Carbohydrate Polymers 88 (2012) 352–360

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Network of sodium hyaluronate with nano-knots junction of poly(amido amine) dendrimer

Toyoko Imae[a,b,*], Shin-ichi Hamaguchi[b]

[a] Graduate Institute of Applied Science and Technology and Department of Chemical Engineering, National Taiwan University of Science and Technology, 43 Keelung Road, Taipei 10607, Taiwan, ROC
[b] Graduate School of Science, Nagoya University, Chikusa, Nagoya 464-8602, Japan

ARTICLE INFO

Article history:
Received 17 October 2011
Received in revised form
18 November 2011
Accepted 13 December 2011
Available online 21 December 2011

Keywords:
Covalent-bonded network
Complexation
Sodium hyaluronate
Poly(amido amine) dendrimer
Coupling reaction
Bead & string network
Sodium poly-L-glutamate

ABSTRACT

Amine-terminated poly(amido amine) (PAMAM) dendrimers have been attached to sodium hyaluronates (NaHAs) by a coupling reaction. The morphology of NaHAs was varied from the common network to the bead & string network, which gave rise to the decrease in viscosity of NaHAs. The bead & string network was more abundant for the covalent network complex than the noncovalent one. The beads, that is, the nano-knots of the network consist of the covalent-bonded NaHA/dendrimer composites, and the strings are NaHA chains. Beads became small and strings decreased in number with decreasing a molecular weight of NaHA. The complexation of sodium poly-L-glutamates (NaPGAs) with PAMAM dendrimers was different in the manner from that of NaHAs with dendrimers. Flexible NaPGAs produced globular composites with dendrimers.

1. Introduction

A hyaluronic acid (HA) is a linear polysaccharide including D-glucuronic acid and N-acetyl-D-glucosamine in a repeating unit (Takayama et al., 1990). Importantly this polysaccharide is a major biomacromolecular component of the intercellular matrix in most connective tissues (e.g. eye vitreous humor, synovial fluid, and cartilage) (Myers, Negami, & White, 1966). Moreover, its unique physicochemical properties such as remarkable viscoelastic property and biocompatibility led the utilization of HA in the pharmaceutical and medical fields (Lim, Martin, Berry, & Brown, 2000; Nimrod, Ezra, Ezov, Nachum, & Parisada, 1992; Peyron, 1993; Smeds et al., 2001; Tirtaatmadja, Boger, & Fraser, 1984). Especially, recent pharmaceutical and medical researchers paid attention to the use of HA in drug delivery systems in addition to the therapeutic products in ophthalmology, dermatology, and osteoarthritis (Cho et al., 2003; Shu, Liu, Palumbo, & Prestwich, 2003). Incidentally, it has been reported that HAs formed the complexes with another biomaterials like liposomes, proteins, other sugars, graft polymers and DNA (Asayama, Nogawa, Takei, Akaike, & Maruyama, 1998; Burns et al., 1996; Yerushalmi, Arad, & Margalit, 1994).

On the other hand, a dendrimer is a highly branched synthetic architecture with many functional terminals. Especially, poly(amido amine) (PAMAM) dendrimer (Beezer et al., 2003; Esfand & Tomalia, 2001; Liu & Fréchet, 1999; Namazi & Adeli, 2005), one of typical dendrimers, has been focused on the interaction with biomacromolecules, such as DNA (Bielinska, Kukowska-Latallo, Johnson, Tomalia, & Baker, 1996; Chen, Turro, & Tomalia, 2000; Kukowska-Latallo et al., 1996; Mitra & Imae, 2004; Ottaviani et al., 1999; Planck, Mechtler, Szoka, & Wagner, 1996; Tang, Redemann, & Szoka, 1996), polypeptides (Imae & Miura, 2003; Leisner & Imae, 2003a, 2003b, 2004), and polysaccharides (Arima, Kihara, Hirayama, & Uekama, 2001; Kihara, Arima, Tsutsumi, Hirayama, & Uekama, 2002; Kihara, Arima, Tsutsumi, Hirayama, & Uekama, 2003). Such researches are valuable, because the PAMAM dendrimer is nontoxic and biocompatible, if adequate terminal groups are selected (Malik et al., 2000). A complexation of sodium hyaluronate (NaHA) with PAMAM dendrimer has been reported by one of authors and her coworkers (Imae, Hirota, Funayama, Aoi, & Okada, 2003). Then the motive force of the complexation was the electrostatic interaction between negatively charged carboxylates in NaHA and positively charged amino terminal groups in PAMAM dendrimer. In addition, the formation of the complex was played an important role by the hydrogen bonding between hydroxyl groups

---

* Corresponding author at: Graduate Institute of Applied Science and Technology, National Taiwan University of Science and Technology, 43 Keelung Road, Taipei 10607, Taiwan, ROC. Tel.: +886 2 2730 3627.
E-mail address: imae@mail.ntust.edu.tw (T. Imae).

0144-8617/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2011.12.015

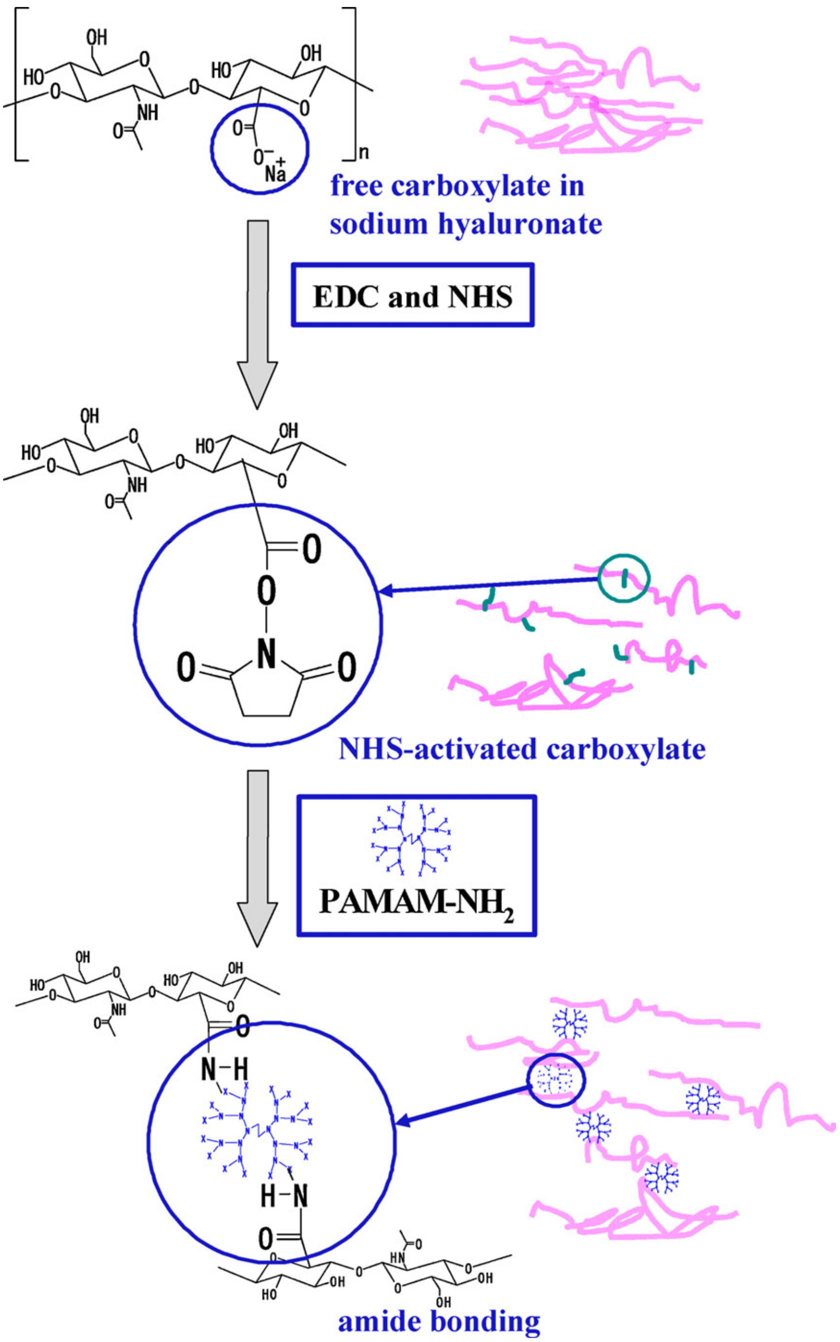

Fig. 1. Schematic illustration of coupling reaction between NaHA and dendrimer.

in NaHA and amide and amino groups in dendrimer so as to overcome the repulsive interaction between charged amino groups in dendrimers. However, on the utilization of the complex, the complex must be chemically stable so that it does not break apart under the variation of conditions (e.g. pH, temperature, ionic strength, etc.).

In the present work, amine-terminated dendrimers were immobilized on NaHAs by means of the chemical reaction, which is well known to form an amide bond (Sheehan, Cruickshank, & Boshart, 1961). By use of this procedure, PAMAM dendrimers were covalent-bonded. The covalent-bond increases the chemical stability of the complex against the variation of conditions, unlike the non-covalent-bonded complexation by only the electrostatic interaction. The composites were investigated by a viscometry and an atomic force microscopy, which enabled us to understand the behavior of the complex both in aqueous solution and on substrate. Moreover, the difference between covalent-bonded and electrostatic (non-covalent-bonded) complexation and the dependence on molecular weight of NaHA were discussed, and the composites were also compared with those of sodium poly-L-glutamate (NaPGA) with dendrimer.

2. Experimental

2.1. Materials

NaHA (repeating unit mass = 401.3) was donated from Shiseido Co., Japan. Among three samples with different molecular weights (1.0, 10 and 20 × 10^5), a sample with the largest molecular weight was mostly used except the examination on the dependency of molecular weight. NaPGA (repeating unit mass = 151.3) was a product from Peptide Institute Inc., Japan. N-hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC), and second generation PAMAM dendrimer with NH_2 terminals (molecular weight = 3256; 16 terminal groups) were purchased from Aldrich Chemical Co. Water was distilled, further purified using a Millipore Milli-Q apparatus, and used throughout the experiments.

2.2. Coupling reaction between NaHA and dendrimer

The procedure of a covalent-crosslinkage between NaHA and dendrimer is schematically illustrated in Fig. 1. The crosslinkage was prepared in accordance with the well-known coupling reaction (Yamazaki & Imae, 2005; Yamazaki et al., 2005): equimolar coupling reagents, EDC and NHS, were added to an aqueous NaHA solution. Then an EDC-activated carboxylate group in NaHA reacted with NHS to form a stable NHS carboxylate. After that, an aqueous dendrimer solution was mixed with a reaction solution, and then the NHS-carboxylate was combined with the terminal amino group of dendrimer. The molar concentration of dendrimer was equal to that of coupling reagents. A concentration of NaHA in the reaction solution was varied like 0.004 wt% (concentration of the repeating unit = 0.1 mM), 0.008 wt% or 0.04 wt%. Then a molar ratio of NaHA residue and dendrimer [NaHA residue/dendrimer] changed.

2.3. Analyses

Dynamic light scattering was measured at 25 °C on an Otsuka Electronics DLS-700 spectrophotometer equipped with an Ar ion laser at a wavelength of 488 nm and loaded an ALV-5000 correlator. The analysis was carried out using Contin method. The apparent diffusion coefficients of NaHA were determined. The viscosity measurements were carried out using an Ubbelohde viscometer in a temperature-regulated water bath (25 °C). A Digital Instruments NanoScope III microscope was used to take atomic force microscopic (AFM) images at room temperature (∼25 °C). Spin-coated films on mica substrates were prepared by spinning solutions for 1 min at 2000 rpm using a spinner Model K-359SD-1 (Kyowa Riken Co., Japan). Dialysis of an aqueous NaHA solution was carried out by using a Visking seamless cellulose tube in excess water (four times over 48 h with each 1000 cm^3 of water).

3. Results and discussion

3.1. Effect of complexation on viscosities of aqueous NaHA solutions

NaHA molecules are estimated to overlap each other at high concentrations. Then an overlapping threshold concentration (c^*) of an aqueous NaHA solution is evaluated from a plot of apparent diffusion coefficient D as a function of NaHA concentration c_{NaHA}. As seen in Fig. 2(A), the apparent diffusion coefficients decreased initially and increased through a minimum with increasing NaHA concentration. Thus a concentration (around 0.06 wt%) at minimum D value was assigned to an overlapping threshold concentration, which is a threshold from “dilute solutions” to “semi-dilute solutions” (de Gennes, 1979; Imae & Ikeda, 1986).

Fig. 2(B) plots the reduced viscosity η_{red} against NaHA concentration. The viscosity of NaHA solutions (NaHA residue/dendrimer) = [1/0]) was proportional to NaHA concentration below 0.04 wt% but deviated from a linearity above it. This indicates that the NaHA solutions below 0.04 wt% are in the category of “diluted solutions”, which was determined from the apparent diffusion coefficients (Fig. 2(A)). Therefore, the NaHA concentrations below 0.04 wt% were chosen for the preparation of composites with dendrimers unless otherwise noted.

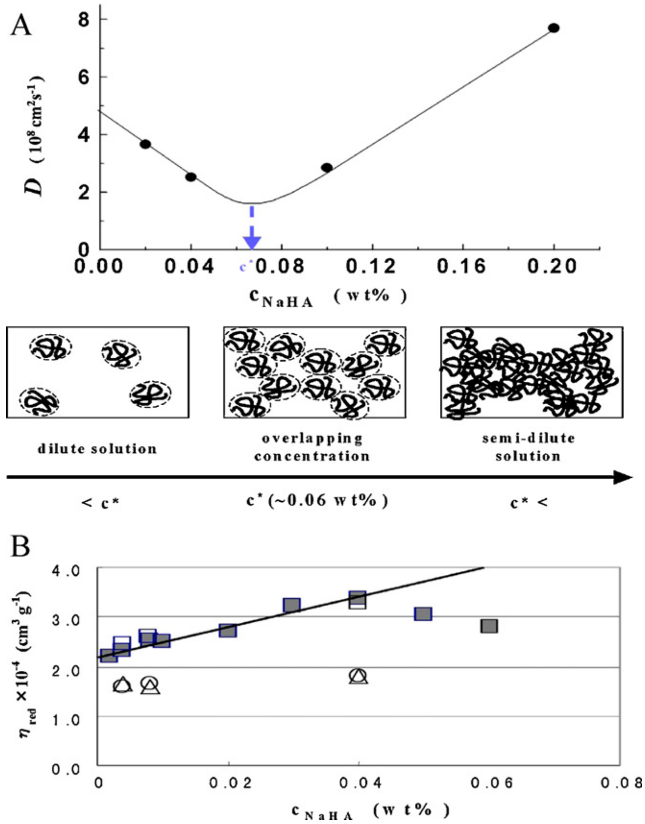

Fig. 2. (A) Apparent diffusion coefficients D of aqueous NaHA solutions as a function of NaHA concentration c_{NaHA}. c^* is an overlap threshold concentration. (B) Reduced viscosities η_{red} of aqueous NaHA solutions with and without coupling reaction with dendrimers as a function of NaHA concentration c_{NaHA}. [NaHA residue/dendrimer]: closed square, [1/0]; open square, [1/0.001] with coupling reaction; open circle, [1/0.01] with coupling reaction; open triangle, [1/0.01] without coupling reaction.

The reduced viscosities of aqueous NaHA solutions with and without coupling reaction with dendrimers are included in Fig. 2(B). While there was no change in the viscosity behavior of dendrimer-immobilized NaHA at a ratio of [1/0.001] in comparison with that at [1/0], drastic decrease occurred at [1/0.01]. This implies that NaHA molecules shrink by the reaction with much amount of dendrimers, namely, the linkage with dendrimer affects the morphology of NaHA. On the other hand, the viscosities of mixture at [1/0.01] without coupling reaction were same as those with coupling reaction. It can be noted that the decrease of viscosity is relative to the ratio of dendrimer to NaHA but independent of the fact whether the composite is networked by the covalent bond or not.

3.2. Morphology of NaHA before and after coupling reaction

The effect of coupling reaction can be confirmed by means of the morphological observation by AFM. Figs. 3 and 4 show AFM images of spin-coated films prepared from aqueous NaHA solutions at 0.004 and 0.04 wt%, respectively, by comparison between complexes without and with coupling reaction. NaHA molecules formed network domains with monomolecular and multimolecular thicknesses for solutions at 0.004 and 0.04 wt%, respectively, according to their section analyses. Because of intermolecular hydrogen bonding, NaHA molecules attract each other to form domains, which were dispersed as network films on a substrate, if the solution is dilute. Larger amount of residues in NaHA molecules were intermolecularly hydrogen-bonded in a concentrated solution, producing larger network domains in comparison with those in a dilute solution.

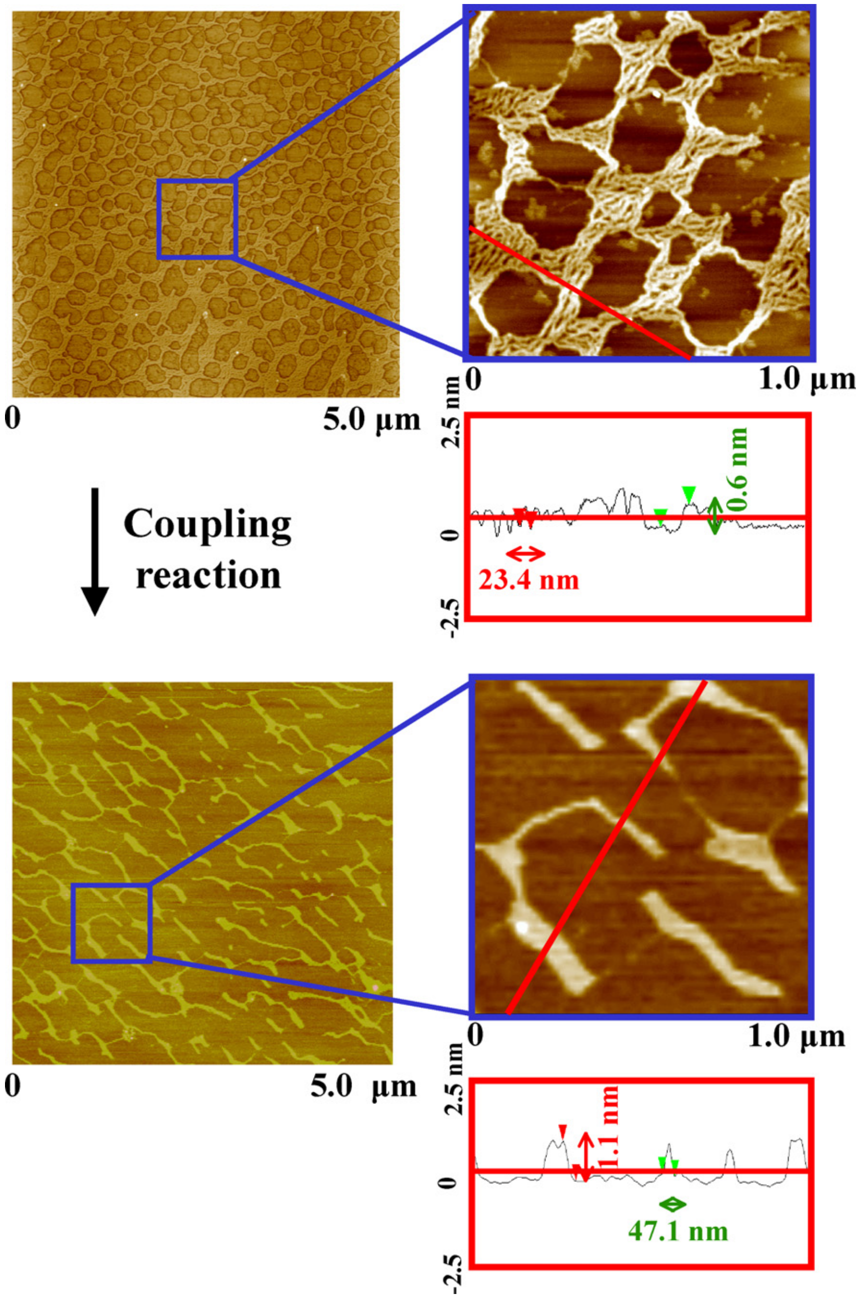

Fig. 3. AFM images and section analyses of NaHA and its coupling-reacted composite with dendrimer at a ratio of [1/0.01]. NaHA concentration = 0.004 wt%.

A drastic change happened, when NaHA molecules in solutions at both low and high NaHA concentrations suffered the coupling reaction with dendrimers at [1/0.01]. As seen in Fig. 3, NaHA domains in a spin-coated film prepared from a 0.004 wt% solution changed to zones with two-fold thickness of monolayer. It was assumed that the zones became dense by strong intermolecular linkages between NaHAs through dendrimers, since the separation between NaHA strings in the zone was not found any more, different from a case of NaHAs free from the cooperation of dendrimers. On the other hand, on a film from a 0.04 wt% solution (Fig. 4), large spherical beads (with about 10 nm height and submicron width) were linked by strings. It can be mentioned that a lot of dendrimers and NaHA molecules form large globules or aggregates after the coupling reaction, and the remaining (free) NaHA chains connects globules (or aggregates).

3.3. *Effects of covalent network and dialysis on morphology of NaHA–dendrimer composites*

Fig. 5 shows an AFM image of a NaHA–dendrimer composite without the coupling reaction (NaHA concentration = 0.04 wt%, a ratio = [1/0.01]). Large globular beads and the strings between them were confirmed. The texture is apparently similar to that of the composite with the coupling reaction (Fig. 4) but the numbers of beads and strings are apparently less, although this difference is not sensitive in viscosity behavior as described above. These results suggest that the electrostatic binding between dendrimers and NaHAs is less abundant than the covalent-bonding between them, indicating the high effectivity of the coupling reaction. Moreover, the formation of globular beads consisting of NaHA–dendrimer composites both with and without coupling reaction gives rise to the decrease in viscosity of NaHA, as seen in Fig. 2(B).

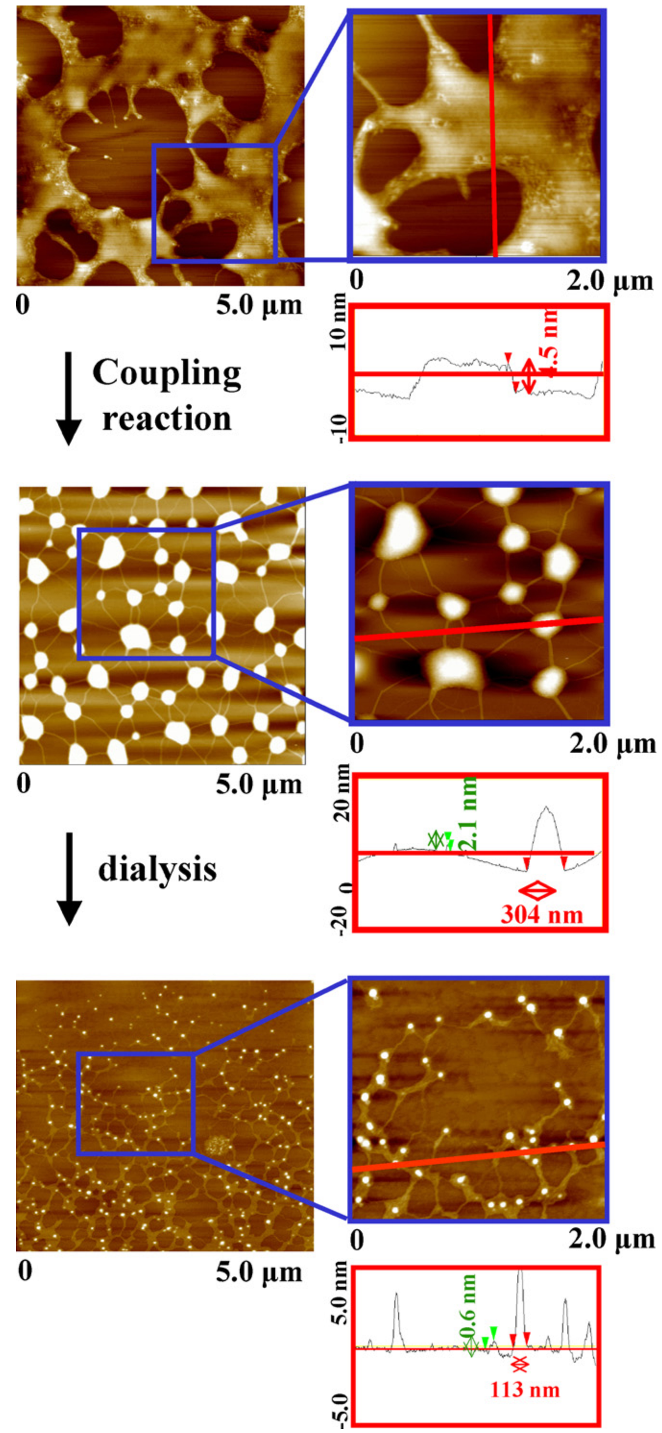

Fig. 4. AFM images and section analyses of NaHA, its coupling-reacted composite with dendrimer at a ratio of [1/0.01], and the dialyzed composite. NaHA concentration = 0.04 wt%.

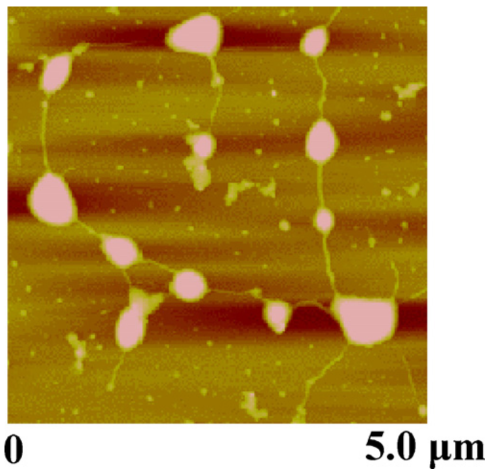

Fig. 5. An AFM image of a noncoupling-reacted composite of HaNA and dendrimer at [1/0.01]. NaHA concentration = 0.04 wt%.

An AFM image of a spin-coated film from a NaHA–dendrimer composite (NaHA concentration = 0.04 wt%, a ratio = [1/0.01], with the coupling reaction) after dialysis is compared to that before dialysis in Fig. 4. The large beads, which were found after the coupling reaction, became smaller after dialysis, although the network texture through strings remained. Instead, small aggregates appeared on the network as nano-knots of the network. This indicates that dendrimers, free from covalent-bonding or bound electrostatically, were removed from the large beads. Then the nano-knots consist of covalent-bonded HaNA–dendrimer composites. The scheme in Fig. 6 illustrates the changing morphologies of NaHA and its composites on the process of coupling reaction and dialysis.

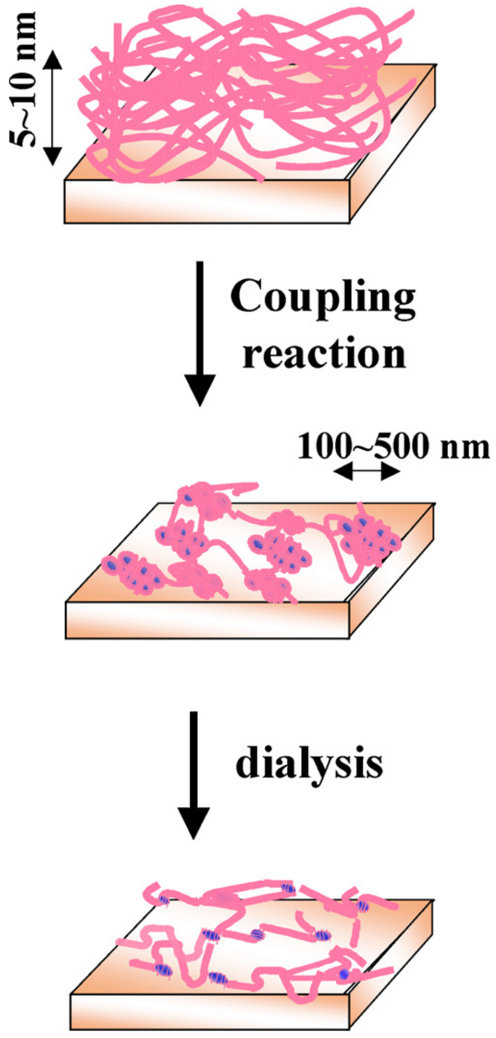

Fig. 6. Schematic illustration of NaHA, its coupling-reacted composite with dendrimer at a ratio of [1/0.01], and the dialyzed composite on substrate. NaHA concentration = 0.04 wt%.

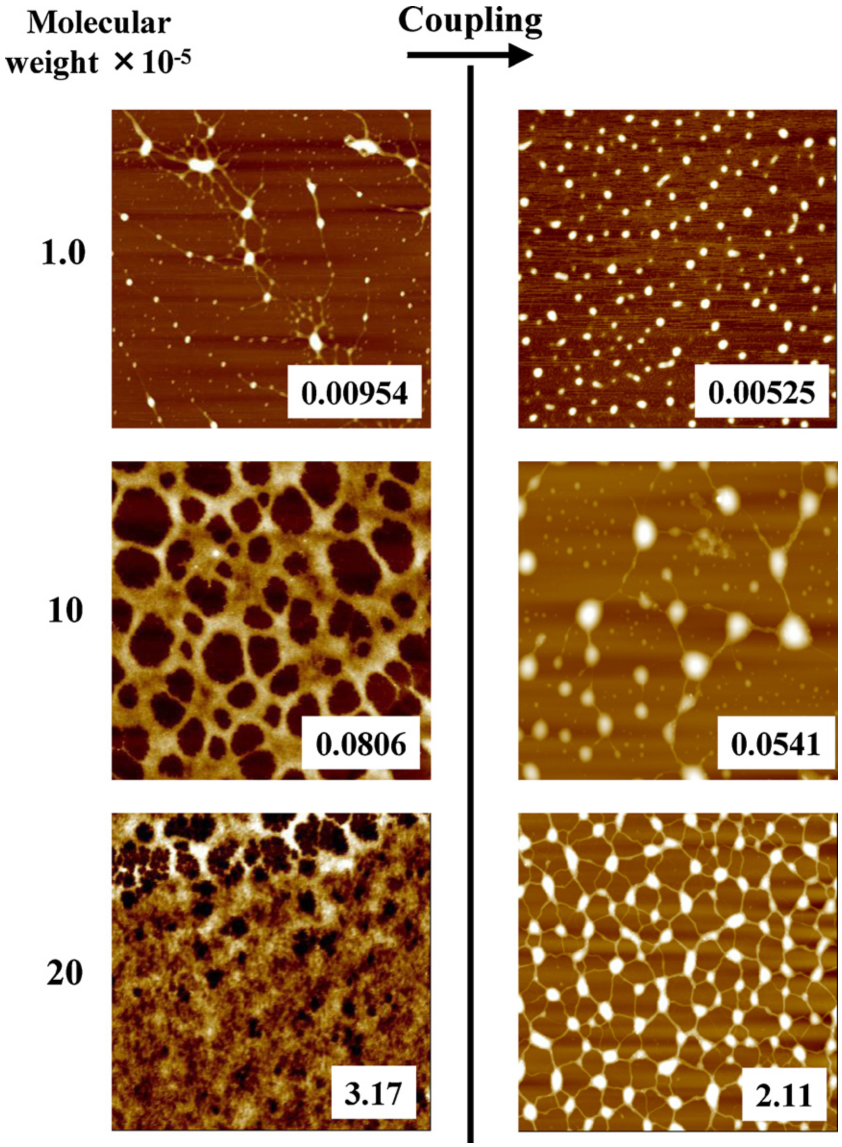

Fig. 7. AFM images of three NaHAs with different molecular weights and their coupling-reacted composites with dendrimer at [1/0.01]. NaHA concentration = 0.04 wt%. Size: 5 μm × 5 μm. Inset: numerical values of reduced viscosities η_{red}.

3.4. Effect of NaHA size on complexation

For three NaHAs with different molecular weights (molecular weight = 1.0×10^5, 1.0×10^6, 2.0×10^6), which were lower than that used in the examination described above (molecular weight = 3.0×10^6), viscosities and AFM images before and after the coupling reaction were compared in Fig. 7. The viscosity η_{red} was in same order between NaHAs of molecular weights 3.0×10^6 and 2.0×10^6, but it drastically decreased with further decrease in molecular weight. Especially, the viscosity of NaHA with the lowest molecular weight (1.0×10^5) was as low as that of solvent (water). Although same tendency on molecular weight dependence of viscosity was preserved even after the coupling reaction with dendrimers, the viscosity after the coupling reaction was always smaller than that before the coupling reaction, as well as a case of the highest molecular weight (3.0×10^6) NaHA (see Fig. 2). It can be noted that regardless of the molecular weight, NaHA in an aqueous solution shrank by the coupling reaction with dendrimer so as to decrease the viscosity.

In AFM images of pristine NaHAs (Fig. 7), the common network structures were visualized for three NaHAs of different molecular weights as well as a case of the highest molecular weight (3.0×10^6) NaHA (Fig. 4). With decreasing molecular weight, the texture changed from the network of large scale with multimolecular thickness to the network of small scale with small-size mesh and monomolecular thickness. The network of the lowest molecular weight (1.0×10^6) NaHA consisted of beads (knots) and strings. It is assumed that the intermolecular interaction of high molecular weight NaHAs is too stronger to overcome the centrifugal force of spin-coating than that of low molecular weight NaHAs.

After the coupling reaction, string-crosslinking beads were observed for all NaHAs of different molecular weights, indicating that the NaHA molecules were coiled by the involvement of dendrimers. The network consisting of beads (aggregates) and strings by a high molecular weight (2.0×10^6) NaHA was in the similar manner to a case of the highest molecular weight (3.0×10^6) NaHA (Fig. 4), but the former bead size was smaller than the latter one. The size of beads and the number of strings became further small with decreasing molecular weight of NaHA. These results are in consistency with the viscosity behavior. It is inferred that NaHA molecules with molecular weight below 1.0×10^6 are too short to

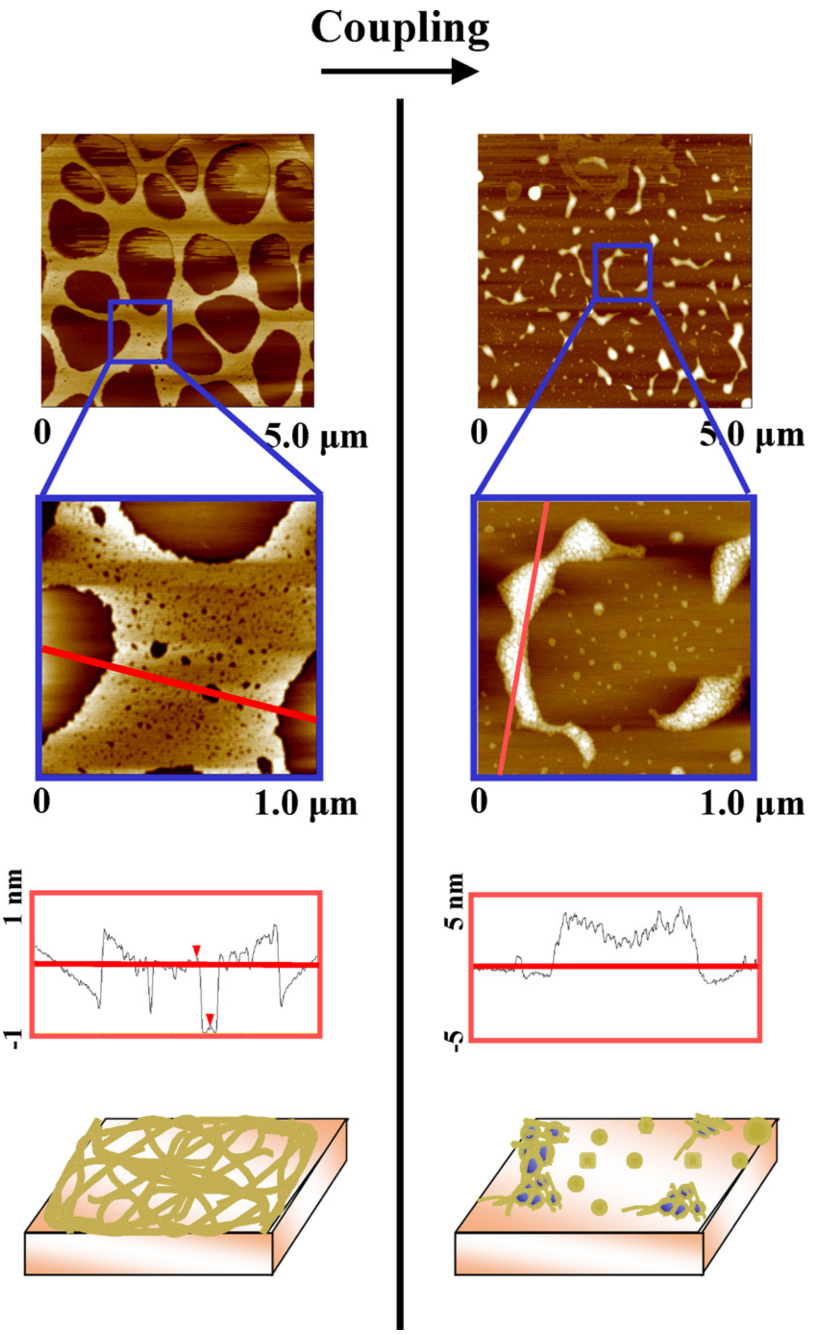

Fig. 8. AFM images, section analyses, and schematic illustration of NaPGA and its coupling-reacted composite with dendrimer at [1/0.01]. NaPGA concentration = 0.04 wt%.

form the network of NaHA–dendrimer beads connected by NaHA strings.

3.5. Comparison with the complexation of sodium poly-L-glutamate with dendrimer

The complexation of biomimetic polymer, NaPGA, with amine-terminated PAMAM dendrimer in water occurs in different manner from a case of the complexation between NaHA and dendrimer (Imae & Miura, 2003). Since NaPGA possesses carboxyl group in the repeating unit as well as NaHA, the motive force of the interaction with dendrimer is the electrostatic attraction between carboxyl group of NaPGA and amine group of dendrimer, but the hydrogen bonding is not expected. Another difference from NaHA–dendrimer composites is the fact that flexible NaPGA chains can penetrate into the interior of dendrimer. This suggests the easy production of globular composites.

In the present work, dendrimers were electrostatically combined on NaPGA and then immobilized by the coupling reaction. An AFM image after the coupling reaction was compared to the result for pristine NaPGA at same conditions, as shown in Fig. 8. The pristine NaPGA molecules formed network domain with many pinholes on a solid substrate. The height (~1 nm) of the flat network indicated that the network sheet consisted of a mono- or bi-molecular layer. Then it is supposed that the network is constructed by the condensed entanglement of NaPGA.

The morphology of a NaHA film remarkably changed after the reaction with dendrimers. In this case, the islands (zones) with various shapes existed, and their heights (~4 nm) were about four times as high as that of pristine NaPGA, indicating that NaPGAs formed complexes with dendrimers. Moreover, it should be noticed that there are many spherical particles besides large polymorphic zones in the NaPGA–dendrimer composite system (Fig. 8). Particles will be complexes consisting of some NaPGAs and some dendrimers, and large zones will be the aggregates of such complexes or the grown complexes. Similar globules were visualized on a transmission electron microscopic image of a NaPGA–dendrimer mixture without the coupling reaction (Imae & Miura, 2003). These characteristics of NaPGA–dendrimer composites at a 0.04 wt% NaPGA concentration are comparable to those of NaHA–dendrimer composites at the lower NaHA concentration (0.004 wt%) but not at the higher NaHA concentration (0.04 wt%), namely, a concentration corresponding to the examination of NaPGA. This supports that flexible NaPGAs are easy to produce globular composites with dendrimers without bridging of extended NaPGAs.

4. Conclusion

In the present work, NaHAs and PAMAM dendrimers have been covalent-crosslinked using a coupling reaction and characteristics of the composites have been investigated. Increasing the ratio of PAMAM dendrimer to NaHA caused decreasing viscosity of the composite, which is irrespective of the coupling reaction. An AFM image on a spin-coated film of NaHA–dendrimer composite at a high NaHA concentration showed drastic change from the common network sheet structure of pristine NaHA to the bead–string network structure of the composites, where beads were crosslinked by strings. The bead–string network became more abundant by the coupling reaction between NaHAs and dendrimers than by only the electrostatic binding between them, indicating the high effectivity of the covalent-network. Furthermore, it was confirmed by the dialysis that there were nano-knots consisting of covalent-bonding between NaHAs and dendrimers in the network structure. Effect of NaHA size on the morphology of composites was investigated, and it was suggested that the length of NaHA molecule played an important role in the size of beads consisting of NaHA–dendrimer composite as well as in the number of strings connecting the beads. It was inferred from the availment of NaPGA instead of NaHA that the aggregates of NaPGA and dendrimer were globules without cross-linkages by strings. This difference on the morphology of composites is ascribed to the conformational characters of flexible NaPGA and semiflexible NaHA (Cleland, 1984; Ghosh, Li, Reed, & Reed, 1990; Imae et al., 2003). It is suggested that the results of the present work can be applied to other linear polymers possessing carboxyl group in the repeating unit, in order to control viscosity and morphology of polymer network. Moreover, the combination of NaHA and PAMAM dendrimer is valuable as biomaterials and applicable as drug delivery systems and biosensors, because both polymers are biocompatible.

Acknowledgements

We are grateful to Shiseido Co. for generous donation of NaHAs, which were key materials of the present work. We also acknowledge Mr. Daisuke Onoshima, Nagoya University, for his kind help on the viscosity measurements.

References

Arima, H., Kihara, F., Hirayama, F., & Uekama, K. (2001). Enhancement of gene expression by polyamidoamine dendrimer conjugates with α-, β-, and γ-cyclodextrins. *Bioconjugate Chemistry*, *12*, 476–484.

Asayama, S., Nogawa, M., Takei, Y., Akaike, T., & Maruyama, A. (1998). Synthesis of novel polyampholyte comb-type copolymers consisting of a poly(L-lysine) backbone and hyaluronic acid side chains for a DNA carrier. *Bioconjugate Chemistry*, *9*, 476–481.

Beezer, A. E., King, A. S. H., Martin, I. K., Mitchel, J. C., Twyman, L. J., & Wain, C. F. (2003). Dendrimers as potential drug carriers: Encapsulation of acidic hydrophobes within water soluble PAMAM derivatives. *Tetrahedron*, *59*, 3873–3880.

Bielinska, A. U., Kukowska-Latallo, J. F., Johnson, J., Tomalia, D. A., & Baker, J. R., Jr. (1996). Regulation of *in vitro* gene expression using antisense oligonucleotides or antisense expression plasmids transfected using starburst PAMAM dendrimers. *Nucleic Acids Research*, *24*, 2176–2182.

Burns, J. W., Skinner, K., Colt, M. J., Burgess, L., Rose, R., & Diamond, M. P. (1996). A hyaluronate based gel for the prevention of postsurgical adhesions: Evaluation in two animal species. *Fertility and Sterility*, *66*, 814–821.

Chen, W., Turro, N. J., & Tomalia, D. A. (2000). Using ethidium bromide to probe the interactions between DNA and dendrimers. *Langmuir*, *16*, 15–19.

Cho, K. Y., Chung, T. W., Kim, B. C., Kim, M. K., Lee, J. H., Wee, R. W., et al. (2003). Release of ciprofloxacin from poloxamer-graft-hyaluronic acid hydrogels in vitro. *International Journal of Pharmaceutics*, *260*, 83–91.

Cleland, R. L. (1984). Viscometry and sedimentation equilibrium of partially hydrolyzed hyaluronate: Comparison with theoretical models of wormlike chains. *Biopolymers*, *23*, 647–666.

de Gennes, P. G. (1979). *Scaling concepts in polymer physics*. Ithaca, London: Cornell Univ. Press.

Esfand, R., & Tomalia, D. A. (2001). A dimensionally scaled comparison of a series of PAMAM dendrimers with a variety of proteins, a typical lipid bilayer membrane and DNA. *Drug Discovery Today*, *6*, 427–436.

Ghosh, S., Li, X., Reed, C. E., & Reed, W. F. (1990). Apparent persistence lengths and diffusion behavior of high molecular weight hyaluronate. *Biopolymers*, *30*, 1101–1112.

Imae, T., & Ikeda, S. (1986). Sphere-rod transition of micelles of tetradecyltrimethylammonium halides in aqueous sodium halide solutions and flexibility and entanglement of long rodlike micelles. *Journal of Physical Chemistry*, *90*, 5216.

Imae, T., & Miura, A. (2003). Binding of poly(amido amine) dendrimer on sodium poly-L-glutamate in aqueous NaCl solution. *Journal of Physical Chemistry B*, *107*, 8088–8092.

Imae, T., Hirota, T., Funayama, K., Aoi, K., & Okada, M. (2003). Binding of poly(amido amine) dendrimer to sodium hyaluronate in aqueous NaCl solution. *Journal of Colloid and Interface Science*, *263*, 306–311.

Kihara, F., Arima, H., Tsutsumi, T., Hirayama, F., & Uekama, K. (2002). Effects of structure of polyamidoamine dendrimer on gene transfer efficiency of the dendrimer conjugate with α-cyclodextrin. *Bioconjugate Chemistry*, *13*, 1211–1219.

Kihara, F., Arima, H., Tsutsumi, T., Hirayama, F., & Uekama, K. (2003). In vitro and in vivo gene transfer by an optimized α-cyclodextrin conjugate with polyamidoamine dendrimer. *Bioconjugate Chemistry*, *14*, 342–350.

Kukowska-Latallo, J. F., Bielinska, A. U., Johnson, J., Spindler, R., Tomalia, D. A., & Baker, J. R., Jr. (1996). Efficient transfer of genetic material into mammalian cells using starburst polyamidoamine dendrimers. *Proceedings of the National Academy of Sciences of the United States of America*, *93*, 4897–4902.

Leisner, D., & Imae, T. (2003a). Interpolyelectrolyte complex and coacervate formation of poly(glutamate acid) with dendrimer studied by light scattering and SAXS. *Journal of Physical Chemistry B*, *107*, 8078–8087.

Leisner, D., & Imae, T. (2003b). Polyelectolyte behavior of an interpolyelectrolyte complex formed in aqueous solution of a charged dendrimer and sodium poly(L-glutamate). *Journal of Physical Chemistry B*, *107*, 13158–13167.

Leisner, D., & Imae, T. (2004). Structural evolution of an interpolyelectrolyte complex of charged dendrimers interacting with poly(L-glutamate). *Journal of Physical Chemistry B*, *108*, 1798–1804.

Lim, S. T., Martin, G. P., Berry, D. J., & Brown, M. B. (2000). Preparation and evaluation of the in vitro drug release properties and mucoadhesion of novel microspheres of hyaluronic acid and chitosan. *Journal of Controlled Release*, *66*, 281–292.

Liu, M., & Fréchet, J. M. J. (1999). Designing dendrimers for drug delivery. *Pharmaceutical Science & Technology Today*, *2*, 393–401.

Malik, N., Wiwattanapatapee, R., Klopsch, R., Lorenz, K., Frey, H., Weener, J. W., et al. (2000). Dendrimers: Relationship between structure and biocompatibility in vitro, and preliminary studies on the biodistribution of I-125-labelled poly(amidoamine) dendrimers in vivo. *Journal of Controlled Release*, *65*, 133–148.

Mitra, A., & Imae, T. (2004). Nanogel formation consisting of DNA and poly(amido amine) dendrimer studied by static light scattering and atomic force microscopy. *Biomacromolecules*, *5*, 69–73.

Myers, R. R., Negami, S., & White, R. K. (1966). Dynamic mechanical properties of synovial fluid. *Biorheology*, *3*, 197–209.

Namazi, H., & Adeli, M. (2005). Dendrimers of citric acid and poly (ethylene glycol) as the new drug-delivery agents. *Biomaterials*, *26*, 1175–1183.

Nimrod, A., Ezra, E., Ezov, N., Nachum, G., & Parisada, B. J. (1992). Absorption distribution, metabolism, and excretion of bacteria-derived hyaluronic acid in rats and rabbits. *Journal of Ocular Pharmacology*, *8*, 161–172.

Ottaviani, M. F., Sacchi, B., Turro, N. J., Chen, W., Jockusch, S., & Tomalia, D. A. (1999). An EPR study of the interactions between starburst dendrimers and polynucleotides. *Macromolecules*, *32*, 2275–2282.

Peyron, J. G. (1993). Intraarticular hyaluronan injections in the treatment of osteoarthritis: State-of-the-art review. *Rheumatology*, *20*, 10–15.

Planck, C., Mechtler, K., Szoka, F. C., & Wagner, E. (1996). Activation of the complent system by synthetic DNA complexs: A potential barrier for intravenous gene delivery. *Human Gene Therapy*, *7*, 1437–1466.

Sheehan, C. J., Cruickshank, P. A., & Boshart, G. L. (1961). Notes—A convenient synthesis of water-soluble carbodiimides. *Journal of Organic Chemistry*, *26*, 2525–2528.

Shu, X. Z., Liu, Y., Palumbo, F., & Prestwich, G. D. (2003). Disulfide-crosslinked hyaluronan–gelatin hydrogel films: A covalent mimic of the extracellular matrix for in vitro cell growth. *Biomaterials*, *24*, 3825–3834.

Smeds, K. A., Pfister-Serres, A., Miki, D., Dastgheib, K., Inoue, M., Hatchell, D. L., et al. (2001). Photocrosslinkable polysaccharides for in situ hydrogel formation. *Journal of Biomedical Materials Research*, *54*, 115–121.

Takayama, K., Hirata, M., Machida, Y., Masada, Y., Sannan, T., & Nagai, T. (1990). Effect of interpolymer complex formation on bioadhesive property and drug release phenomenon of compressed tablet consisting of chitosan and sodium hyaluronate. *Chemical & Pharmaceutical Bulletin*, *38*, 1993–1997.

Tang, M. X., Redemann, C. T., & Szoka, F. C., Jr. (1996). *In vitro* gene delivery by degraded polyamidoamine dendrimers. *Bioconjugate Chemistry*, *7*, 703–714.

Tirtaatmadja, V., Boger, D. V., & Fraser, J. R. E. (1984). The dynamic and steady shear properties of synovial fluid and of the components making up synovial fluid. *Rheologica Acta*, *23*, 311–321.

Yamazaki, T., & Imae, T. (2005). Preparation of dendrimer SAM on Au substrate and adsorption/desorption of poly-L-glutamate on the SAM. *Journal of Nanoscience and Nanotechnology*, *5*, 1066.

Yamazaki, T., Imae, T., Sugimura, H., Saito, N., Hayashi, K., & Takai, O. (2005). Photolithographic patterning of dendrimer monolayers and pattern-selective adsorption of linear macromolecules. *Journal of Nanoscience and Nanotechnology*, *5*, 1792.

Yerushalmi, N., Arad, A., & Margalit, R. (1994). Molecular and cellular studies of hyaluronic acid-modified liposomes as bioadhesive carriers for topical drug delivery in wound healing. *Archives of Biochemistry and Biophysics*, *313*, 267–273.