Available online at www.sciencedirect.com
ScienceDirect
Carbohydrate Polymers 70 (2007) 251–257
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

Mechanical properties and degradation behaviors of hyaluronic acid hydrogels cross-linked at various cross-linking densities

Oju Jeon [a], Su Jin Song [a], Kee-Jung Lee [a], Moon Hyang Park [b], Soo-Hong Lee [d], Sei Kwang Hahn [e], Sungjee Kim [f], Byung-Soo Kim [c,*]

[a] *Department of Chemical Engineering, Hanyang University, 17 Haengdang-dong, Seongdong-gu, Seoul 133-791, Republic of Korea*
[b] *Department of Pathology, Hanyang University, 17 Haengdang-dong, Seongdong-gu, Seoul 133-791, Republic of Korea*
[c] *Department of Bioengineering, Hanyang University, 17 Haengdang-dong, Seongdong-gu, Seoul 133-791, Republic of Korea*
[d] *CHA Stem Cell Institute, Pochon CHA University, Seoul 135-081, Republic of Korea*
[e] *Department of Materials Science and Engineering, Pohang University of Science and Technology (POSTECH), Pohang, Kyungbuk 790-784, Republic of Korea*
[f] *Department of Chemistry, Pohang University of Science and Technology (POSTECH), Pohang, Kyungbuk 790-784, Republic of Korea*

Received 17 March 2006; received in revised form 16 March 2007; accepted 4 April 2007
Available online 12 April 2007

Abstract

The effect of cross-linking density of hyaluronic acid (HA) hydrogels on mechanical properties and degradation behaviors has been investigated. HA hydrogels were prepared by the covalent cross-linking of HA with poly(ethylene glycol)-diamine with two different molecular weights at various cross-linking densities. The elastic modulus increased gradually as the theoretical cross-linking density of HA hydrogels increased from 0% to 20%. However, as the theoretical cross-linking density increased above 20%, the elastic modulus decreased. At a theoretical cross-linking density of 20%, the elastic modulus increased as the molecular weight of the cross-linking molecule decreased. *In vitro* degradation rates of HA hydrogels decreased as the molecular weight of the cross-linking molecule decreased at a theoretical cross-linking density of 20%. The degradation rate of the cross-linked HA hydrogels decreased with increases in the theoretical cross-linking density from 0% to 20%. However, there was no significant difference in the degradation rate as the theoretical cross-linking density increased above 30%. With controllable mechanical properties and degradation rates, the developed HA hydrogels would be further investigated for various medical applications.

Keywords: Cross-linking; Degradation; Elastic modulus; Hyaluronic acid; Hydrogel; Poly(ethylene glycol)

1. Introduction

Hydrogels have gained attention over the years as cell transplantation vehicles for the regeneration of a variety of tissues (Gwak et al., 2004; Jeon, Ryu, Chung, & Kim, 2005; Kong, Kaigler, Kim, & Mooney, 2004; Ryu et al., 2005; Yamamoto, Sakakibara, Nishimura, Komeda, & Tabata, 2003). Hydrogels are highly swollen, insoluble networks that can be used to entrap cells (Peppas, Bures, Leobandung, & Ichikawa, 2000). The high equilibrium swelling promotes nutrient diffusion into the gel and cellular waste removal out of the gel, while the insolubility provides the structural integrity necessary for tissue growth. Hydrogels are ideal materials for applications of soft tissue engineering due to their similarity to the body's own highly hydrated composition (Brook, 1980, chap. 4; Park, Shalaby, & Park, 1975; Park et al., 1993). It is of great interest to create hydrogels with controlled mechanical properties for biomedical applications. They must possess the mechanical strength and flexibility sufficient to withstand compressional forces from the surrounding tissues *in vivo* without deformation or collapse (de Goot et al., 1997). In addition,

* Corresponding author. Tel.: +82 2 2220 0491; fax: +82 2 2291 0838.
E-mail address: bskim@hanyang.ac.kr (B.-S. Kim).

0144-8617/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2007.04.002

the mechanical properties of materials to which cells adhere can profoundly affect the function of the cells (Ingber, Karp, Plopper, Hansen, & Mooney, 1993).

Hyaluronic acid (HA) is a particularly attractive hydrogel material for biomedical applications (Pouyani & Prestwich, 1994; Rosier & O'Keefe, 2000). It is an important component of the extracellular matrix (ECM) of the human body. It has a high capacity for lubrication, and water sorption and retention. These properties have allowed HA to be applied in ophthalmic surgery as a viscoelastic material (Juzych, Parrow, Shin, Swendris, & Ramocki, 1992) and in orthopaedic surgery for treatment of articular cartilage defects (Guarda-Nardini, Tito, Staffieri, & Beltrame, 2002). HA also plays a critical role as a signaling molecule in cell motility (Hall & Turley, 1995), cell differentiation (Huang et al., 2003), wound healing (Sasaki & Watanabe, 1995), and cancer metastasis (Wernicke et al., 2003). However, poor mechanical properties and rapid degradation of HA limit broader ranges of clinical applications.

To improve the mechanical properties and control the degradation rate, HA can be chemically modified or cross-linked. Chemical modification of HA typically involves the carboxylic acid groups and/or the alcohol groups of its backbone. The carboxylic acid or alcohol groups have been modified by esterification (Campoccia

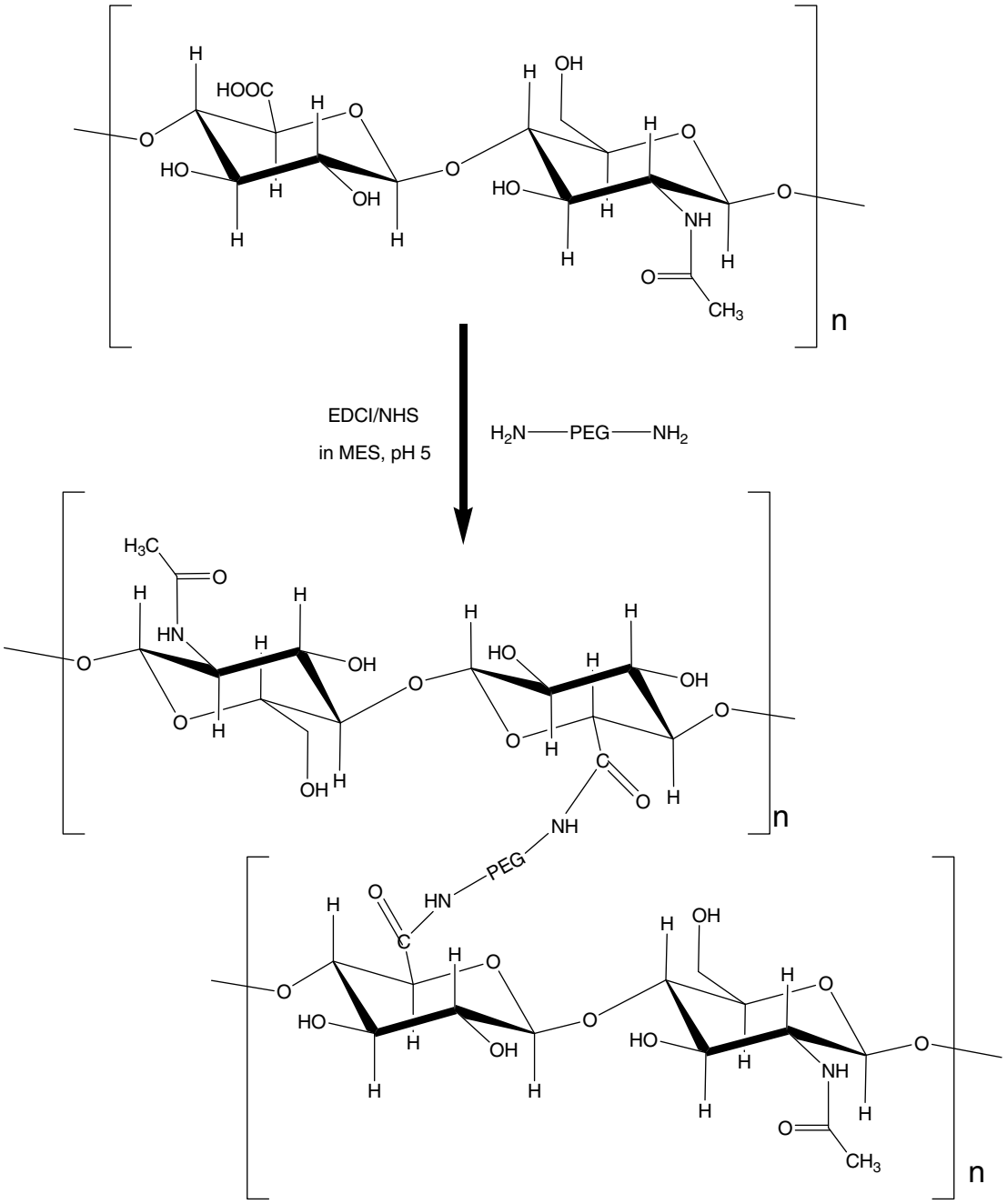

Fig. 1. Reaction scheme for cross-linking of HA with PEG-diamine.

et al., 1998) and by cross-linking with dihydrazide (Vercruysse, Marecak, Marecek, & Prestwich, 1997), dialdehyde (Bulpitt & Aeschlimann, 1999; Luo, Kirke, & Prestwich, 2000), divinyl sulfone (Ramamurthi & Vesely, 2002), diglycidyl ethers (Tomihata & Ikada, 1997), or disulfide (Shu, Liu, Luo, Roberts, & Prestwich, 2002) cross-linkers. However, no study has reported on the effect of cross-linking density and the molecular weight of the cross-linker [poly(ethylene glycol) (PEG)] on mechanical properties and degradation behaviors of the cross-linked HA hydrogels. The present study shows that the mechanical properties and degradation rate of cross-linked HA hydrogels can be controlled with the cross-linking density and molecular weight of the cross-linking molecule. HA was covalently cross-linked using PEG-diamine at various cross-linking densities. PEG was chosen as the cross-linking molecule because it is biocompatible and hydrophilic. PEG is soluble in aqueous solutions and commercially available at various molecular weights. The flexible PEG chains are thought to provide elasticity to the hydrogels, while the stiff hyaluronic acid chains provide mechanical strength.

2. Experimental

2.1. Materials

Fermentation-derived HA (molecular weight $= 1.5 \times 10^6$ Da) was purchased from LG Life Sciences, Inc. (Seoul, Korea). 1-Ethyl-3-[3-(dimethylamino)propyl]carbodiimide (EDC), and *N*-hydroxysuccinimide (NHS) were purchased from Aldrich (Milwaukee, WI, USA). Morpholinoethanesulfonic acid (MES) and trifluoroacetic acid (TFA) were purchased from Sigma (St. Louis, MO, USA). The hyaluronidase (HAase) was purchased from Sigma (499 U/mg, bovine testes isolated).

2.2. Synthesis of covalently cross-linked HA

Five grams of PEG-diamine was dissolved in 100 ml of methylene chloride, stirred at 25 °C for 3 h, and dried over Na_2SO_4. The Na_2SO_4 was removed by filtering. Methylene chloride was evaporated and dried in a vacuum oven at room temperature for 24 h.

HA was cross-linked with PEG-diamines with two different molecular weights, 1000 and 2000 Da. HA (1 w/v%) was dissolved in a buffer solution of 50 mM MES and 0.2 M NaCl. The pH was adjusted to 5.5. NHS (4.35 g, 38 mmol) and EDC (14.57 g, 76 mmol) were added to 15 ml of 1% (w/v) HA solution to activate the carboxyl groups on the HA backbone. The solution was stirred to obtain homogeneity and the PEG-diamines were added. The reaction was maintained at room temperature for 12 h. The solution was cast on a Teflon plate. After drying in air at room temperature for 24 h, the HA films were cut into 1×1 cm^2 pieces and placed in double distilled water to remove unreacted materials and byproducts.

2.3. Determination of cross-linking efficiency

The ninhydrin assay was utilized to determine the degree of effective cross-linking and unreacted amino groups during the coupling reaction (Romberg et al., 2005). Briefly, a small amount of dried hydrogel was suspended in 5 ml of 1 M sodium acetate buffer (pH 5) and

Table 1
Cross-linking efficiency (%) of HA hydrogels cross-linked with PEG1000 (molecular weight = 1000 Da) or PEG2000 (molecular weight = 2000 Da)

Theoretical cross-linking density (%)[a]	PEG1000			PEG2000		
	Unreacted	Single-end anchorage	Double-end anchorage	Unreacted	Single-end anchorage	Double-end anchorage
10	0.2	6.4	92.4	0.2	9.7	90.1
20	0.3	8.9	90.8	0.2	10.1	89.7
30	0.3	13.0	86.7	0.6	15.7	83.7
40	1.4	12.3	86.3	1.8	14.9	83.3
50	2.7	12.2	85.1	4.1	14.5	81.4

[a] Cross-linking density was theoretically calculated based on a 1% (w/w) hyaluronic acid solution and molecular weight of the repeat unit ($M_0 = 395$) and expressed as mol%.

Table 2
Cross-linking efficiency, swelling ratio, and elastic moduli of HA hydrogels cross-linked at various cross-linking densities

Theoretical cross-linking density (%)	PEG1000			PEG2000		
	Cross-linking efficiency	Swelling ratio	Elastic modulus	Cross-linking efficiency	Swelling ratio	Elastic modulus
10	92.4	153 ± 8	39.9 ± 6.7	90.1	172 ± 7	36.6 ± 3.6
20	90.8	97 ± 6	66.3 ± 4.1	89.7	108 ± 19	51.0 ± 4.2
30	86.7	121 ± 9	35.6 ± 1.1	83.7	284 ± 17	39.3 ± 1.8
40	86.3	232 ± 13	33.4 ± 3.1	83.3	332 ± 34	26.8 ± 4.3
50	85.1	274 ± 15	20.7 ± 3.4	81.4	309 ± 21	24.8 ± 3.1

ninhydrin reagent was added. The mixture was kept in boiling water for 20 min. After incubation, 75 ml of a water/ethanol mixture (1/1, v/v) was added and the reaction mixture was cooled to room temperature for 2 h in a dark place. Ninhydrin reacted with free amino groups and created a water-soluble blue compound. The amount of free amino groups in the hydrogel was determined by measuring the UV absorbance of the supernatant at 570 nm. Glycine was the reference molecule. The number of unreacted amino groups of PEG was determined in the hydrogels both before and after they were leached in water. The number of amino groups of the unleached hydrogels corresponds to single-end anchorage and unreacted amino groups of PEG. Subtraction of single-end anchorage and unreacted amino groups of PEG from the total amount of added amino groups reveals the extent of the effective cross-linking reaction. The number of amino groups in the leached hydrogels corresponds to single-end anchorage. Subtraction of single-end anchorage in the leached hydrogels from single-end anchorage and unreacted amino groups of PEG in the unleached hydrogels results in the amount of unreacted PEG-diamine that has not participated in the cross-linking reaction (Eiselt, Lee, & Mooney, 1999).

2.4. Equilibrium swelling properties

The cross-linked HA hydrogels were placed in deionized water at 25 °C for 3 days. Assuming that the network swells uniformly in all directions, the equilibrium swelling ratio can be defined as a ratio of the weight of swollen hydrogel to the weight of dry hydrogel. The weight of dry gel was determined by drying the gel in a vacuum at 50 °C.

2.5. Measurement of mechanical properties

Mechanical properties of the cross-linked HA hydrogels were determined using a mechanical tester (Instron, Model 5567, Instron Corp.). The cross-linked HA films were cut into disks (1.0 cm in radius) and kept in water at 25 °C for 3 days. Uniaxial compression tests were performed on the swollen HA hydrogels at 25 °C using a crosshead speed of 1.0 cm/min and a load cell of 100 N. Each compressive test was performed for less than 1 min to avoid loss of water during measurement. The experiments were performed in quintuplicate. The shear modulus (G) was obtained from the slope of a plot of elastic stress (σ) versus $\lambda - \lambda^{-2}$, where λ is the extension ratio of HA hydrogels (Eq. 1) (Treloar, 1975). All plots were linear over the range of strain covered and the ratio of E (elastic modulus) to G was approximately three in all cases. Therefore, it was assumed that HA hydrogels are rubbery materials. The number average molecular weight between the cross-links (M_{crl}) was calculated according to (Eq. 2).

$$\sigma = G\left(\lambda - \frac{1}{\lambda^2}\right) \tag{1}$$

$$M_{crl} = \frac{cRT}{G} \tag{2}$$

T is the temperature (298 K) at which the modulus was measured, c is the concentration of HA (g/m^3) in the cross-linking solution, and R is the gas constant (8.3145 J mol^{-1} K^{-1}).

2.6. In vitro degradation of cross-linked HA

The *in vitro* degradation of cross-linked HA hydrogels was performed by incubating the hydrogels with HAase at 37 °C. For *in vitro* degradation studies, the cross-linked HA hydrogels were cut into rectangles with dimensions of 0.5×0.5 cm^2, placed in a 200 μl solution of HAase (100 U/ml in 30 mM citric acid, 150 mM Na_2HPO_4, 150 mM NaCl, pH 6.3), and incubated at 37 °C with orbital agitation at 150 rpm. At various time points, the supernatant was withdrawn and fresh buffer was replenished. The collected supernatant was then diluted 100 times in distilled water saturated with benzoic acid and analyzed for uronic acid content according to the carbazole assay (Bitter & Muir, 1962) using D-glucuronic acid lactone as the standard. The percent degradation was calculated by dividing the amount of uronic acid released at a given time point by the final amount of uronic acid collected when the hydrogel was completely degraded.

2.7. Statistical analysis

All quantitative data were expressed as means ± standard deviation. Statistical analysis was performed with unpaired Student's t-tests using Instat Software (Instat 3.0, GraphPad Software Inc., San Diego, CA, USA). A value of $p < .05$ was considered statistically significant.

3. Results and discussion

3.1. Synthesis and characterization of cross-linked HA hydrogels

To examine how the molecular weight of the cross-linking molecules would affect the cross-linking efficiency of the HA hydrogels, HA was covalently cross-linked with either PEG1000 (molecular weight = 1000 Da) or PEG2000 (molecular weight = 2000 Da) (Fig. 1). The theoretical cross-linking density varied from 10% to 50%. Cross-linking density was calculated theoretically on the basis of the concentration of PEG added to the HA solution (Table 1). The cross-linking efficiency of HA was determined by quantifying the amount of unreacted PEG-diamine and the amount of single-end reacted PEG-diamine (Tables 1 and 2). The amino groups of PEG-diamine were covalently bonded to HA with an overall cross-linking efficiency of higher than 80% for both types of PEG-diamine.

Increasing the theoretical cross-linking density from 10% to 50% resulted in a decrease in the cross-linking efficiency of HA hydrogels cross-linked with either PEG1000 or PEG2000 (Table 2). This may be attributed to increased steric hindrance. Therefore, the accessibility of the PEG-diamine to the HA chain decreases as the theoretical cross-linking density increases.

3.2. Degree of swelling and mechanical properties

The swelling ratio of HA hydrogels cross-linked with PEG1000 or PEG2000 decreased as the theoretical cross-linking density increased from 10% to 20% (Table 2 and Fig. 2). In general, the swelling ratio of materials decreases as the degree of cross-linking increases (Treloar, 1975). However, increasing the cross-linking density from 20% to 40% resulted in increased swelling ratios (Fig. 2). This was likely due to an abundance of intramolecular cross-linking at high cross-linking densities.

The elastic moduli of the cross-linked HA hydrogels varied depending on the molecular weight of the cross-linking molecules (PEG-diamines) and the cross-linking densities (Table 2 and Fig. 3). The elastic modulus increased with increasing cross-linking density from 10% to 20%. Decreasing the molecular weight of the cross-linking molecules also caused the elastic moduli to increase. However, at cross-linking densities higher than 20%, the modulus gradually decreased regardless of the molecular weight of the PEG-diamine. This was likely due to a high degree of intramolecular cross-linking at high cross-linking densities.

Next, the effect of cross-linking density on M_{crl} was examined. Increasing the cross-linking density from 10% to 20% resulted in decreases in the M_{crl} of cross-linked HA hydrogels (Fig. 4), which is in accordance with the theory of rubber-elasticity (Treloar, 1975). However, the M_{crl} gradually increased as the cross-linking density of the HA hydrogel increased above 20%. This behavior is inconsistent with the theory of rubber elasticity. The theory is only applicable to systems of short cross-linking molecules (Eiselt et al., 1999). PEG is a flexible molecule and typically forms elastic gels. In this study, the elastic deformation

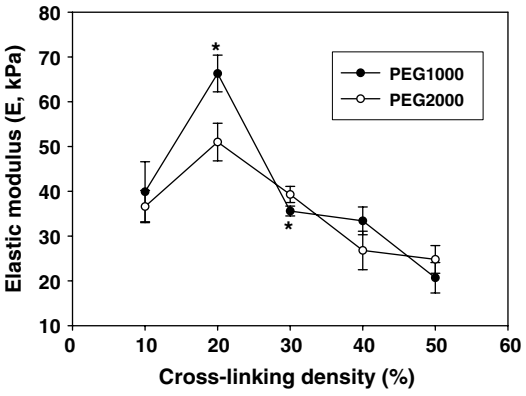

Fig. 3. Elastic modulus in compression of HA hydrogels cross-linked with PEG1000 or PEG2000 as a function of the theoretical cross-linking density. Values represent means ± standard deviation ($n = 5$). $*p < .05$ compared with PEG2000.

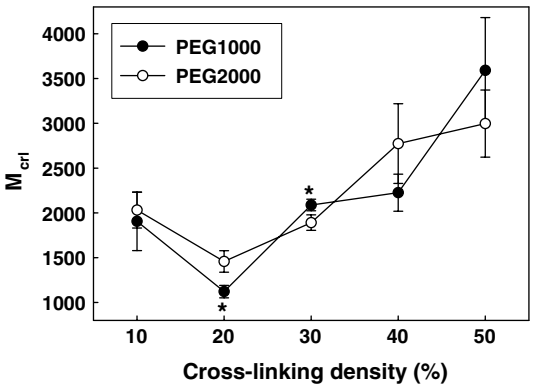

Fig. 4. The number average molecular weight between cross-links (M_{crl}) of HA hydrogels cross-linked with PEG1000 or PEG2000 as a function of the theoretical cross-linking density. Values represent means ± standard deviation ($n = 5$). $*p < .05$ compared with PEG2000.

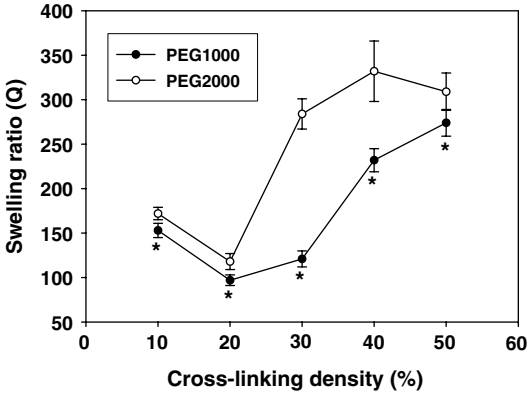

Fig. 2. Equilibrium swelling ratio of HA hydrogels cross-linked with PEG1000 or PEG2000. Values represent means ± standard deviation ($n = 5$). $*p < .05$ compared with PEG2000.

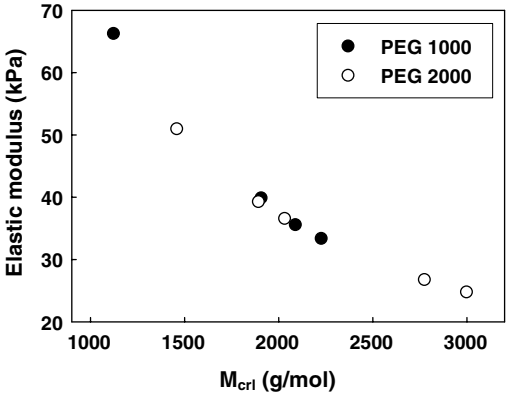

Fig. 5. The number average molecular weight between cross-links (M_{crl}) of HA hydrogels cross-linked with PEG1000 or PEG2000 as a function of the elastic modulus. Values represent mean ($n = 5$).

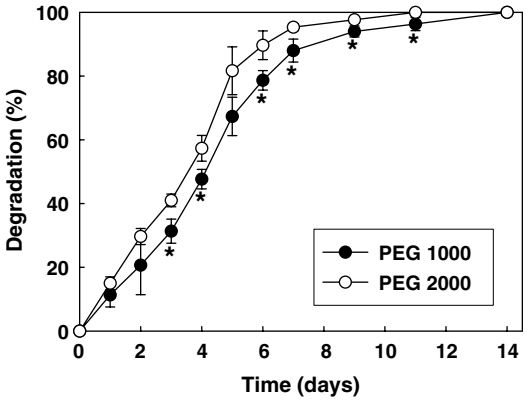

Fig. 6. *In vitro* degradation of cross-linked HA hydrogels at a theoretical cross-linking density of 20%. The degradation experiments were performed in a 100 U/ml hyaluronidase at 37 °C. Values represent means ± standard deviation ($n = 5$). $*p < .05$ compared with PEG2000.

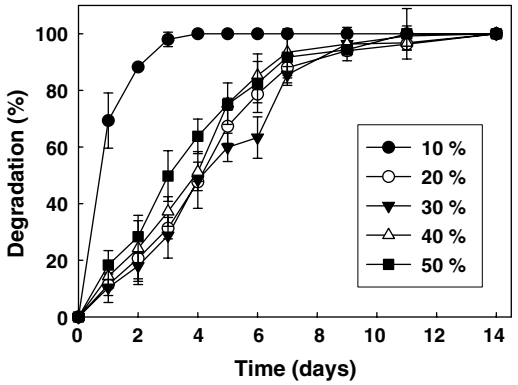

Fig. 7. *In vitro* degradation of HA hydrogels cross-linked with PEG1000 at various theoretical cross-linking densities. Values represent means ± standard deviation ($n = 5$).

observed during the compression of gels with high PEG content is likely caused by the less rigid PEG molecule being deformed rather than deformation of the stiff HA. There is currently no theory that adequately addresses the mechanical properties of hydrogels cross-linked with large molecules (Eiselt et al., 1999).

Elastic moduli decreased as the M_{crl} of cross-linked HA hydrogels increased (Fig. 5). Nearly identical elastic moduli were obtained from HA hydrogels cross-linked with molecules of different molecular weight (PEG1000 and PEG2000) (Fig. 5). This result suggests that mechanical properties of the cross-linked HA hydrogels are affected not by molecular weight of the cross-linking molecules but by M_{crl}.

3.3. Degradation of cross-linked HA hydrogels

To mimic *in vivo* conditions, the degradation of cross-linked HA hydrogel by HAse was examined. It was found to be dependent on molecular weight of the cross-linking molecule and cross-linking density. HA hydrogels cross-linked with PEG1000 degraded at a slower rate than those with PEG2000 (Fig. 6). The swelling ratios of HA hydrogels cross-linked with PEG2000 was higher than that of HA hydrogels cross-linked with PEG1000. The higher degradation rate of HA hydrogels cross-linked with PEG2000 is possibly due to increased interactions of the highly-swelled hydrogel with enzyme solution. As theoretical cross-linking density increased from 10% to 20%, the degradation rate of the cross-linked hydrogels decreased (Fig. 7). However, HA hydrogels with theoretical cross-linking densities of 20–50% had similar degradation rates, with complete degradation occurring after 14 days, which suggests that similar extents of interchain cross-links were present among HA hydrogels with theoretical cross-linking densities of 20–50%.

4. Conclusions

HA hydrogels were modified using covalent cross-linking with PEG-diamines of different molecular weights. The mechanical properties and degradation rates of the cross-linked HA hydrogels were controlled by varying the molecular weight of the cross-linking molecules and the cross-linking density. The HA hydrogels developed in this study have controllable mechanical properties and degradation rates, and may have broad biomedical applications, such as matrices for cell transplantation and drug delivery.

Acknowledgement

This work was supported by a grant (No. R01-2006-000-10647-0) from the Basic Research Program of the Korea Science & Engineering Foundation.

References

Bitter, T., & Muir, H. M. (1962). A modified uronic acid carbazole reaction. *Analytical Biochemisty, 4*, 330–334.

Brook, S. D. (1980). *In properties of biomaterials in physiological environment*. Boca Raton, FL: CRC Press.

Bulpitt, P., & Aeschlimann, D. (1999). New strategy for chemical modification of hyaluronic acid: preparation of functionalized derivatives and their use in the formation of novel biocompatible hydrogels. *Journal of Biomedical Materials Research, 47*, 152–169.

Campoccia, D., Doherty, P., Radice, M., Brun, P., Abatangelo, G., & Williams, D. F. (1998). Semisynthetic resorbable materials from hyaluronan esterification. *Biomaterials, 19*(23), 2101–2127.

de Goot, J. H., Zijlstra, F. M., Kuiipers, H. W., Pennings, A. J., Klompmaker, J., Veth, R. P. H., et al. (1997). Meniscal tissue regeneration in porous 50/50 copoly(L-lactide/ε-caprolactone) implants. *Biomaterials, 18*(8), 613–622.

Eiselt, P., Lee, K. Y., & Mooney, D. J. (1999). Rigidity of two-component hydrogels prepared from alginate and poly(ethylene glycol)-diamines. *Macromolecules, 32*, 5561–5566.

Guarda-Nardini, L., Tito, R., Staffieri, A., & Beltrame, A. (2002). Treatment of patients with arthrosis of the temporomandibular joint by infiltration of sodium hyaluronate: a preliminary study. *European Archives Otorhinolaryngology, 259*(5), 279–284.

Gwak, S. J., Choi, D. H., Paik, S. S., Lee, E. Y., Lee, K. S., & Kim, B. S. (2004). Stable hepatocyte transplantation using fibrin matrix. *Biotechnology Letters, 26*, 505–508.

Hall, C. L., & Turley, E. A. (1995). Hyaluronan: RHAMM mediated cell locomotion and signaling in tumorigenesis. *Journal of Neurooncology, 26*(3), 221–229.

Hoffman, A. S. (1975). In R. L. Kronenthal, Z. Oser, & E. Martin (Eds.), *Polymers in medicine and surgery* (pp. 33–44). New York: Plenum Press.

Huang, L., Cheng, Y. Y., Koo, P. L., Lee, K. M., Qin, L., Cheng, J. C., et al. (2003). The effect of hyaluronan on osteoblast proliferation and differentiation in rat calvarial-derived cell cultures. *Journal of Biomedical Material Research, 66*(4), 880–884.

Ingber, D., Karp, S., Plopper, G., Hansen, L., & Mooney, D. (1993). *Physical forces and the mammalian cell*. New York: Academic Press, Chapter 2.

Jeon, O., Ryu, S. H., Chung, J. H., & Kim, B. S. (2005). Control of basic fibroblast growth factor release from fibrin gel with heparin and concentrations of fibrinogen and thrombin. *Journal of Controlled Release, 105*, 249–259.

Juzych, M. S., Parrow, K. A., Shin, D. H., Swendris, R. P., & Ramocki, J. M. (1992). Adjunctive viscoelastic therapy for postoperative ciliary block. *Ophthalmic Surgery, 23*, 784–788.

Kong, H. J., Kaigler, D., Kim, K., & Mooney, D. J. (2004). Controlling rigidity and degradation of alginate hydrogels via molecular weight distribution. *Biomacromolecules, 5*, 1720–1727.

Luo, Y., Kirke, K. R., & Prestwich, G. D. (2000). Cross-linked hyaluronic acid hydrogel films: new biomaterials for drug delivery. *Journal of Controlled Release, 69*(1), 169–184.

Park, K., Shalaby, W. S. W., & Park, H. (1993). *Biodegradable hydrogels for drug delivery*. Lancaster: Technomic Pub.

Peppas, N. A., Bures, P., Leobandung, W., & Ichikawa, H. (2000). Hydrogels in pharmaceutical formulations. *European Journal of Pharmaceutics and Biopharmaceutics, 50*(1), 27–46.

Pouyani, T., & Prestwich, G. D. (1994). Functionalized derivatives of hyaluronic acid oligosaccharides-drug carriers and novel biomaterials. *Bioconjugate Chemistry, 5*, 339–347.

Ramamurthi, A., & Vesely, I. (2002). Smooth muscle cell adhesion on crosslinked hyaluronan gels. *Journal of Biomedical Material Research, 60*(1), 195–205.

Romberg, B., Metselaar, J. M., de Vringer, T., Motonaga, K., Kettenes-van den Bosch, J. J., Oussoren, C., et al. (2005). Enzymatic degradation of liposome-grafted poly(hydroxyethyl *L*-glutamine). *Bioconjugate Chemistry, 16*, 767–774.

Rosier, R. N., & O'Keefe, R. J. (2000). Hyaluronic acid therapy. *Instructional Course Lectures, 49*, 495–502.

Ryu, J. H., Kim, I. K., Cho, S. W., Cho, M. C., Hwang, K. K., Piao, H., et al. (2005). Implantation of bone marrow mononuclear cells using injectable fibrin matrix enhances neovascularization in infarcted myocardium. *Biomaterials, 26*, 319–326.

Sasaki, T., & Watanabe, C. (1995). Stimulation of osteoinduction in bone wound healing by high-molecular hyaluronic acid. *Bone, 16*(1), 9–15.

Shu, X. Z., Liu, Y., Luo, Y., Roberts, M. C., & Prestwich, G. D. (2002). Disulfide cross-linked hyaluronan hydrogels. *Biomacromolecules, 3*(6), 1304–1311.

Tomihata, K., & Ikada, Y. (1997). Preparation of cross-linked hyaluronic acid films of low water content. *Biomaterials, 18*(3), 189–195.

Treloar, L. R. G. (1975). *Physics of rubber elasticity;*. Oxford: Clarendon Press.

Vercruysse, K. P., Marecak, D. M., Marecek, J. F., & Prestwich, G. D. (1997). Synthesis and *in vitro* degradation of new polyvalent hydrazide cross-linked hydrogels of hyaluronic acid. *Bioconjugate Chemistry, 8*(5), 686–694.

Wernicke, M., Pineiro, L. C., Caramutti, D., Dorn, V. G., Raffo, M. M., Guixa, H. G., et al. (2003). Breast cancer stromal myxoid changes are associated with tumor invasion and metastasis: a central role for hyaluronan. *Modern Pathology, 16*(2), 99–107.

Yamamoto, M., Sakakibara, Y., Nishimura, K., Komeda, M., & Tabata, Y. (2003). Improved therapeutic efficacy in cardiomyocyte transplantation for myocardial infarction with release system of basic fibroblast growth factor. *Artificial Organs, 27*, 181–184.