to the HA solution. Then, the HA solution was placed in a solution hopper attached to the Masterflex L/S tubing pump(Cole Parmer, USA) and fed into a syringe equipped with a 14-gage metal needle at a flow rate of 0.005 mL/min to 0.010 mL/min. Microbeads were fabricated by supplying compressed air with a pressure of 34.475 Pa along the HA solution nozzle. The nozzle was enclosed by a delivery tube with a diameter of 6 mm[4, 11, 13, 16]. Microbeads were collected from a solution mixture of 0.2 vol % of DVS($\geq$ 98 %, Sigma and Aldrich, Germany) in 2-methyl-1-propanol (99 %, Aldrich), followed by a stirring process (200 rpm ~ 300 rpm) for 5 h at room temperature. Then, the crosslinked microbeads were screened through a 200 mesh sieve. Afterwards, the microbeads were immersed in distilled water for 0.5 h and ethanol for 0.5 h to remove impurities such as divinyl

sulfone and 2-methyl-1-propanol. After 3-time cleaning in ethanol to eliminate an unreacted residual crosslinker, the microbeads were then dried for 2 h at 60℃ in a vacuum of 20 torr. The as-dried microbeads were examined by using SEM(S-3000H, Hitachi, Japan) to elucidate the morphology, the porosity and the size of the beads.

2.2 The measurement of swelling property

The swelling test was performed. Firstly, the weight of the as-dried beads was measured. Then, the beads were immersed for 24 h in a phosphate buffered saline solution(NaH_2PO_4) with various pH conditions(2, 4, 6, 10) and distilled water(pH 7.4). The as-treated beads were then screened through a 3.0 μm membrane filter(Advantec, Japan). After measuring the weight of the as-treated beads, the swelling ratio(S) was determined by using equation (1)[17],

$$S(\%) = \frac{W_s - W_d}{W_d} \times 100 \quad (1)$$

where W_s and W_d are the weight of swollen and as-dried beads, respectively.

3. RESULTS AND DISCUSSION

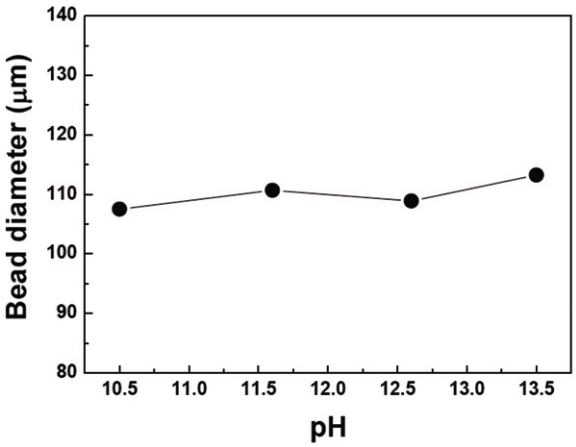

Fig. 3. Diameter of HA microbeads crosslinked in different pH.

The size of the as-dried HA microbeads obtained from HA solutions having various pH conditions is shown in Fig. 3. The value of pH was achieved by adjusting the NaOH to the HA solution[13]. No dramatic variation of the bead diameter prepared in the pH range of 10 to 14 is observed, suggesting that the bead size is not a function of pH.

Surface morphology was examined by using SEM, as shown in Fig. 4. No beads were formed when the HA solution was crosslinked below pH 9 because DVS (crosslinker) is a basic solution[16]. As pH rose, the smoothness and the network of the beads becomes high and dense due to higher crosslinking, as depicted in Figs. 4 and 5.

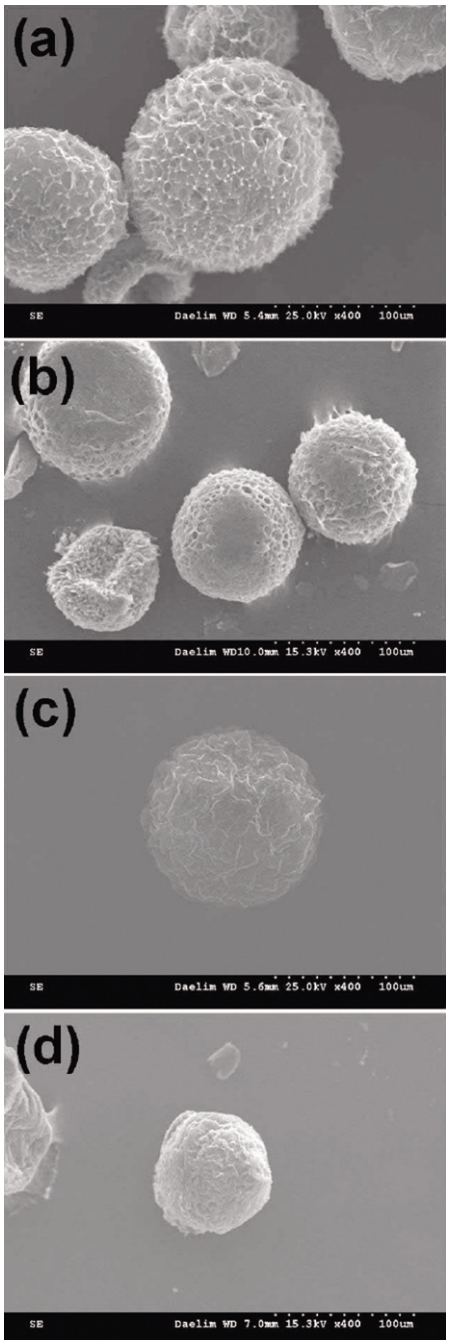

Fig. 4. SEM images of HA microbeads prepared at (a) pH 10.5~11.0, (b) pH 11.5~12.0, (c) pH 12.5~13.0 and (d) pH 13.5~14.0.

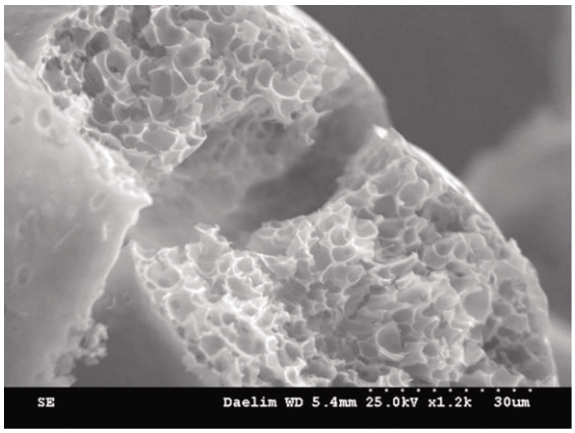

Fig. 5. SEM image of the cross-section of the microbeads prepared at pH 13.5. Note that the porous network of the beads becomes dense at higher pH.

The microbeads were immersed for 24 h in NaH_2PO_4 with different pH conditions(2, 4, 6, 10) and distilled water, respectively. After filtering and drying the microbeads immersed in the buffered solution, the swelling properties were evaluated by measuring the weight of the beads. The images of the as-dried microbeads and the swollen microbeads are shown in Fig. 6. This image indicates that the microbeads were swollen from 910 % (pH 2) up to 1750 % (pH 7.4). The swelling increased dramatically after raising the pH from 4 to 6, as depicted in Fig. 7. It is likely due to the presence of the carboxyl functional group in the side chains. The ionization of the carboxyl group(-COOH) gives rise to anionic carboxylate(CH_3COO-). The electrostatic repulsion between the negatively charged groups results in dramatic swelling behavior. The highest swelling ratio is observed when the beads are immersed in distilled water(pH 7.4). However, it decreased with increasing pH(pH 10), as displayed in Fig. 7. The low swelling ratio of the microbeads below pH 6(acidic) and above pH 10(basic) may be due to the lack of ionization regarding the carboxyl groups. The highest level of swelling was detected near pH 7 ~ 8(neutral). Thus, a pH-responsive smart hydrogel for drug release systems can only be possible if the HA hydrogel membrane is designed to release the entrapped drug at a certain value of pH in a controlled manner.

Drug release generally involves the simultaneous absorption of water as well as desorption of the drug via a swelling controlled mechanism[17-19]. The rate-controlling factor mediating drug delivery is the resistance of the polymer to an increase in volume and change in shape. The beauty of hydrogels for controlled drug delivery

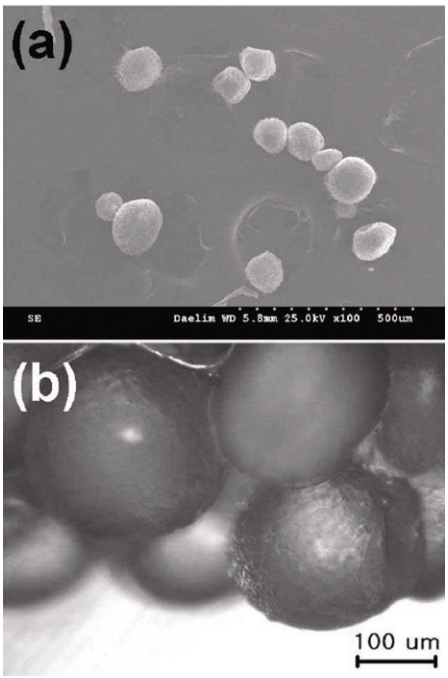

Fig. 6. (a) SEM image of dried HA microbeads prepared at pH 11.5 and (b) optical photography of swollen HA microbeads in distilled water.

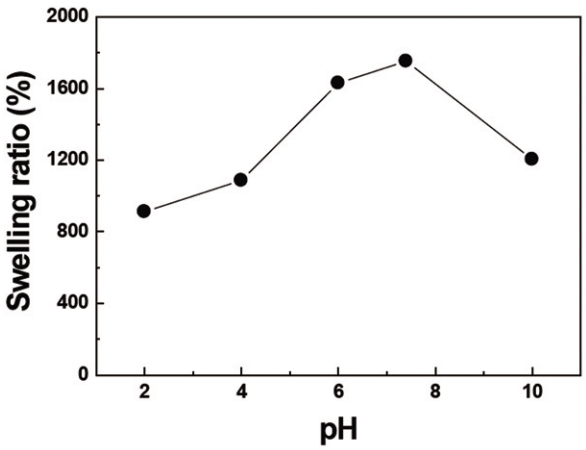

Fig. 7. Swelling ratio of HA microbeads as a function of pH for a phosphate buffered solution.

lie in the near constant release rates[18, 21]. However, it warrants further studies.

4. CONCLUSIONS

HA hydrogels crosslinked with DVS were synthesized by immersing the microbeads into a phosphate buffered solution. The smoothness and the dense porous network of HA microbeads are observed since the degree of

crosslinking increased with raising the pH of the HA precursor solution. The low swelling ratio in the pH range of 2 to 4(acidic region) increased dramatically when the pH was higher than 6(neutral region). However, it decreased when the pH was in basic regime due to the lack of ionization of the carboxyl groups. From the results, a pH-responsive HA hydrogel membrane can be designed to release the entrapped drug in a controlled manner for smart drug delivery systems.

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REFERENCES

[1] I. Tomatsu, Ke Peng, and A. Kros, "Photoresponsive hydrogels for biomedical applications", *Adv. Drug Delivery Rev*., vol. 63, pp. 1257-1266, 2011.

[2] A.K. Bajpai, S.K. Shukla, S. Bhanu, and S. Kankane, "Responsive polymers in controlled drug delivery", *Prog. Polymer Sci*., vol. 33, pp. 1088-1118, 2008.

[3] H. Omidian, J. G. Rocca, and K. Park, "Advances in superporous hydrogels", *J. Control. Release*, vol. 102, pp. 3-12, 2005.

[4] J.T. Kim, D.Y. Lee, and J.H. Choi, "Preparation and characterization of hyaluronic acid microbeads", *Biomater. Res*., vol. 14, pp. 157-160, 2010.

[5] J. Kopecek and J. Yang, "Hydrogels as smart biomaterials", *Polymer Intl*., vol. 56, pp. 1078-1098, 2007.

[6] A.C. Jen, M.C. Wake, and A.G. Mikos, "Review : Hydrogels for cell immobilization", *Biotech, Bioeng*., vol. 50, pp. 357-364, 1996.

[7] W.E. Hennink and C.F. Van Nostrum, "Novel crosslinking methods to design hydrogels", *Adv. Drug Deliv. Rev*., vol. 54, pp. 13-36, 2002.

[8] A.S. Hoffman, "Hydrogels for biomedical applications", *Adv. Drug Deliv. Rev*., vol. 54, pp. 3-12, 2002.

[9] Y. Qiu and K. Park, "Environment-sensitive Hydrogels for Drug Delivery", *Adv. Drug Deliv. Rev*., vol. 53, pp. 321-339, 2001.

[10] Y. Shin, K.S. Kim and B. Kim, "Loading behavior of pH-responsive P(MAA-co-EGMA) hydrogel microparticles for intelligent drug delivery applications", *Polymer*, vol. pp. 421-426, 2008.

[11] S. Choi and J. Lee, "Thermoresponsive graft copolymers of hyaluronic acid", *Polymer,* vol. 35, pp. 223-227, 2011.

[12] J.T. Kim and J.H. Choi, "Production and evaluation of hyaluronic acid gel for soft tissue augmentation", *Biomater. Res*., vol. 13, pp. 105-108, 2009.

[13] J. Kim, J. Choi, and D.Y. Lee, "Pyrogenicity of hyaluronic acid hydrogel cross-linked by divinyl sulfone for soft tissue augmentation", *Natural Sci*., vol. 2, pp. 764-768, 2010.

[14] G.D. Prestwitch, D.M. Marecak, and J.F. Marecek, "Controlled chemical modification of hyaluronic acid: synthesis, applications, and biodegradation of hydrazide derivatives", *J. Control. Rel*., vol. 53, pp. 93-103, 1998.

[15] S.N. Park, H.J. Lee, K.H. Lee, and H. Suh, "Characterization of porous collagen/hyaluronic acid scaffold modified by 1-ethyl-3-(3-dimethyl-aminopropyl) carbodimide cross-linking", *Biomat*., vol. 22, pp. 1403-1415, 2002.

[16] J.T. Kim, D.Y. Lee, and J.H. Choi, "Implanting test of re-hydrated hydrogels by hyaluronic acid microbeads", *Biomat. Res*., vol. 15, pp. 125-128, 2011.

[17] P. Gupta, K. Vermani, and S. Garg, "Hydrogels : from controlled release to pH-responsive drug delivery", *Drug Delivery Today*, vol. 7, pp. 569-579, 2002.

[18] K.V.R. Rao and K.P. Devi, "Swelling controlled-release systems : recent developments and applications", *Int. J. Pharm*., vol. 48, pp. 1-13, 1988.

[19] E.J. Oh, S. Kang, B. Kim, G. Jiang, I.H. Cho, and S.K. Hahn, "Control of the molecular degradation of hyaluronic acid hydrogels for tissue augmentation", *J. Biomed. Mater. Res*., vol. 86A, pp. 685-693, 2008.

[20] C.E. Schante, G. Zuber, C. Herlin, and T.F. Vandamme, "Chemical modifications of hyaluronic acid for the synthesis of derivatives for a broad range of biomedical applications", *Carbohydrate Polymer*., vol. 85, pp. 469-489, 2011.

[21] G.D. Prestwich and K.P. Vercruysse, "Therapeutic applications of hyaluronic acid and hyaluronan derivatives", *Pharm. Sci. Technol. Today*, vol. 1, pp. 42-43, 1998.

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