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A gel-forming poly-L-guluronic acid produced from no guluronate-rich marine algae using new hydrolysis method: test for endovascular embolization

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Abstract To prepare a gel-forming poly-L-guluronic acid (Poly-G) from no guluronate-rich *Laminaria japonica*, a new hydrolysis method was employed with a lower HCl concentration (0.025–0.15 M) and a shorter treatment time (5 min). The Poly-Gs were set to measure purity, presence of poly-L-guluronic block, molecular weight distribution, polymer yield, viscosity, and compressive gel strength. Finally, the Poly-G was tested to embolize the renal vascular system by using a rabbit model and angiography. Optimized Poly-G could be selected with respect to wt% concentration, polymer yield, gel-forming stability, viscosity, and gel strength as an endovascular embolizing agent. Overall, 0.4–0.6% of 0.03 M-Poly-G obtained from acid treatment with 0.03 M of HCl had molecular weights greater than 80 kDa, and the best gelling capacity with an injectable viscosity (30–120 cP). It was successfully delivered into the vascular bed of a rabbit kidney and was shown angiographically to embolize the renal vascular system.

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1 Introduction

Alginic acids are heteropolysaccharides composed of varying ratios of β-D-mannuronic (M) and α-L-guluronic acid (G) residues. Generally, the monomers form a block copolymer with homopolymeric regions of poly-β-D-mannuronate (M-blocks) and poly-α-L-guluronic acid (G-blocks) and heteropolymeric regions (MG-blocks). The wide range of pharmaceutical applications of alginates is mainly attributed to their gel-forming capacity. Alginates are used in bioengineering in various ways: adjuvants such as drug delivery agents, polymer films, cell encapsulations, wound dressings, surgical sponges, and embolization agents [1–4]. In addition, specific applications of alginate gels require specific compositions; high-G alginate, for example, was shown to be more desirable for vascular embolization [5, 6]. Release rates of drugs from alginate gels correlate with carbohydrate composition and varying amounts of cross-linking with calcium ions. Moreover, composition-controlled alginate produced using the conventional Haug method often fails to meet the mechanical properties of the gel required for certain applications. Therefore, instead of using the defragmentation method, it is simply advantageous to use intrinsically specific composition-rich algae species for obtaining G- or M-homopolymer. However, such algae species are often available only in specific regions. The G contents of alginates from *L. japonica* typically does not exceed 40% [7]. Therefore *L. japonica* is not typically G-rich algal species. Since the structural nature and biocompatibilities of the alginate gels strongly correlate with composition (M- or G-blocks) and molecular weight, controlled preparation of alginate gel from well-characterized algae species is preferable to control purity, block composition, and molecular weight. In this regard, it is useful to produce gel-forming Poly-G acid

from no G-rich algal species that are available locally. In this study, we examine new manufacturing methods to prepare composition-controlled gel-forming alginates from *L. japonica* found on the coast of the Korean Peninsula, and test the feasibility of utilizing them in endovascular embolization agent.

2 Materials and methods

2.1 Production of Poly-G alginate by weak acid hydrolysis

Alginate was extracted with 1% Na_2CO_3 from dried *L. japonica* and purified by repeated precipitation by EtOH [8]. A mild acid hydrolysis method was employed to obtain Poly-G homopolymer from the alginate solution. Instead of using 0.3 M HCl in extensive hydrolysis by the Haug method [9] or partial hydrolysis by the Boyd method [10],each 10 g of extracted alginate was dissolved in a series of weak acid (0.025, 0.03, 0.07, 0.1, and 0.15 M HCl) solutions and heated for 5 min. The pH of each reacted solution was then adjusted to 2.75 by adding 0.1 M NaOH, and its insoluble portion (Poly-G) was neutralized by adding NaOH. A spoonful of active carbon was added to the reacted solution to remove residual polyphenolic compounds, stirred extensively, and then filtered with a 0.2-μm membrane filter. Thus, various batches of alginate solution were produced using different concentrations of HCl. Finally, all the alginate solutions were subjected to dialysis and then freeze-dried. To compare our mild acid hydrolysis method with the Haug method, hydrolysis was performed using 0.3 M HCl, and the hydrolyzed sample was heated for 5–25 min to produce Poly-G solution. Three of alginate solutions of each Poly-G batch were made for various tests (Tables 1, 2, 3).

2.2 Spectrophotometric measurements

The absorption spectrum of the purified alginate was measured in the range of 200–400 nm with a quartz sample holder and a UV spectrophotometer (Shimazu UV-1601). The fluorometric method [7] was used to detect polyphenols and other impurities with a Perkin-Elmer LS 50B fluorescence spectrometer. Excitation was performed at 366 nm and emission spectra were recorded at 400–600 nm. The fluorescence spectra were compared with those of a commercial alginate (Sigma, US).

2.3 Protein determination

The protein assay was performed using the Bio-Rad protein assay—a modified Bradford method [11]. Bradford reagent (200 μl) was added to 800 μl alginate sample solutions. After 10 min of incubation, the absorbance of the sample was read at 595 nm. For the quantitative evaluation of protein content, bovine serum albumin (Sigma, US) was used as the standard solution for calibration at 0–25 μg/ml. The concentration of the measured alginate solution was 0.1%.

2.4 CD measurements

Sampled sodium alginate solutions (80 μl) from each batch of 2% Poly-G were prepared in 2 ml of deionized water and adjusted to pH 7 by the addition of 1 M NaOH. CD spectra were obtained at 25°C between 190 and 250 nm by using a Jasco J-810 spectrometer and quartz cuvettes with a path length of 1 mm (Hellma, Germany). The CD spectra were compared with those reported in a previous study by Morris et al. [12]. In the CD spectrum of native alginate, the peak and trough were measured and the percentage of mannuronate in the alginate was calculated [12]:

$$\%\text{mannuronate} \approx 27 \cdot \frac{\text{peak}}{\text{trough}} + 40. \quad (1)$$

2.5 HPLC study

Each 25-μl sodium alginate solution was sampled from the same batch of Poly-G for CD measurement. Injected samples were eluted through a gel permeation HPLC column (Ultrahydrogel 250; Waters) with 0.1 M

Table 1 Polymer yield of Poly-G solutions produced by the mild acid hydrolysis method with different HCl concentrations (%)

Alginate solution (%)	Control	0.025 M-Poly-G	003 M-Poly-G	0.05 M-Poly-G	0.07 M-Poly-G	0.10 M-Poly-G	0.15 M-Poly-G
2	39.1 ± 0.3	62.7 ± 0.6	58.0 ± 0.5	47.1 ± 0.4	47.1 ± 0.7	46.3 ± 0.3	45.5 ± 0.4
3		56.7 ± 0.8	57.6 ± 0.5	55.6 ± 0.6	54.1 ± 0.3	–	–
4		58.8 ± 0.5	60.6 ± 0.6	59.7 ± 0.4	60.3 ± 0.5	–	–
5		55.9 ± 0.6	60.6 ± 0.4	61.8 ± 0.5	64.4 ± 0.4	–	–
6		57.4 ± 0.4	60.4 ± 0.3	61.8 ± 0.6	64.4 ± 0.6	–	–

Data are shown as mean ± SD, $n = 3$

–: not measured due to incapable of gelling

Table 2 Viscosity of Poly-G solutions produced by the mild acid hydrolysis method with different HCl concentrations (cP)

Solution (%)	Control	0.025 M-Poly-G	0.03 M-Poly-G	0.05 M-Poly-G	0.07 M-Poly-G	0.10 M-Poly-G	0.15 M-Poly-G
1	357.5 ± 2.5	4.0 ± 0.2	3.9 ± 0.3	3.2 ± 0.4	2.9 ± 0.2	2.5 ± 0.2	1.9 ± 0.2
2	380.9 ± 1.2	12.1 ± 1.1	10.0 ± 0.5	5.4 ± 0.3	5.4 ± 0.4	4.6 ± 0.3	3.2 ± 0.3
3		24.0 ± 0.6	16.5 ± 0.6	11.9 ± 0.5	9.4 ± 0.3	7.0 ± 0.3	4.4 ± 0.2
4		32.5 ± 0.8	39.8 ± 1.1	16.2 ± 0.6	15.6 ± 0.5	9.6 ± 0.4	7.3 ± 0.3
5		99.5 ± 1.8	61.4 ± 1.2	36.2 ± 0.8	26.9 ± 0.7	9.2 ± 0.5	9.1 ± 0.3
6		115.8 ± 1.2	100.8 ± 2.4	42.0 ± 0.7	39.6 ± 0.5	18.6 ± 0.5	15.3 ± 0.4
7		293.3 ± 1.5	217.0 ± 1.8	55.0 ± 0.5	57.0 ± 0.8	26.8 ± 0.8	24.6 ± 0.5
8		367.4 ± 2.3	254.4 ± 1.7	70.9 ± 0.7	60.7 ± 0.7	35.0 ± 1.1	30.4 ± 0.4

Data are shown as mean ± SD, $n = 3$

Table 3 Forty percent compressive deflection strength of Poly-G solutions produced by the mild acid hydrolysis method with different HCl Concentrations (kPa)

Alginate solution (%)	0.025 M-Poly-G	0.03 M-Poly-G	0.05 M-Poly-G	0.07 M-Poly-G
4	48.7 ± 0.6	38.3 ± 1.0	21.1 ± 0.8	30.0 ± 0.9
6	45.3 ± 1.0	77.4 ± 1.1	28.1 ± 0.7	39.0 ± 0.8

Data are shown as mean ± SD, $n = 3$

ammonium phosphate/0.3 M NaCl buffer (adjusted to pH 4.0 by phosphoric acid) and a flow rate of 0.5 ml/min for 40 min. The UV detection wavelength was set to 210 nm.

To compare the molecular weight of 1% 0.03 M-Poly-G with that of a number of standard Dextran samples with known molecular weight (40–120 kDa), both the Poly-G solutions and the standard Dextran samples were eluted separately under the same flow condition as described above.

2.6 Viscosity measurements

The apparent viscosity of 1–10% Poly-G solutions was measured at 25°C by using a Brookfield Digital Rheometer with spindle rotation set to 20 rpm. All alginate solutions were filtered through 0.8-μm Millipore filters prior to the determination of viscosity.

2.7 Measurements of polymer yields

Calcium alginate samples were polymerized by mixing 17 ml of liquid alginate with 17 ml of 0.68 M calcium chloride dehydrate in a 100-ml beaker [total volume (V_t) of 34 ml] at 25°C. The alginate ionically cross-linked to form a disk-shaped gel sample. Polymer yield is a measure of the percent of alginate that cross-links into a gel. The polymer gel volume was determined by subtracting the liquid alginate and calcium chloride volume remaining (V_r) after polymerization from the initial total volume (V_i). The resulting polymer yield was calculated by the following equation:

$$\text{PolymerYield}(\%) = 100 \cdot \frac{V_t - V_r}{V_t}. \quad (2)$$

2.8 Compression tests

Compression tests were conducted at 25°C with a material-testing Instron machine (INSTRON 4411-standard). Four percent and six percent alginate gel samples (1–2 cm thick, 2.5–3.5 cm in diameter) were placed between the Instron cylindrical load cell with a constant 2.03 cm diameter and a metal plate. The gel sample was compressed at a set compression rate (1.3 cm/s^{-1}) to the maximum force of 2200 N. All gel samples tested had surface areas greater than that of the cylindrical load cell to facilitate the calculation of pressure from the force reading divided by the load cell surface area. The polymer compressive strengths were determined by calculating the polymer-resistive pressure at either 40% or 60% compression for each sample. Compression is calculated by measuring the initial gel thickness (t_i) and the gel thickness after compression (t_f), as shown in the following equation:

$$\text{Compression}(\%) = 100 \cdot \frac{t_i - t_f}{t_i}. \quad (3)$$

2.9 Endovascular occlusion test

The alginate with optimal material characteristics was injected by two methods into the renal vessels of the kidneys of New Zealand white rabbits (4 kg) to determine the extent of endovascular occlusion. Two rabbits for each injecting method were anesthetized with ketamine and the alginate was delivered by a staged arterial injection through

a single microcatheter. Three milliliters of 6% Poly-G was injected first, followed by 0.25 ml of saline and 3 ml of 0.68 M $CaCl_2$. The flows mixed in the kidney and polymerized. In the second method, the alginate was delivered by a bi-directional injection. Both renal vessels were catheterized. Three milliliters of 6% 0.03 M-Poly-G was injected retrograde through the renal vein, and 3 ml of 0.68 M $CaCl_2$ was injected through the renal artery. The flows met in the kidney and polymerized. Both methods were evaluated with angiography by injecting a contrast agent. Animal experiments were conducted in accordance with the guidelines set by the policies of the animal research committee of the Catholic University Hospital of Daegu, Korea.

3 Results

3.1 Production of Poly-G

The yield of alginate was 10–13%, which is comparable with that (10–15%) obtained by other methods [8, 13]. The protein content in the extracted alginate, determined by the Bradford assay, was below 0.2%. Figure 1 shows the results of fluorescence measurements at different steps of extraction and purification. Fluorometric analysis revealed that polyphenolic compounds were removed significantly with multiple alcoholic precipitation steps and further removal by an active carbon filtering process during the preparation of Poly-G alginate. The corresponding fluorescence intensity of purified alginates was considerably less than that of a commercial alginate, as shown in Fig. 1.

Six batches of Poly-G solution (0.025 M-Poly-G, 0.03 M-Poly-G, 0.05 M-Poly-G, 0.07 M-Poly-G, 0.1 M-Poly-G, and 0.15 M-Poly-G) were obtained by the mild acid hydrolysis method with different HCl concentrations. The prefixes represent the concentration of HCl used for hydrolysis. In addition, five Poly-G solutions and five Poly-M solutions (poly-L-mannuronic alginate) were prepared by the Haug method with 0.3 M HCl and different acid treatment times (5–25 min): Poly-G solutions, 5 m-Poly-G, 10 m-Poly-G, 15 m-Poly-G, 20 m-Poly-G, and 25 m-Poly-G; Poly-M solutions, 5 m-Poly-M, 10 m-Poly-M, 15 m-Poly-M, 20 m-Poly-M, and 25 m-Poly-M. The prefixes represent the acid treatment time (min) used for hydrolysis.

3.2 HPLC analysis

Poly-G or Poly-M produced by using longer heating time showed an increase in the peak portion of smaller molecular weight that eluted with longer retention time in this column as shown in Fig. 2. A similar trend was observed for Poly-Gs produced by stronger HCl concentrations. A total of 0.03 M-Poly-G was eluted slightly faster than standard Dextran samples with molecular weight 80 kDa, as shown in Fig. 2c and d. Indeed, 0.03 M-Poly-G was eluted much faster than standard Dextran samples with molecular weight 40 kDa.

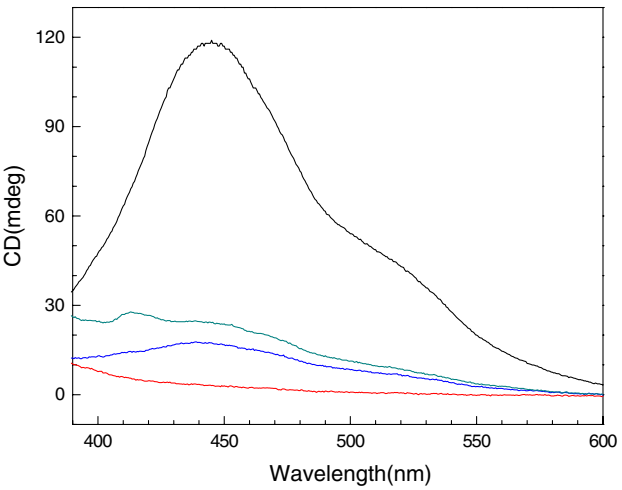

Fig. 1 Fluorescence measurements of Sigma alginate and laboratory purified alginates. After final active carbon filtering, Poly-Gs showed less fluorescence than crude alginate, alginate in the intermediate step of purification, and a commercial alginate. Black, green, blue, and red indicate laboratory purified alginate, alginate from the intermediate step, commercial alginate, and carbon-filtered alginate, respectively. *Black* laboratory purified alginate, *green* intermediate step, *blue* commercial alginate, *red* carbon filtering (*color online*)

3.3 CD measurements

Native alginate from *L. japonica* was composed of 75% mannurate, as calculated from its CD spectrum (Fig. 3d) and Eq. 1. CD spectra of Poly-Gs are shown in Fig. 3a and compared with typical spectra (Fig. 3b, c) of Poly-G or Poly-M produced by the Haug method. All Poly-G spectra obtained by acid treatment were negative, confirming the presence of Poly-G. The trough depth at around 215 nm of the Poly-G spectra increased with the increase in the molar concentration of treated acid. In comparison with the HPLC spectra, 0.15 M-Poly-G or 25 m-Poly-G that had elution peaks at longer retention time showed deeper trough in its CD spectra than 0.025 M-Poly-G or 5 m-Poly-G, respectively (Fig. 3a, b). In contrast, 25 m-Poly-M that had elution peak at longer retention time showed shallower trough in its CD spectra than 5 m-Poly-M (Fig. 3c).

3.4 Polymer yield and gel formation

Although all Poly-G solutions were polymerized at various concentrations, gel formation occurred only for Poly-Gs within a concentration range, depending on the molar

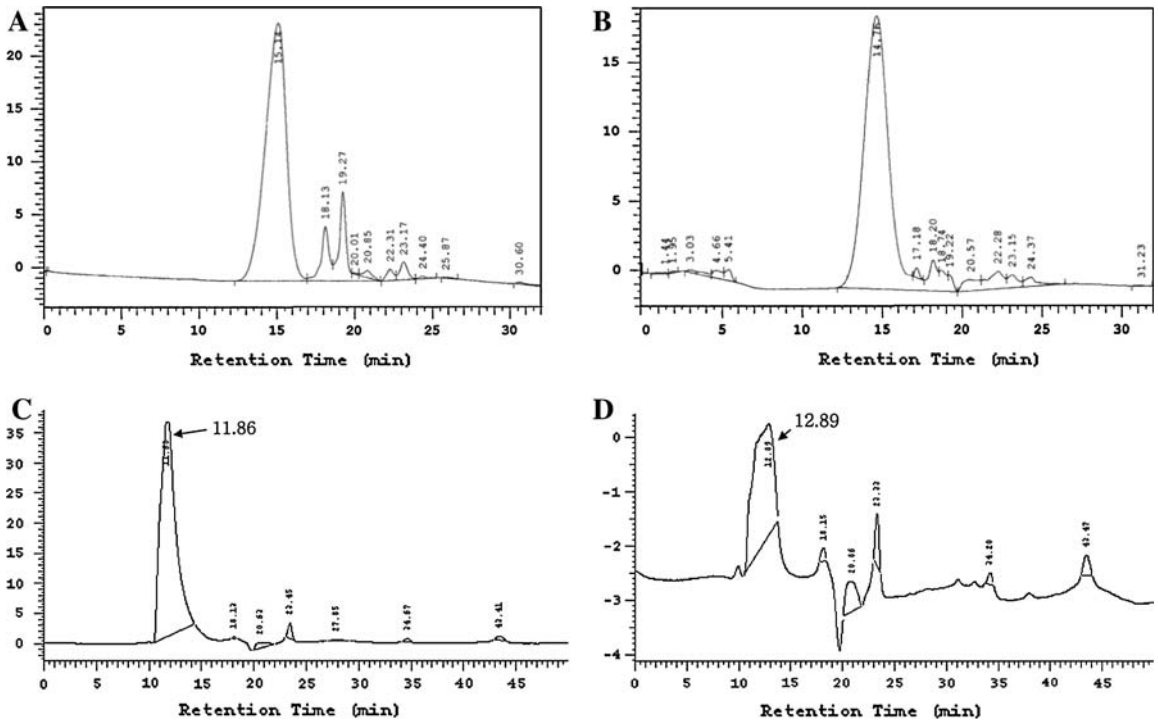

Fig. 2 HPLC elution profiles of 25 m-Poly-G (**a**) and 5 m-Poly-G (**b**) samples injected through a gel permeation HPLC column (Ultrahydrogel 250; Waters), eluted with 0.1 M ammonium phosphate/0.3 M NaCl pH 4.0 buffer, and a flow rate of 0.5 ml/min for 40 min. The UV detection wavelength was set to 210 nm. A total of 0.03 M-Poly-G alginate (**c**) was eluted slightly faster than an 80-kDa Dextran MW marker (**d**), which indicates that 0.03 N-Poly-G may have molecular weights greater than 80 kDa

concentration of treated HCl and weight percent concentration of the alginate solution. The results are summarized in Table 1. When the polymer yield of a Poly-G was less than 50%, it failed to form a gel with a consistent shape. Stable gel formation occurred mostly in 0.03 M-Poly-G and 0.025 M-Poly-G (4–6% concentration). Poly-Gs produced by the Haug method did not form gel with a consistent shape, as shown in Fig. 4a, while 0.03 M-Poly-G showed stable gel formation (Fig. 4b).

3.5 Gel strength and viscosity

The strength of the Poly-G gels was compared at the compression levels of 40% and 60% (Table 2) within the usable range (4.0–6.0%). High G acid alginates form stable gels with consistently high strength according to the concentration. 0.03 M-Poly-G showed the highest gel strength with 6% concentration at both compression levels. However, 0.025 M-Poly-G exhibited the highest strength among all Poly-Gs with 4% concentration at the 40% compression level. Indeed, it also exhibited the highest strength with 6% concentration at the 60% compression level. The Poly-Gs with concentrations higher than 6% became very viscous to use for injection, as shown in Table 3. Poly-Gs obtained from the acid treatment showed lower viscosity than untreated crude alginate. Poly-G gels had the highest strength across their usable range (4.0–6.0%).

3.6 Renal arterial embolization in rabbits

In artery–vein sequential injection, the renal artery and the aorta leading to the right kidney were first ligated, and their blood flow was blocked. A contrast agent was then injected through the aorta to observe patent blood flow to the left kidney, as shown in Fig. 5a. Next, Poly-G and $CaCl_2$ were injected through the renal vein and the renal artery, respectively, as shown in Fig. 5b. Finally, the contrast agent was injected after removing the ligation in the aorta. Both kidneys were visualized, as shown in Fig. 5c, indicating incomplete obstruction of the left kidney.

In the sequential artery injection method, Poly-G and $CaCl_2$ were injected through the artery sequentially via a single catheter (Fig. 5d). Re-injection of the contrast agent after removing the ligation in the aorta showed blood flow to the right kidney but no blood flow to the left kidney, thereby making it invisible in angiography (Fig. 5e). This signifies a complete blockage of blood flow due to gel formation in the vascular bed of the left kidney.

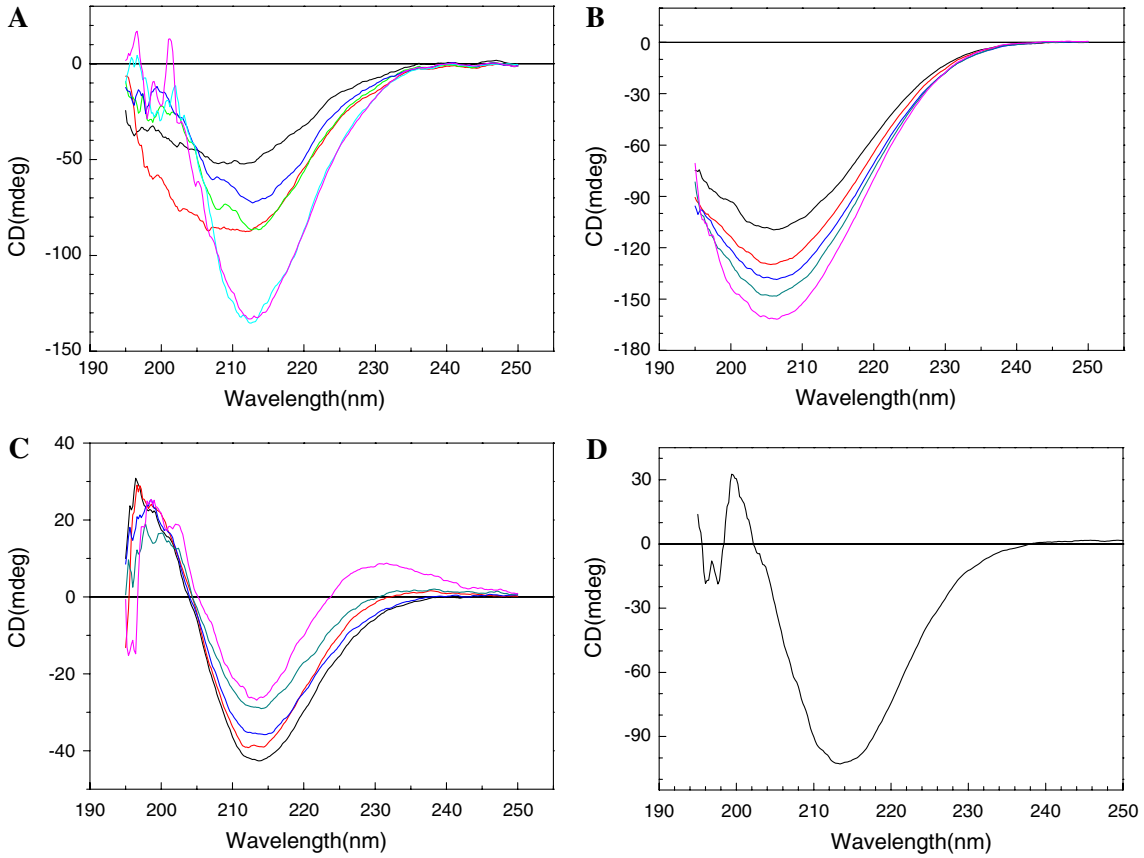

Fig. 3 CD spectra of Poly-Gs obtained using the mild acid (0.015–0.15 M HCl) hydrolysis method (**a**), and Poly-G; (**b**) and Poly-M (**c**) obtained using the Haug method with a treatment time of 5–25 min. The *black*, *red*, *green*, *blue*, *sky blue*, and *pink* sections of (**a**) indicate 0.025 M-Poly-G, 0.03 M-Poly-G, 0.05 M-Poly-G, 0.07 M-Poly-G, 0.1 M-Poly-G, and 0.15 M-Poly-G, respectively. The *black*, *red*, *blue*, *green*, and *pink* sections of (**b**) indicate 5 m-Poly-G, 10 m-Poly-G, 15 m-Poly-G, 20 m-Poly-G, and 25 m-Poly-G, respectively. The *black*, *red*, *blue*, *green*, and *pink* sections of (**c**) indicate 5 m-Poly-M, 10 m-Poly-M, 15 m-Poly-M, 20 m-Poly-M, and 25 m-Poly-M, respectively. Both Poly-Gs showed completely negative bands, while the Poly-M showed typical positive and negative bands. Poly-Gs with low molecular weights were produced by treatment with a stronger acid or longer treatment time and exhibited a deeper trough (around 210 nm) in their CD spectra (**b**, **c**). In contrast, Poly-Ms with low molecular weights produced by longer treatment times exhibited a shallower trough (around 210 nm) in their CD spectra (**c**). The CD spectrum of native alginate (**d**) showed a peak and a trough and their G/M ratio was expected to be 0.33 (*color online*)

4 Discussion

4.1 Poly-G production

The study results indicate that it is possible to produce ultra-pure gelling Poly-G alginate with a comparable yield from no G-rich *L. japonica.* The two types of block sequences present in alginates showed quite different CD behaviors; the spectrum of poly-L-guluronic is entirely negative, whereas that of poly-D-mannuronate had both positive and negative bands (Fig. 2). The simple pyranose rings contain only ether chromophores, and electronic transitions occur at wavelengths shorter than 200 nm. CD spectra in this range are not easily accessible, and until recently, required special prototype instruments for detection [14]. For carbohydrates carrying unsaturated substituents, CD absorption bands can generally be detected at wavelengths longer than 200 nm. Alginates have carboxylate as the intrinsic chromophore and hence exhibit CD around 210 nm corresponding to the $n\pi^*$ transition of the COO^- group. Spectra of intact alginates peak at ~198 nm and trough at ~215 nm, with relative magnitudes varying with their compositions. Our Poly-Gs demonstrated a typical negative band of poly-L-guluronic acid with trough around 213 nm. The peak around ~198 nm was not observed in CD spectra of the Poly-Gs. Although the ratio of peak height to trough depth representing the ratio of mannuronate to guluronic acid could not be determined, the presence of poly-G blocks could be easily monitored with the appearance of a negative band in the CD spectra. The trough depth in these spectra may represent a difference in the molecular weight distribution of each Poly-G obtained from the treatment of alginates with different HCl concentrations [15]. A Poly-G with deeper trough depth

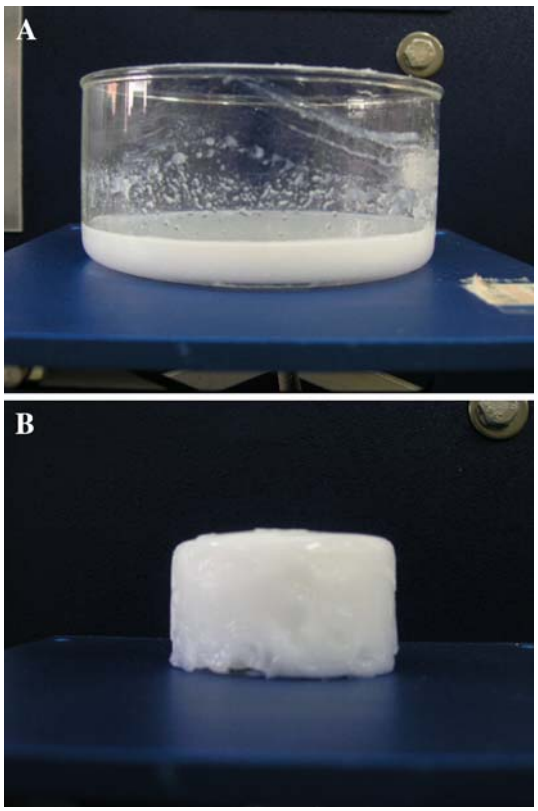

Fig. 4 Gel formation of a Poly-G (15 m-Poly-G) obtained by the Haug method (**a**) and a Poly-G (0.03 M-Poly-G) obtained by mild acid hydrolysis (**b**). All the Poly-Gs produced by the Haug method exhibited a grain-like gel that did not form a consistent shape, while the Poly-G obtained by mild acid hydrolysis demonstrated a gel formation with a consistent shape

showed elution peaks at longer retention time in HPLC. This indicates that acid treatment of alginates with stronger acid or longer hydrolysis time would produce more portions with smaller molecular weights. Poly-G obtained by hydrolysis with stronger acid exhibited a relatively narrow and deep trough, while the trough became broader as the concentration of HCl decreased, as shown in Fig. 3a. Poly-G obtained by the conventional Haug method with longer treatment time showed deeper trough depth in its CD spectra, resulting in more portions with smaller molecular weight. In contrast, Poly-M obtained by the conventional Haug method with longer treatment time exhibited shallower trough as containing more portions with smaller molecular weight, which was consistent with the HPLC results. Therefore, CD spectra of alginate solutions would be a convenient method to monitor the block composition or the molecular distribution of an alginate homopolymer.

4.2 Polymer yield and gelling

Although most Poly-Gs showed typical negative CD spectra, indicating the presence of poly-L-guluronic block, they did not all form gels. Gelling of Poly-G was highly sensitive to the HCl concentration used when treating the alginate with acid. When the polymer yield was less than 50%, gel formation did not occur. This was observed either when the HCl concentration of the acid treatment was greater than 0.1 M or when weight concentration of 0.05–0.07 M-Poly-G solution was less than 3%. Even though gel was formed using 0.05–0.07 M-Poly-Gs, their appearance looked like grain rather than a consistent block-like shape, as demonstrated in Fig. 4. Therefore, actual gel formation with a consistent shape was obtained only by 4–6% alginate solution from the 0.03–0.025 M-Poly-Gs.

In order to inject alginate solution for an embolization application, it is necessary to have its viscosity in the range of 30–120 cP [5, 6]. This requirement was met in the 0.03–0.025 M-Poly-G alginates, as shown in Table 2. The viscosity is affected not only by the molecular weight of the alginate but also by the flexibility of the polymer chains. The flexibility of the chains is determined by the chemical composition (i.e., block structure), because parts of the chains containing predominantly G blocks are less flexible than those containing predominantly M-blocks, which are in turn stiffer than areas of roughly alternating M- and G-blocks [16]. High-G acid alginates form more stable gels with consistently higher strengths with respect to concentration than high-M acid alginates [5]. The differential viscosities of all the Poly-G solutions in this study suggest the dependency of alginate viscosity more on molecular weight distribution rather than on block structure, as indicated in a previous study [6]. A Poly-G produced by a higher concentration of HCl shows less viscosity compared to a Poly-G produced by a lower concentration of HCl, as demonstrated in Table 2. Therefore, a Poly-G produced by stronger acid contained more portions of the alginate with smaller molecular weights than a Poly-G obtained by a weaker acid, which was consistent with the HPLC and CD spectra studies. Since 0.03 M-Poly-G eluted slightly faster than an 80-kDa Dextran molecular marker, the appropriate molecular weight distribution of gelling Poly-G would be greater than 80 kDa.

4.3 Gel Strength and kidney endovascular occlusion

Renal arterial embolization is one of the effective therapeutic methods for types of hemorrhagic disease of kidney and symptom control of renal cancer [17, 18]. It could avoid unnecessary nephrectomy and increase the success of operation. Calcium alginate turned out to be good embolization agents in experimental aneurysm model [19–21] as well as local chemoembolization [22] with biocompatibility and long-term stability. Therefore we performed renal arterial embolization to investigate the effect of alginate gels in this field.

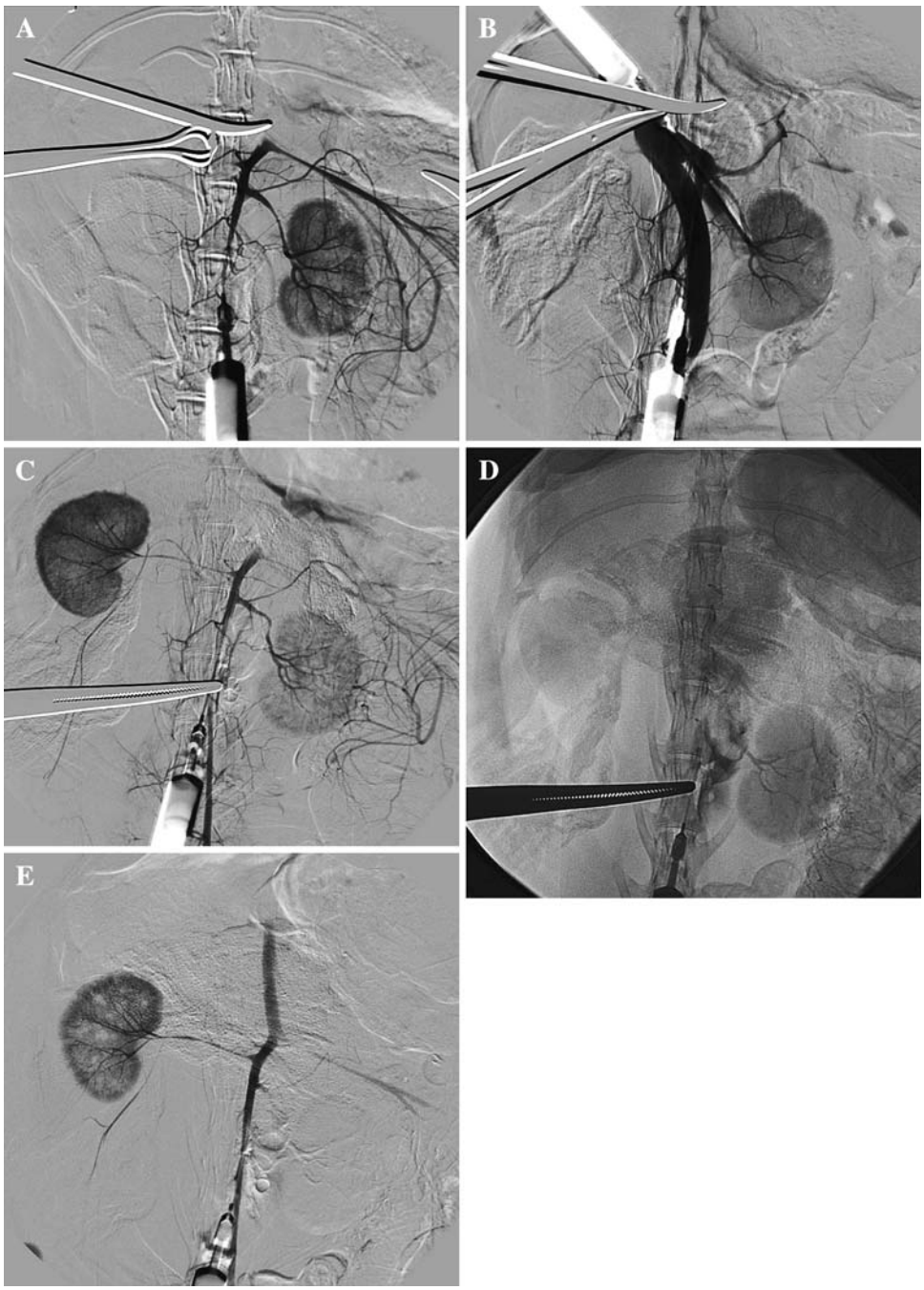

Fig. 5 Angiography of endovascular embolization with 6% 0.03 M-Poly-G. **a** In the sequential artery–vein injection method, the renal artery and the aorta leading to the right kidney were first ligated, and their blood flow was blocked. The contrast agent was then injected through the aorta to observe patent blood flow to the left kidney. **b** Next, Poly-G and $CaCl_2$ were injected through the renal vein and the renal artery, respectively. **c** Finally, the contrast agent was injected after removing the ligation in the aorta. Both kidneys were visualized, indicating incomplete obstruction of the left kidney. **d** In the sequential artery injection method, Poly-G and $CaCl_2$ were injected through the artery sequentially via a single catheter. **e** Re-injection of the contrast agent after removing the ligation in the aorta showed blood flow to the right kidney but no flow to the left kidney, thereby making it invisible in angiography. This signifies a complete blockage of blood flow due to gel formation in the vascular bed of the left kidney

The data in Tables 3 and 4 showed a similar molecular weight dependence existing with regard to the gel strength of all Poly-G alginates. The number of cross-links per volume unit that determines gel strength may increase as the molecular weight of the Poly-G increases. Both gel strength and viscosity of the Poly-G solution increased with an increase in the alginate concentration. Therefore, two properties should be considered in order to ensure the injectability of a Poly-G solution for endovascular application. Indeed, high polymer yield is important for complete endovascular occlusions. The higher the polymer yield, the greater the resulting polymer gel volume and the more effective the resulting endovascular occlusion. Utilizing the optimal concentration of alginate with respect to viscosity, the optimal compressive strength and the polymer yield of the Poly-G were compared and selected. Overall, 0.4–0.6% of 0.03 M-Poly-G had the highest strength and best gelling capacity with an injectable viscosity. However, selected alginate solutions were chosen based on the mechanical and rheology tests performed at room temperature which did not reflect the gel strength in vivo exactly. Therefore, it should be considered that the gel may be less viscous or has weaker

Table 4 Sixty percent compressive deflection strength of Poly-G solutions produced by the mild acid hydrolysis method with different HCl Concentrations (kPa)

Alginate solution (%)	0.025 M-Poly-G	0.03 M-Poly-G	0.05 M-Poly-G	0.07 M-Poly-G
4	107.0 ± 1.2	87.3 ± 0.8	49.3 ± 0.7	41.0 ± 0.6
6	124.2 ± 1.1	154.2 ± 1.6	55.8 ± 0.4	61.6 ± 0.4

Data are shown as mean ± SD, $n = 3$

gelling strength at the environment with body temperature when testing 0.6% of 0.03 M-Poly-G for preliminary endovascular occlusion. Six percent of 0.03 M-Poly-G was successfully delivered using single lumen catheter from the renal vessels into the vascular bed of a rabbit kidney and polymerized, as shown in Fig. 5. Before embolization, the renal arterial angiogram displayed a good image of the trunk and the branch of the renal artery, while after embolization, most of the artery image in the embolized kidney disappeared, depending on the injection method. Bi-directional injection did not produce complete occlusion; major trunks and small branches were still seen, as shown in Fig. 5c. Conversely, sequential arterial injection completely blocked all flow out of the kidney model and the kidney was not seen (Fig. 5e). In the bi-directional method, each flow of $CaCl_2$ and Poly-G that was injected in the artery and vein, respectively, was supposed to reach the kidney. Therefore there may be a slight delay in polymerization compared to the sequential arterial injection method, resulting in incomplete polymerization throughout the vascular bed and a view of the kidney in the angiogram. An ideal embolic agent should yield rapid and effective embolization, have the ability to reach and fill distal vasculature targeted for embolization, be easy to prepare and use, biocompatible, and cost effective. Since the Poly-G was non-adhesive, a large volume of alginate could be delivered from micro catheters to the vessel system for more complete occlusion without the concern of catheter being glued to the vessel wall. However, because of lack of adhesive properties and because of its softness, alginate gel can be carried by blood flow, escaping into parent artery or another organ in particular when there is delay in gelling timing. This point may require more accurate delivery system of endovascular injecting alginate solution for safety concern in potential clinical practice.

5 Conclusions

Ultra-pure gel-forming poly-L-guluronic acid could be prepared from specific algal species containing no G-rich alginate. Determination of CD spectra was a useful and simple technique to monitor the presence of Poly-G. The new method was used with a considerably lower HCl concentration and a shorter treatment time to establish the gelling capacity of poly-L-guluronic acid while maintaining a certain range of their molecular weight distribution by preventing extensive acidic fragmentation. It was possible to select optimized Poly-Gs with respect to the percentage of concentration, polymer yield, gel-forming stability, viscosity, and gel strength suitable for a preliminary endovascular embolizing agent. Selected 6% 0.03 M-Poly-G alginate was successfully used to embolize the renal vascular system through stable gel formation. This result may demonstrate that alginate embolization is likely to provide minimally invasive vascular therapy to hemorrhaging, cancer as well as vessel damage including aneurysm.

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