Self-degradation of tissue adhesive based on oxidized dextran and poly-L-lysine

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Abstract

We have developed a low-toxicity bioadhesive based on oxidized dextran and poly-L-lysine. Here, we report that the mechanical properties and degradation of this novel hydrogel bioadhesive can be controlled by changing the extent of oxidation of the dextran and the type or concentration of the anhydride species in the acylated poly-L-lysine. The dynamic moduli of the hydrogels can be controlled from 120 Pa to 20 kPa, suggesting that they would have mechanical compatibility with various tissues, and could have applications as tissue adhesives. Development of the hydrogel color from clear to brown indicates that the reaction between the dextran aldehyde groups and the poly-L-lysine amino groups may be induced by a Maillard reaction via Schiff base formation. Degradation of the aldehyde dextran may begin by reaction of the amino groups in the poly-L-lysine. The gel degradation can be ascribed to degradation of the aldehyde dextran in the hydrogel, although the aldehyde dextran itself is relatively stable in water. The oxidized dextran and poly-L-lysine, and the degraded hydrogel showed low cytotoxicities. These findings indicate that a hydrogel consisting of oxidized dextran and poly-L-lysine has low toxicity and a well-controlled degradation rate, and has potential clinical applications as a bioadhesive.

Keywords: biodegradation, bioadhesive, hydrogel, dextran, poly-L-lysine

Introduction

Many studies have focused on surgical tissue adhesives for joining tissues together; typically, these adhesives are composed of synthetic or biological compounds, or their combinations (Li et al., 2014; Lim, Kim & Park, 2012). Cyanoacrylates are very common synthetic glues, which rapidly polymerize on contact with water or blood (Doraiswamy, Baig, Hammett & Hutton, 2003). Cyanoacrylates have high adhesive strength; however, they cause systemic inflammatory responses (Ramond, Valla, Gotlib, Rueff & Benha-Moou, 1986) and have poor handling properties (Bhasin, Sharma, Prasad & Singh, 2000); high cytotoxicities have also been reported (Bhatia, Arthur, Chenault & Kodokian, 2007). Fibrin glue, a biological adhesive, is widely used in clinical applications and consists of two components: a highly purified human fibrinogen with factor XIII and a human thrombin solution. Fibrin sealants have the advantages of biocompatibility and biodegradability, compared with synthetic sealants. Some complications associated with fibrin glue have been reported, such as serious bleeding diatheses (Ortel et al., 1994), weak adhesion (MacGillivray, 2003), and risk of infection (Canonico, 2003).

Recently, aldehyde-containing polysaccharides have been extensively studied. Periodate easily and effectively oxidizes 1,2-diol groups in polysaccharides and introduces aldehyde groups under gentle conditions [e.g., Malaprade oxidation (Malaprade, 1928)], and aldehyde groups can easily react with amino species in aqueous media.

In our previous study, we described the synthesis of novel low-cytotoxicity bioadhesives using ε-poly(L-lysine) (PL) and dextran containing aldehyde units, obtained by Malaprade oxidation (Hyon, Nakajima, Sugai & Matsumura, 2014; Araki et al., 2009; Takagi et al., 2013; Naitoh et al., 2013). Hydrogels were easily formed by the reaction between the aldehyde and amino groups, leading to the formation of a Schiff base and multiple crosslinking points, and these hydrogels showed high adhesive strength against living tissue. The gelation time could be controlled by the amount of aldehyde introduced into the dextran and by controlling the residual amino groups of the PL by an acylation reaction.

Degradation control is one of the key issues in biomaterials for tissue regeneration. There have been many studies of biodegradable polymers for biomedical applications, especially bioadhesives (Czech et al., 2013). In a previous study, we did not focus on the degradability of our oxidized dextran-based adhesives; we did not expect the hydrogels to degrade rapidly under physiological conditions, because hydrolysis of the crosslinking points is slow. However, we found that the hydrogels degraded rapidly. In this study, we focused on degradation control of the hydrogel-based bioadhesive, proposed a possible mechanism, and evaluated the hydrogel mechanical properties and cytotoxicities of the hydrogel and degraded portions.

Fibrin glue or activated polycarboxylic esters with *N*-hydroxysuccnimide (Taguchi, et al., 2004) should be prepared in solution just before an operation, because their components are unstable in

aqueous media. If adhesives in the form of aqueous solutions are required, their stability is important, to prevent adhesion failure. The stabilities of oxidized dextran and acylated PL in aqueous media were therefore also investigated.

Materials and methods

Materials

Dextran with a molecular weight of 70 kDa was obtained from the Meito Sangyo Co., Ltd. (Nagoya, Japan). PL (4 kDa, 25 wt% aqueous solution, free base) was obtained from the JNC Corp. (Tokyo, Japan). Sodium periodate, acetic anhydride (AA), succinic anhydride (SA), dextrin, and other chemicals were purchased from Nacalai Tesque, Inc., (Kyoto, Japan), and used without further purification unless otherwise stated.

Oxidation of dextran with periodate

Aldehyde dextran was prepared by the oxidation of dextran with sodium periodate, according to the method reported in our previous study (Hyon, Nakajima, Sugai & Matsumura, 2014). The aldehyde content of the dextran was evaluated by simple iodometry.

Acylation of PL by anhydrides

PL, an oligomer of L-lysine, has about 30 primary amino groups per molecule. To control gelation, some of the amino groups were acylated by adding AA or SA, according to the method detailed in

92 our previous report (Hyon, Nakajima, Sugai & Matsumura, 2014).

93 **Rheological measurements on hydrogels**

94 Rheological measurements were conducted using a strain-controlled rheometer (Rheosol G5000,
95 UBM Co., Ltd., Kyoto, Japan). A cone–plate geometry with a cone diameter of 40 mm and an angle
96 of 2° (truncation 60 μm) was used. The hydrogels for the rheological studies were prepared as
97 follows. Aqueous aldehyde dextrans (20 wt%, 1 mL), oxidized to various degrees with periodate,
98 were mixed with 1 mL of 10 wt% aqueous acylated PL containing AA or SA using a vortex mixer.
99 The mixture (1 mL) was loaded onto the plate using a micropipette within 1 min of mixing. The
100 dynamic viscoelastic properties (dynamic storage modulus G' and loss modulus G'') of the hydrogels
101 10 min after loading were determined using oscillatory deformation experiments performed from
102 0.01 to 10 Hz at 25 °C.

103 ***In vitro* gel degradation**

104 Dextran–PL hydrogels with different compositions were prepared, and their degradations in
105 phosphate buffer saline (PBS) were compared. Aqueous aldehyde dextran with various oxidation
106 ratios (20 wt%, 1 mL) and 1 mL of 10 wt% aqueous PL containing various amounts of AA were put
107 in a glass tube (16 mm diameter). After curing for 2 min at 25 °C, followed by vortex mixing, PBS
108 (3 mL) was added, and the tube was sealed. The degradation was observed for a given period at
109 37 °C.

Quantitative gel degradation was also evaluated in PBS. An aliquot (0.5 mL) of aqueous 20 wt% aldehyde dextran and 0.5 mL of 10 wt% acylated PL were put in a centrifuge tube (15 mL capacity, the same as those used for cell culture), and gelation was allowed to proceed for 2 min at 25 °C via vortex mixing. After the addition of 10 mL of PBS, the tube was tightly sealed and incubated at 37 °C with gentle rotation (10 rpm). After a given period of time, the supernatant was removed, and the remaining gel was rinsed with distilled water, followed by lyophilization (24 h) and vacuum drying (50 °C for 24 h). The weight of the remaining hydrogel was recorded against the incubation periods. Triplicate readings were taken for each sample (n = 3).

Cytotoxicity testing

The cytotoxicities of aldehyde dextran, PL, and the dextran–PL hydrogel were evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method (Mark, Belov, Davay, Davay & Kidman, 1992). L929, an established mouse cell line, which has often been selected for cytotoxicity tests, was used, and cultured in Eagle's minimum essential medium (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) supplemented with 0.15% w/v hydrogen bicarbonate, 0.03% w/v L-glutamine, and 10 vol% fetal bovine serum. Cell culture was carried out at 37 °C under 5% CO_2 in a humidified incubator. Cultured L929 cells in the logarithmic growth state were trypsinized and suspended in culture medium at a concentration of 1.0×10^4 cells/mL. After addition of 0.1 mL of the suspension to a 96-well tissue culture plate, the cells were incubated for 3 d at

37 ℃, and then 0.1 mL of culture medium, containing different concentrations of test substances, were added to each well, followed by further incubation for 2 d. After discarding the medium and rinsing the cells three times with 0.2 mL of PBS, 0.1 mL of MTT solution (90 mg of MTT dissolved in 100 mL of culture medium) were added to the culture and incubated at 37 ℃ for 5 h. The formazan crystals in the culture plate were dissolved in 0.1 mL of dimethyl sulfoxide, and the absorbance at 540 nm was recorded using a microplate reader (Versa Max, Molecular Device Japan K.K., Tokyo, Japan). The cytotoxicity was represented as the concentration of the test compound that caused a 50% reduction in MTT uptake by a treated cell culture compared with the untreated control culture (IC_{50}).

All the test substances were dissolved in distilled water and filtration-sterilized with a membrane filter of pore size 0.22 μm, followed by dilution with the culture medium, prior to addition to the cell culture. The degradation solutions for the dextran–PL hydrogel tests were prepared as follows: equal volumes of aqueous 20 wt% aldehyde dextran and 10 wt% acylated PL solution were mixed, and the hydrogel was prepared using a dual syringe device. After curing for 2 min, the hydrogel was crushed using a triturator and put in a glass vial. A four-fold weight of distilled water was added to the vial, and degradation was allowed to proceed at 37 ℃ for 4 d, followed by filtration sterilization. This degradation solution contained 3 wt% of the solutes (weight ratio of aldehyde dextran/PL = 2/1). For comparison, equal volumes of aqueous 4 wt% aldehyde dextran and 2 wt% acylated PL solution

were also mixed, and the reaction was allowed to proceed at 37 °C for 4 d; no gelation occurred, although the same amounts of the solutes were used for the reaction and the gel degradation.

Solution stability of aldehyde dextran and PL

The stabilities of the aldehyde dextran and PL in aqueous solution were evaluated by examining the gelation time change after different storage periods. After filtration sterilization, 20 w/w% aldehyde dextran (22.6% oxidation) and 10 w/w% PL with 21% substitution by SA were separately stored in brown glass ampoules (5 mL capacity) at 4 and 25 °C. After a given time period, the gelation time of the mixture was measured as follows. The mixture of aqueous aldehyde dextran and acylated PL easily formed a hydrogel, and the gelation time was evaluated using a simple stirring method. An aliquot (0.5 mL) of 20 w/w% of the aqueous aldehyde dextran was added to a glass tube (diameter 16 mm) and incubated for 10 min at 37 °C, and then 0.5 mL of 10 w/w% acylated PL solution at 37 °C were added to the tube. At this mixing ratio, the pH of the mixture was around 7 in all cases. The period of time until a small magnetic stirring bar (4 mm × 10 mm) was stopped by gel formation was recorded (the stirring speed was 280 rpm, using a Mighty Magnetic Stirrer M-12G6, Koike Precision Instruments Co., Ltd., Hyogo, Japan). Triplicate readings were taken for each sample (n = 3).

Statistical analysis

All data are expressed as the mean ± standard deviation. Student's t-test was used for comparison

of two groups.

Results and discussion

Oxidation of dextran and acylation of PL

The results for the oxidation of dextran using sodium periodate and the acylation of PL using AA and SA are shown in Figure 1(A) and (B), respectively. Nearly linear increases in aldehyde introduction and acylation were observed with increasing periodate concentration and anhydride concentration, respectively. These results are in good agreement with those in our previous report (Hyon, Nakajima, Sugai & Matsumura, 2014). The oxidation (aldehyde introduction) per glucose unit was controlled between 5% and 40%. Acylation was slightly suppressed when SA was used instead of AA and the reacted amino group ratio (degree of substitution by acylation) was controlled between 10 and 40 mol%; *x*%OxDex denotes an aldehyde dextran with *x*% oxidation and PLAA*y*% and PLSA*z*% denote PL with *y* mol% substitution with AA and *z* mol% substitution with SA, respectively, for example, the hydrogel formed from 22.6%OxDex and PLAA10% is described as the 22.6%OxDex–PLAA10% hydrogel.

Rheological measurements

The hydrogel strengths were investigated by performing rheological tests on the various hydrogels. Figure 2(A) shows the effects of acylation on the storage moduli of various hydrogels. The dynamic

moduli of hydrogels obtained from mixtures of 22.6%OxDex and PLAA10%, PLAA25%, or PLAA37% were measured. The *G*' and *G*" values were both higher for the lower acylation ratio. The storage moduli were controlled between 2.5 and 20 kPa. The amount of amino groups probably decreased with increasing acylation ratio, therefore the number of crosslinking points decreased, leading to a decrease in the storage modulus. The effect of different degrees of acylation on the dynamic modulus is shown in Figure 2(B). A comparison of the dynamic moduli of the hydrogels formed by PLAA and PLSA showed that the *G*' and *G*" values of 22.6%OxDex–PLAA25% and 22.6%OxDex–PLAA37% were larger than those of 22.6%OxDex–PLSA21% and 22.6%OxDex–PLSA33%, although the acylation ratio was higher in PLAA than PLSA. These results suggest that intermolecular interactions between amino groups and carboxyl groups might reduce the reactive non-dissociated amino groups, leading to fewer crosslinking points. Figure 3(C) shows the effect of the acylation degree of 15.1%OxDex–PLAA hydrogels. Similar to the results shown in Figure 3(A), a higher acylation ratio resulted in lower dynamic moduli. Figure 3(D) shows the effect of dextran oxidation on the dynamic moduli. This graph shows that the dynamic moduli increase with increasing oxidation. These results clearly indicate that increasing the number of crosslinking points improves the mechanical properties of the hydrogels. The storage modulus was controlled between 100 Pa and 20 kPa by controlling dextran oxidation and PL acylation. These values are consistent with the mechanical properties of tissue-engineered hydrogels and the extracellular matrix

(Even-Ram, Artym & Yamada, 2006), suggesting that these hydrogels could be used as mechanically compatible tissue adhesives.

In vitro gel degradation

Ideally, a tissue adhesive should rapidly degrade *in vivo* after the wound-healing process. Degradation control is therefore very important in developing adhesives. The dextran–PL gel degradation as a function of time was observed at 37 °C in PBS, and the results are shown in Figure 3; the sealed tubes were put on an experimental table, and photographs were taken from a bird's-eye view. In this study, 1 mL of 10 wt% aqueous PLAA1037wt% or PLSA33wt% was mixed with 1 mL of 20 wt% 22.6%OxDex. Development of the dextran–PL gel color from clear to brown was observed within a day, which could be ascribed to a Maillard reaction involving Schiff base formation between the aldehyde groups of the dextran and the primary amino groups of the PL (Shen, Tseng & Wu, 2007; Huang, Soliman, Rosen & Ho, 1987). After one week, degradation of the 22.6%OxDex–PLAA37% hydrogel had progressed, and the gel was completely degraded within two weeks (arrow). In contrast, when SA was used (22.6%OxDex–PLSA33% hydrogel), the degradation was far slower than that with AA, and approximately six weeks were required for complete degradation. This delay in the degradation was due to intermolecular ionic crosslinking of the PL molecules acylated with SA. Slower degradation was accomplished by using a lower acylation ratio with AA, and the hydrogel was not degraded, even after 10 weeks, when 22.6%OxDex–PLAA10%

218 and 22.6%OxDex–PLAA20% were selected.

219 The results of quantitative degradation studies are summarized in Figure 4. For each sample, the
220 standard deviation of the data (n = 3) was smaller than the plot symbols (circle, triangle, and square).
221 The effect of test-tube rotation during incubation on the degradation of 22.6%OxDex–PLAA25% is
222 shown in Figure 4(A). After 8 d with rotation, the remaining gel weight was less than 60%. In
223 contrast, without rotation, more than 75% of the gel remained, suggesting that degradation was
224 accelerated by rotation, probably because it led to effective diffusion of the degraded hydrogel into
225 the PBS. Although the composition of the hydrogel was the same as that in Figure 3 (22.6%OxDex–
226 PLAA25%), more degradation was observed after incubation with rotation. In other words, the *in*
227 *vitro* gel degradation depended considerably on the experimental conditions, as well as the hydrogel
228 composition.

229 Figure 4(B) shows a comparison of gels with AA and SA. When AA was selected, after 4 d of
230 rotating incubation, 71.4% and 3.7% of the hydrogel remained in 22.6%OxDex–PLAA25% and
231 22.6%OxDex–PLAA37%, respectively. In contrast, 85.0% and 38.7% of the hydrogel remained, at
232 the same concentrations, when SA was used, i.e., 22.6%OxDex–PLSA21% and 22.6%OxDex–
233 PLSA33%, respectively. SA retarded hydrogel degradation as a result of intermolecular interactions
234 between the PL molecules, which had primary amino and carboxyl groups introduced by acylation.
235 A slightly opaque hydrogel was observed under these conditions, as shown in Figure 3 (0 d,

236 rightmost), suggesting polyion complexation of the acylated PL molecules with SA.

237 Figure 4(C) shows the effects of dextran oxidation and aldehyde introduction on gel degradation;
238 11.6–39.9%OxDex–PLAA25% hydrogels were used. When dextran with the lowest oxidation
239 degree (11.6%) was mixed with the PLAA25%, the hydrogel almost disappeared within 4 d under
240 rotation. An increase in the remaining fraction was associated with an increase in the extent of
241 oxidation, and a slower degradation was associated with a higher extent of dextran oxidation.

242 The effects of AA concentration on acylation and subsequent gel degradation were also
243 investigated [data shown in Figure 4(D)]; 22.6%OxDex–PLAA10–39% hydrogels were used. For
244 the 22.6%OxDex–PLAA10% hydrogel, approximately 80% of the hydrogel remained after 8 d. In
245 contrast, 96% of the hydrogel was degraded within 4 d when the 22.6%OxDex–PLAA39% hydrogel
246 was used. Although the degradation profiles were almost the same as those shown in Figure 4(C), a
247 significantly narrower range of anhydride concentrations was required for a wide range of
248 degradation; this might be ascribed not only to the differences in acylation and the decrease in the
249 amino group content of the PL, but also to the increase in amino group dissociation induced by
250 acetic acid, a byproduct of the acylation, which suppresses the crosslinking reaction. This analysis
251 can also be used to explain the mechanical properties of these hydrogels (Figure 2).

252 **Cytotoxicity**

253 The cytotoxicity of the dextran–PL gel degradation products was also evaluated, and the results

254 are given in Table 1. A non-gelating mixture consisting of 22.6%OxDex and PLAA25% was also
255 investigated. The IC_{50} of the gel degradation products was 9000 ppm; this value was almost in the
256 same order of those of aldehyde dextran (22.6%OxDex; 5000) and PLAA25% (9200), indicating
257 that the degradation products also showed very low cytotoxicity. Because the mobilities of the
258 aldehyde dextran and PL molecules were considerably suppressed in the hydrogels, it is likely that
259 the amounts of aldehyde and amino groups remaining in the hydrogels were higher than those in the
260 non-gelating mixture. However, almost the same IC_{50} values were found, regardless of gelation,
261 suggesting again that the aldehyde groups in the dextran and the amino groups in the PL have low
262 cytotoxicities.

263 **Stabilities of aldehyde dextran and PL in aqueous media**

264 Aldehyde dextran and PL were dissolved in water, and their stabilities were evaluated based on
265 the gelation time change during storage. The gelation point can often be determined by the crossing
266 point of *G*' and *G*", but, in our case, because some hydrogels formed within 1 min of mixing the
267 oxidized dextran and acylated PL, we chose a conventional stirring method (see the section Solution
268 stabilities of aldehyde dextran and PL, in the Materials and methods) instead of rheological
269 measurements. The results are shown in Figure 5. At 25 °C, the gelation time gradually increased
270 with storage time, and a 15.5 s delay was observed after 12 months (from 11.8 to 27.3 s). In contrast,
271 only a small delay of around 1 s (from 11.8 to 12.9 s) was seen for samples stored at 4 °C, even after

12 months of preservation, suggesting that 22.6%OxDex and PLSA21% have excellent stabilities in aqueous media, at least at that temperature. The amide bonds in the acylation region and lysine repeating units in the PL molecules are relatively stable in water, so the gelation time change was probably caused by changes in the aldehyde dextran; this will be discussed later.

Facile control of degradation of the hydrogels prepared from aldehyde dextran and PL is one of the important properties of this adhesive. In the present work, various degradation speeds were obtained by changing the extent of oxidation of the dextran or the concentration of anhydride species in PL acylation, as shown in Figure 4. Of course, other reaction factors such as the molecular weights of the dextran and PL, the solution concentration, and the pH also greatly affect the gelation and degradation properties. Nevertheless, there are still some limitations associated with the application of these changes. It is therefore important to control the hydrogel properties over a broad range with a small number of factors. Our adhesive has been examined for different applications such as ocular surface reconstruction in ophthalmology (Takaoka et al., 2008; Takaoka et al., 2009; Tsujita et al., 2012), prevention of alveolar air leakage in lung surgery (Araki et al., 2007), and tissue regeneration in orthopedic (Yamamoto, Fujibayashi, Nakajima, Sugai, Hyon & Nakamura, 2008; Kazusa et al., 2013) and cardiovascular surgery (Kamitani et al., 2013), and as carriers for the sustained release of drugs (Morishima et al., 2010; Takeda et al., 2011; Togo et al., 2013) and genes in cardiovascular surgery.

290 Development of the hydrogel from clear to brown, shown in Figure 3, indicates that the reaction
291 between the aldehydes of the dextran and the amino groups of the PL might be based on a Maillard
292 reaction via Schiff base formation, similar to the reaction between glutaraldehyde and food proteins
293 (Gerrard, Brown & Fayle, 2003). Schiff base formation is generally reversible under acidic or basic
294 conditions, but the colorization continued even after gel degradation and decolorization was no
295 longer observed, as indicated in Figure 3. It is therefore likely that the degradation reaction of the
296 hydrogel is independent of gelation, the crosslinking reaction, and color development.
297 The aqueous aldehyde dextran and PL solutions were quite stable for at least 12 months at 4 °C
298 (Figure 5). Usually, the amide bonds in peptide bonds in proteins are very stable at neutral pH and
299 37 °C, and the high thermal stability of PL has been reported previously (Hiraki, 1995); these facts
300 suggest that degradation of PL in the hydrogel was unlikely to proceed in PBS at 37 °C. In addition,
301 the browning reaction was sustained even after gel degradation occurred, as mentioned above. These
302 findings suggest that gel degradation could be ascribed to degradation of the aldehyde dextran in the
303 hydrogel, although the aldehyde dextran itself was relatively stable in water. The degradation
304 profiles of the hydrogels after storage for 12 months at 4 °C were quite similar to those for the
305 degradation of the fresh hydrogels (data not shown). The degradation of aldehyde dextran, therefore,
306 might begin with the reaction between the aldehyde groups in the dextran and the amino groups in
307 the PL. We have therefore used the phrase “self-degradation” in the title of this work to express this

unique property of this aldehyde dextran–PL hydrogel. The molecular mechanisms of the hydrogel degradation are currently being studied and will be reported in the near future.

Disclosures

The authors have no conflicts of interest to declare.

References

Araki, M., Tao, H., Nakajima, N., Sugai, H., Sato, T., Hyon, S. H., et al. (2007). Development of new biodegradable hydrogel glue for preventing alveolar air leakage. *The Journal of Thoracic and Cardiovascular Surgery, 134*,1241-1248.

Araki, M., Tao, H., Sato, T., Nakajima, N., Nagayasu, T., Nakamura, T., et al. (2009). Development of a new tissue-engineered sheet for reconstruction of the stomach. *Artifitial Organs,* 33, 818-826.

Bhasin, D. K., Sharma, B. C., Prasad, H., & Singh, K. (2000). Endoscopic removal of sclerotherapy needle from gastric varix after n-butyl-2-cyanoacrylate injection. *Gastrointestinal Endoscopy, 51*, 497-498.

Bhatia, S. K., Arthur, S. D., Chenault, H. K., & Kodokian, G. K. (2007). Interactions of polysaccharide-based tissue adhesives with clinically relevant fibroblast and macrophage cell lines. *Biotechnology Letters, 29*, 1645-1649.

Canonico, S. (2003). The use of human fibrin glue in the surgical operations. *Acta BioMedica, 74*, Suppl 2, 21-25.

Czeck, Z., Wilpiszewskab, K., Tyliszczakc, B., Jiangd, X., Baid, Y., and Shaod, L. (2013). Biodegradable self-adhesive tapes with starch carrier. *International Journal of Adhesion and Adhesives, 44*, 195-199.

Doraiswamy, N. V., Baig, H., Hammett, S., & Hutton, M. (2003). Which tissue adhesive for wounds? *Injury, 34*, 564-567.

Even-Ram, S., Artym, V., & Yamada, M. (2006). Matrix control of stem cell fate. *Cell, 126*, 645-647.

Gerrard, J. A., Brown, P. K., & Fayle, S. E. (2003). Maillard crosslinking of food proteins II: the reactions of glutaraldehyde, formaldehyde and glyceraldehyde with wheat proteins in vitro and in

situ. *Food Chemistry,80*, 35-43.

Hiraki, J. (1995). Basic and applied studies on epsilon polylysine. *Journal of Antibacterial and Antifungal Agents, 23*, 349-354.

Huang, T. C., Soliman, A. A., Rosen, R. T., & Ho, C. T. (1987). Studies on the Maillard browning reaction between aspartame and glucose. *Food Chemistry, 24*, 187-196.

Hyon, S. H., Nakajima, N., Sugai, H., & Matsumura, K. (2014). Low cytotoxic tissue adhesive based on oxidized dextran and epsilon-poly-L-lysine. *Journal of Biomedical Materials Research Part A*, in press (DOI:10.1002/jbm.a.34923).

Kamitani, T., Masumoto, H., Kotani, H., Ikeda, T., Hyon, S. H., & Sakata, R. (2013). Prevention of Retrosternal Adhesion by Novel Biocompatible Glue Derived from Food Additives" *The Journal of Thoracic and Cardiovascular Surgery, 146*, 1232-1238.

Kazusa, H., Nakasa, T., Shibuya, H., Ohkawa, S., Kamei, G., Adachi, N., et al., (2013). Strong adhesiveness of a new biodegradable hydrogel glue, LYDEX, for use on articular cartilage. *Journal of Applied Biomaterials & Functional Materials, 11*, 180-186.

Li C. X., Wang, T., Hu, L. H., Wei, Y. H., Liu, J. N., Mu, X. Y., et al., (2014). Photocrosslinkable bioadhesive based on dextran and PEG derivatives. *Materials Science & Engineering C-Materials for Biological Applications, 35*, 300-306.

Lim, J. I., Kim, J. H., & Park, H. K. (2012). The adhesive properties of prepolymerized allyl 2-cyanoacrylate/poly L-3,4-dihydroxyphenylalanine for use as bioglue. *Materials Letters, 81*, 251-253.

MacGillivray, T. E. (2003). Fibrin sealants and glues. *Journal of Cardiac Surgery, 18*, 480-485.

Malaprade, L. (1928). Action of polyalcohols on periodic acid. *Bulletin de la Societe Chimique de France, 43*, 683.

Mark, D. C., Belov, L., Davay, M. W., Davey, R. A., & Kidman, A. D. (1992). The MTT cell vaiability assay for cytotoxicity testing in multidrug-resistant human leukemic cells. *Leukemia Research, 16,* 1165-1173.

Morishima, M., Marui, A., Yanagi, S., Nomura, T., Nakajima, N., Hyon, S. H., et al. (2010). Sustained release of vancomycin from a new biodegradable glue to prevent methicillin-resistant *Staphylococcus aureus* graft infection. *Interactive Cardiovascular and Thoracic Surgery, 11*, 52-55.

Naitoh, Y., Kawauchi, A., Kamoi, K., Soh, J., Okihara, K., Hyon, S. H., et al., (2013) Hemostatic effect of new surgical glue in animal partial nephrectomy models. *Urology, 81*, 1095-1100.

Ortel, T. L., Charles, L. A., Keller, F. G, Marcom, P. K., Oldham Jr, H. N., Kane, W. H, et al. (1994). Topical thrombin and acquired coagulation factor inhibitors: clinical spectrum and laboratory diagnosis. *American Journal of Hematology, 45*, 128-135.

Ramond, M. J., Valla, D., Gotlib, J. P., Rueff, B., & Benha-Moou, J.P. (1986). Endoscopic obturation of esophagogastric varices with bucrylate. I .Clinical study of 49 patients. *Gastroenterologie Clinique et Biologique, 10*, 575-579.

Shen, S. C., Tseng, K. C., & Wu, J. S. B. (2007). An analysis of Maillard reaction products in ethanolic glucose glycine solution. *Food Chemistry, 102*, 281-287.

Taguchi, T., Saito, H., Uchida, Y., Sakane, M., Kobayashi, H., Kataoka, K., et al., (2004). Bonding of soft tissues using a novel tissue adhesive consisting of a citric acid derivative and collagen. *Materials Science and Engineering: C, 24*, 775-780.

Takagi, K., Araki, M., Fukuoka, H., Takeshita, H., Hidaka, S., Nanashima, A., et al., (2013). Novel powdered anti-adhesion material: Preventing postoperative intra-abdominal adhesions in a rat model. *International Journal of Medical Sciences, 10*, 467-474.

Takaoka, M., Nakamura, T., Sugai, H., Bentley, A. J., Nakajima, N., Fullwood, N. J., et al. (2008).

Sutureless amniotic membrane transplantation for ocular surface reconstruction with a chemically defined bioadhesive. Biomaterials, **29**, 2923-2931.

Takaoka, M., Nakamura, T., Sugai, H., Bentley, A. J., Nakajima, N., Yokoi, N., et al. (2009). Novel sutureless keratoplasty with a chemicallydefined bioadhesive. *Investigative Ophthalmology & Visual Science, 50*, 2679-2685.

Takeda, T., Shimamoto, T., Marui, A., Saito, N., Uehara, K., Minakata, K., et al., (2011). Topical application of a biodegradable disc with amiodarone for atrial fibrillation. *Annals of Thoracic Surgery, 91,* 734-739.

Togo, Y., Takahashi, K., Saito, K., Kiso, H., Huang, B., Tsukamoto, H., et al., (2013). Aldehyded dextran and ε-poly(L-lysine) hydrogel as nonviral gene carrier. *Stem Cells International, Article ID 634379.*

Tsujita, H., Brennan, A. B., Plummer, C. E., Nakajima, N., Hyon, S. H., Barrie, L. P., et al., (2012). An *ex vivo* model for suture-less amniotic membrane transplantation with a chemically defined bioadhesive. *Current Eye Research, 37*, 372-380.

Yamamoto, Y., Fujibayashi, S., Nakajima, N., Sugai, H., Hyon, S. H., & Nakamura, T. (2008). Biodegradable adhesive(LYDEX) with hydroxyapatite granules. *Key Engineering Materials, 361-363*, 575-578.

Figure Captions

Figure 1. Effect of periodate concentration on dextran oxidation (A) (a 20 g sample of dextran in 80 mL of water and 0–10 g of sodium periodate in 40 mL of water were mixed, and the reaction was allowed to proceed at 50 °C for 1 h), and the effect of anhydride concentration on Pl acylation (B) (10 wt% PL was reacted with 0–4 wt% AA (open circles) or SA (closed circles) at 50 °C for 1 h).

Figure 2. Dynamic moduli of various dextran–PL hydrogels: *G*' and *G*" of (A) 22.6%OxDex and various AA-substituted PL hydrogels; (B) 22.6%OxDex and PLAA25%, PLSA21%, PLAA37%, and PLSA33% hydrogels; (C) 15%OxDex and various AA-substituted PL hydrogels; and (D) various OxDex percentages and PLAA10% hydrogels.

Figure 3. Degradation of dextran–PL hydrogel at 37 °C in PBS. One mL of 20 wt% aqueous aldehyde dextran (22.6%OxDex) was mixed with 1 mL of 10 wt% PLAA10–37% or PLSA33%. Arrows mark the completion of degradation.

Figure 4. Quantitative dextran–PL gel degradation at 37 °C in PBS: (A) effect of rotating incubation on degradation speed; (B) comparison of effects of different anhydride species used in PL acylation on gel degradation; (C) effect of dextran oxidation on gel degradation; and (D) effect of AA concentration used in PL acylation on gel degradation.

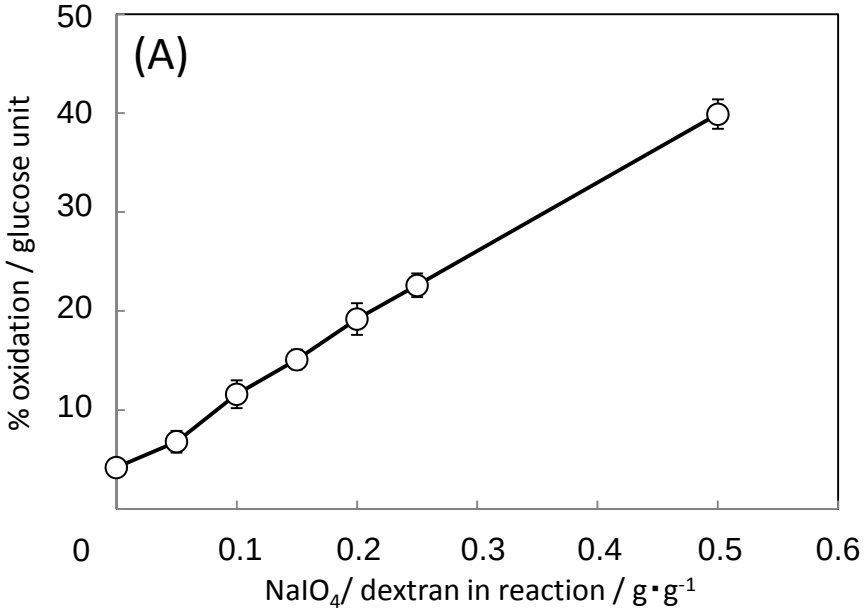
Figure 5. Gelation time change over long storage times at 4 and 25 °C. Separately stored 20 w/w% aldehyde dextran (22.6% oxidation) and 10 w/w% PLSA21% were mixed and the gelation time was measured at 37 °C. ***$P < 0.001$.

Table 1. Cytotoxicities to L929 cells of dextran–PL gel degradation products

material	IC50 / ppm[a)]		
22.6%OxDex	5100	±	100
PLAA25%	9200	±	200
gel degradation	9000	±	100
no gelation mixture[b)]	8500	±	300

[a)] Data = average ± standard deviation (n = 8 × 8).

[b)] Aqueous 4 w/w% 22.6%OxDex and 2 w/w% PLAA25% solution was mixed at the same volume and the reaction was performed at 37 °C for 4 d.


(A)
% oxidation / glucose unit
50
40
30
20
10
0
0.1
0.2
0.3
0.4
0.5
0.6
NaIO4/ dextran in reaction / g･g-1

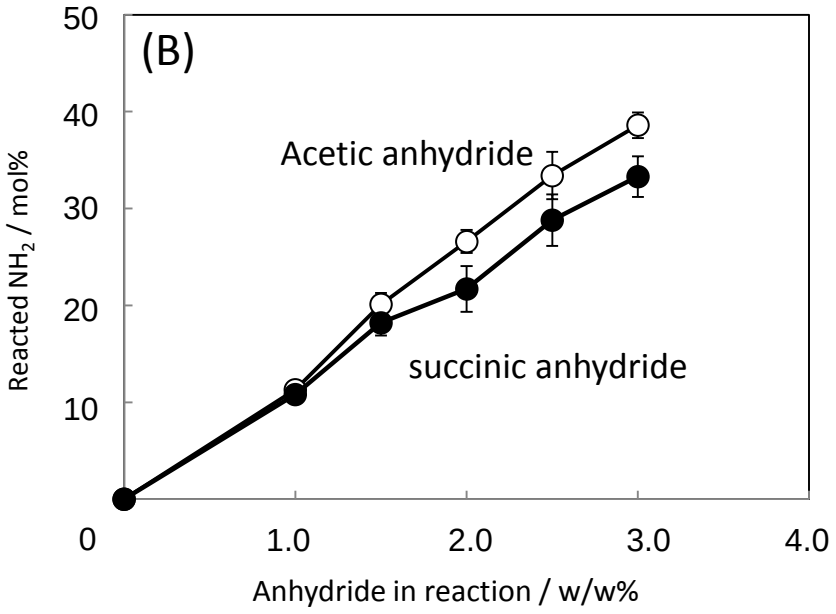

(B)
Reacted NH2 / mol%
50
40
30
20
10
0
1.0
2.0
3.0
4.0
Acetic anhydride
succinic anhydride
Anhydride in reaction / w/w%

Figure 1

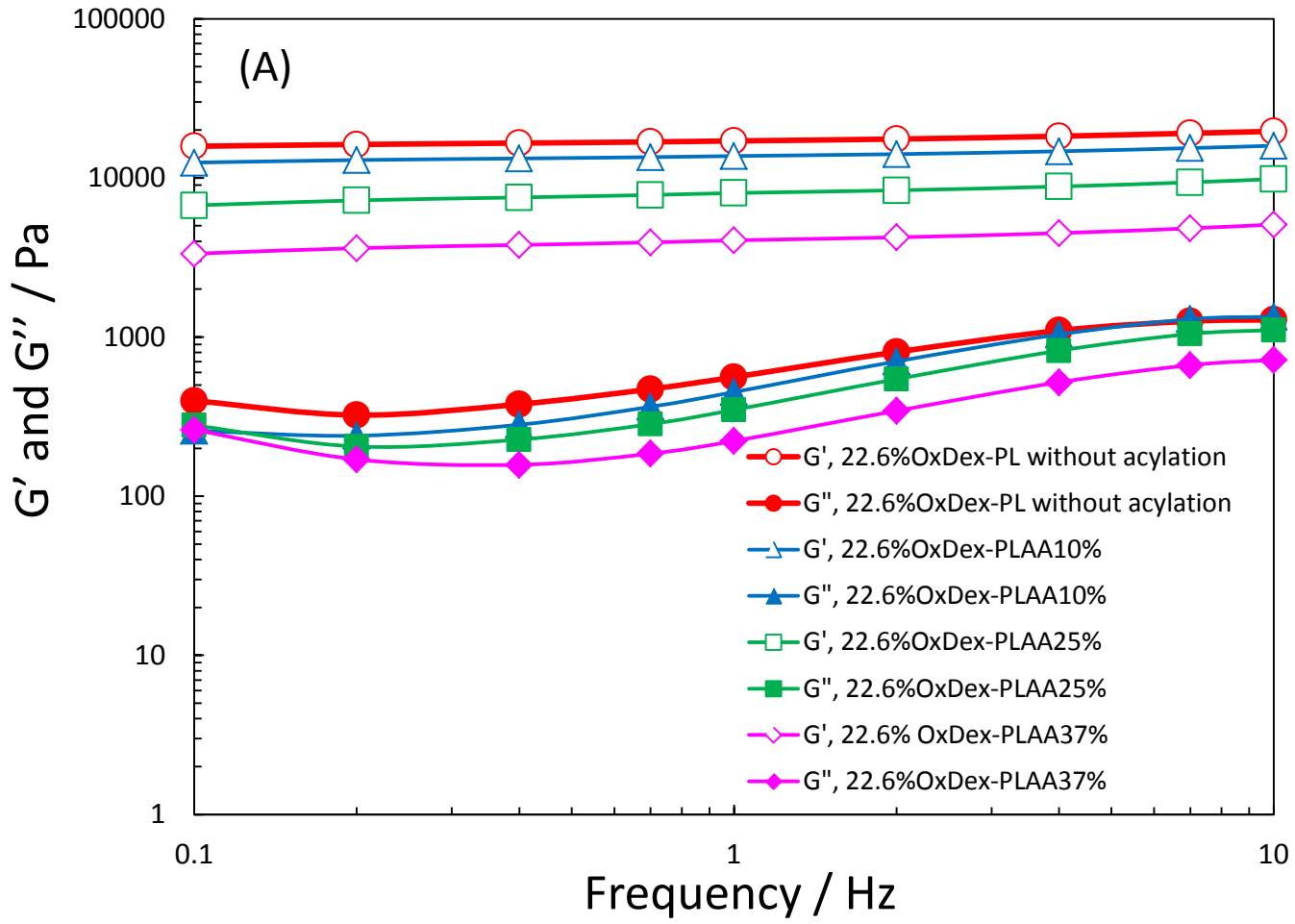

(A)
100000
10000
1000
100
10
1
0.1
1
10
G' and G'' / Pa
Frequency / Hz
G', 22.6%OxDex-PL without acylation
G", 22.6%OxDex-PL without acylation
G', 22.6%OxDex-PLAA10%
G", 22.6%OxDex-PLAA10%
G', 22.6%OxDex-PLAA25%
G", 22.6%OxDex-PLAA25%
G', 22.6% OxDex-PLAA37%
G", 22.6%OxDex-PLAA37%

Figure 2A

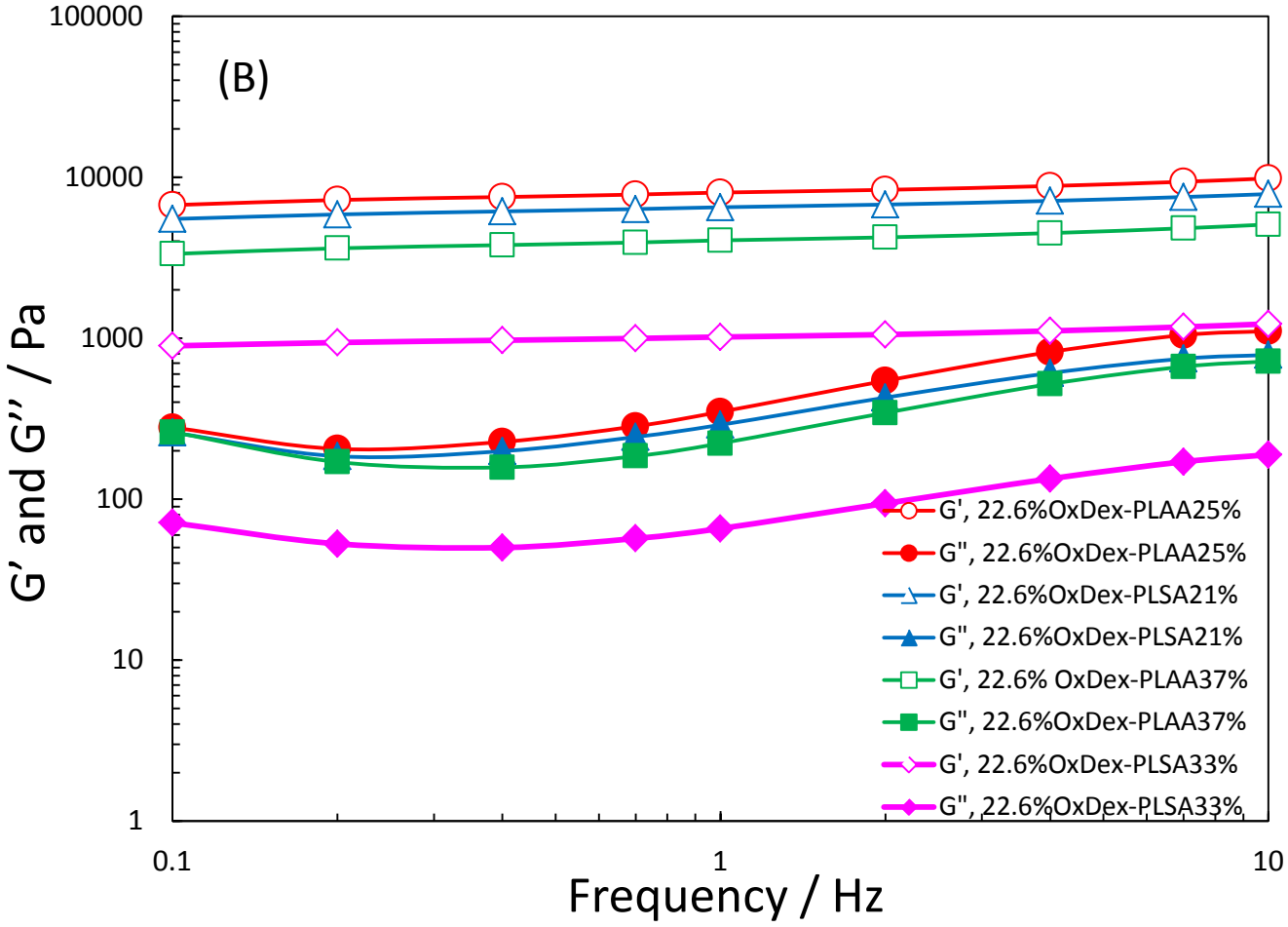

(B)
100000
10000
1000
100
10
1
G’ and G’’ / Pa
0.1
1
10
Frequency / Hz
G', 22.6%OxDex-PLAA25%
G", 22.6%OxDex-PLAA25%
G', 22.6%OxDex-PLSA21%
G", 22.6%OxDex-PLSA21%
G', 22.6% OxDex-PLAA37%
G", 22.6%OxDex-PLAA37%
G', 22.6%OxDex-PLSA33%
G", 22.6%OxDex-PLSA33%

Figure 2B

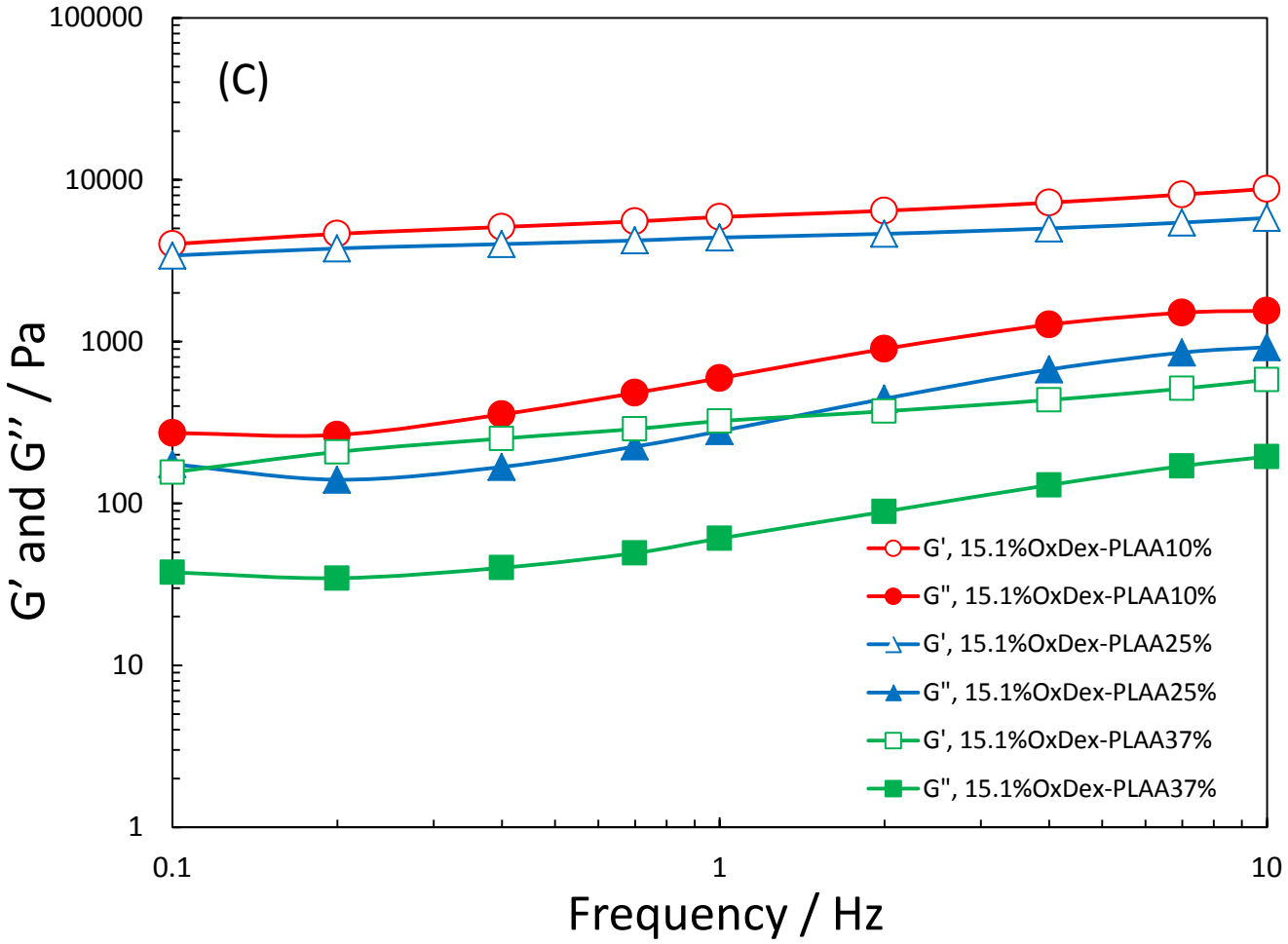

(C)
100000
10000
1000
100
10
1
G’ and G’’ / Pa
0.1
1
10
Frequency / Hz
G', 15.1%OxDex-PLAA10%
G", 15.1%OxDex-PLAA10%
G', 15.1%OxDex-PLAA25%
G", 15.1%OxDex-PLAA25%
G', 15.1%OxDex-PLAA37%
G", 15.1%OxDex-PLAA37%

Figure 2C

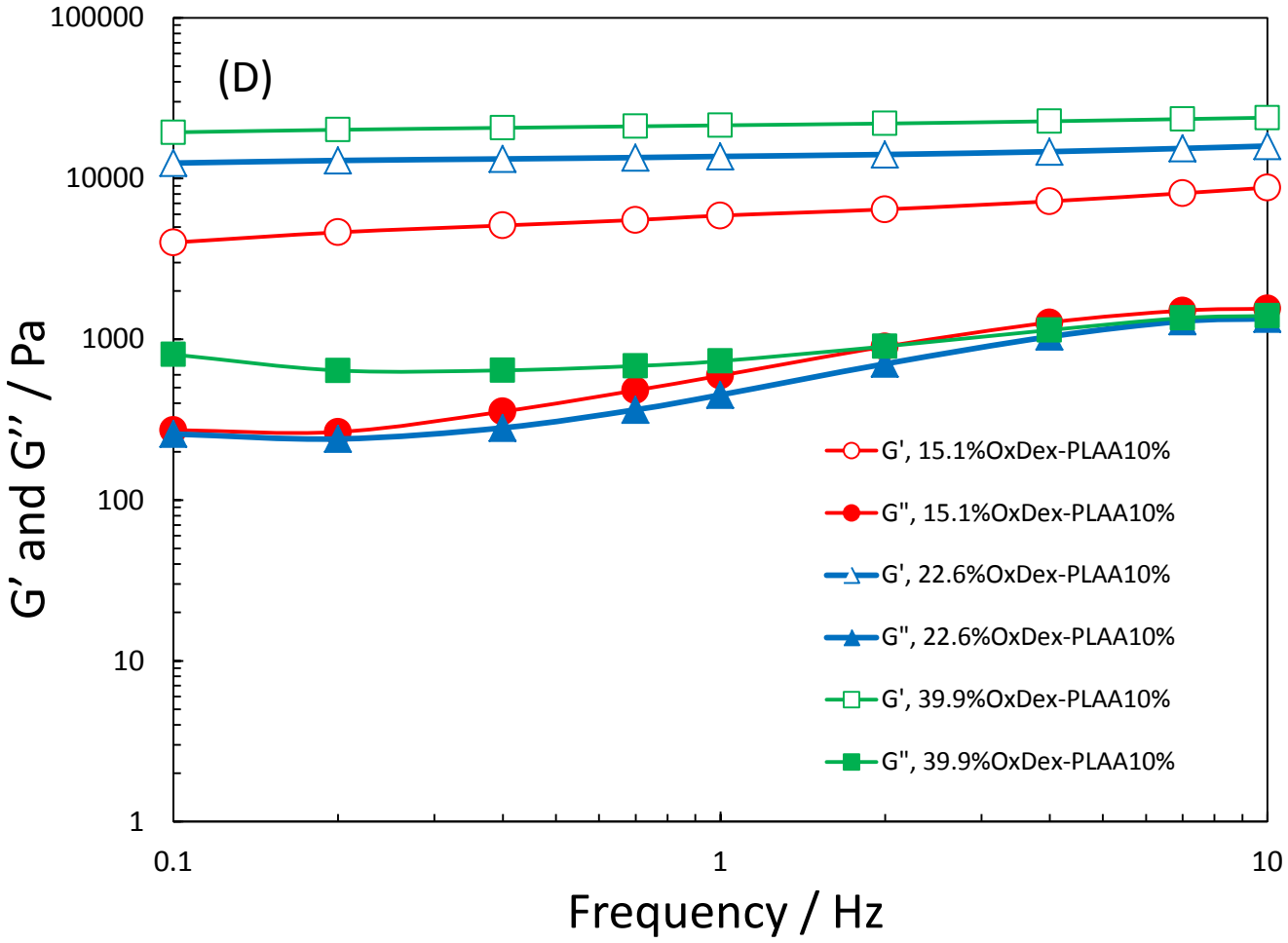

(D)
100000
10000
1000
100
10
1
0.1
1
10
G’ and G’’ / Pa
Frequency / Hz
G', 15.1%OxDex-PLAA10%
G", 15.1%OxDex-PLAA10%
G', 22.6%OxDex-PLAA10%
G", 22.6%OxDex-PLAA10%
G', 39.9%OxDex-PLAA10%
G", 39.9%OxDex-PLAA10%

Figure 2D

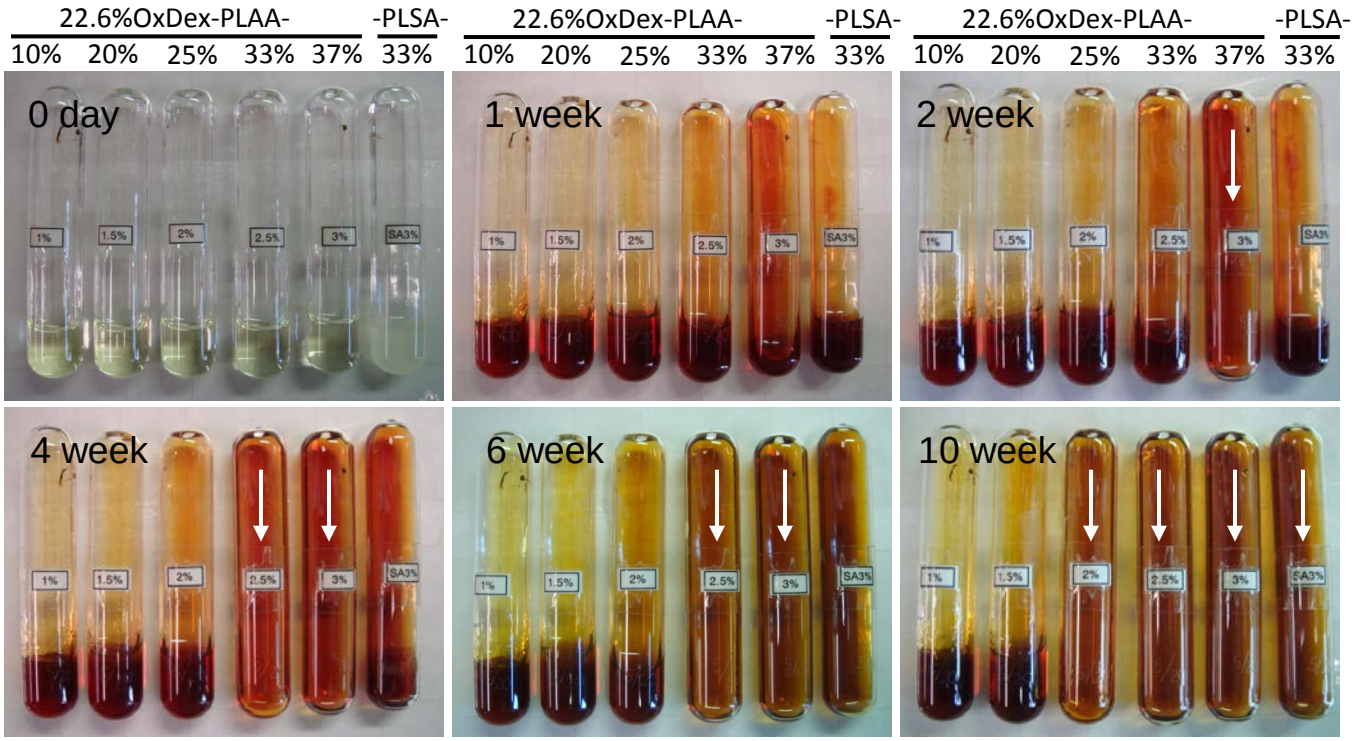

22.6%OxDex-PLAA-
-PLSA-
10% 20% 25% 33% 37% 33%
0 day
1 week
2 week
4 week
6 week
10 week
1%
1.5%
2%
2.5%
3%
SA3%

Figure 3

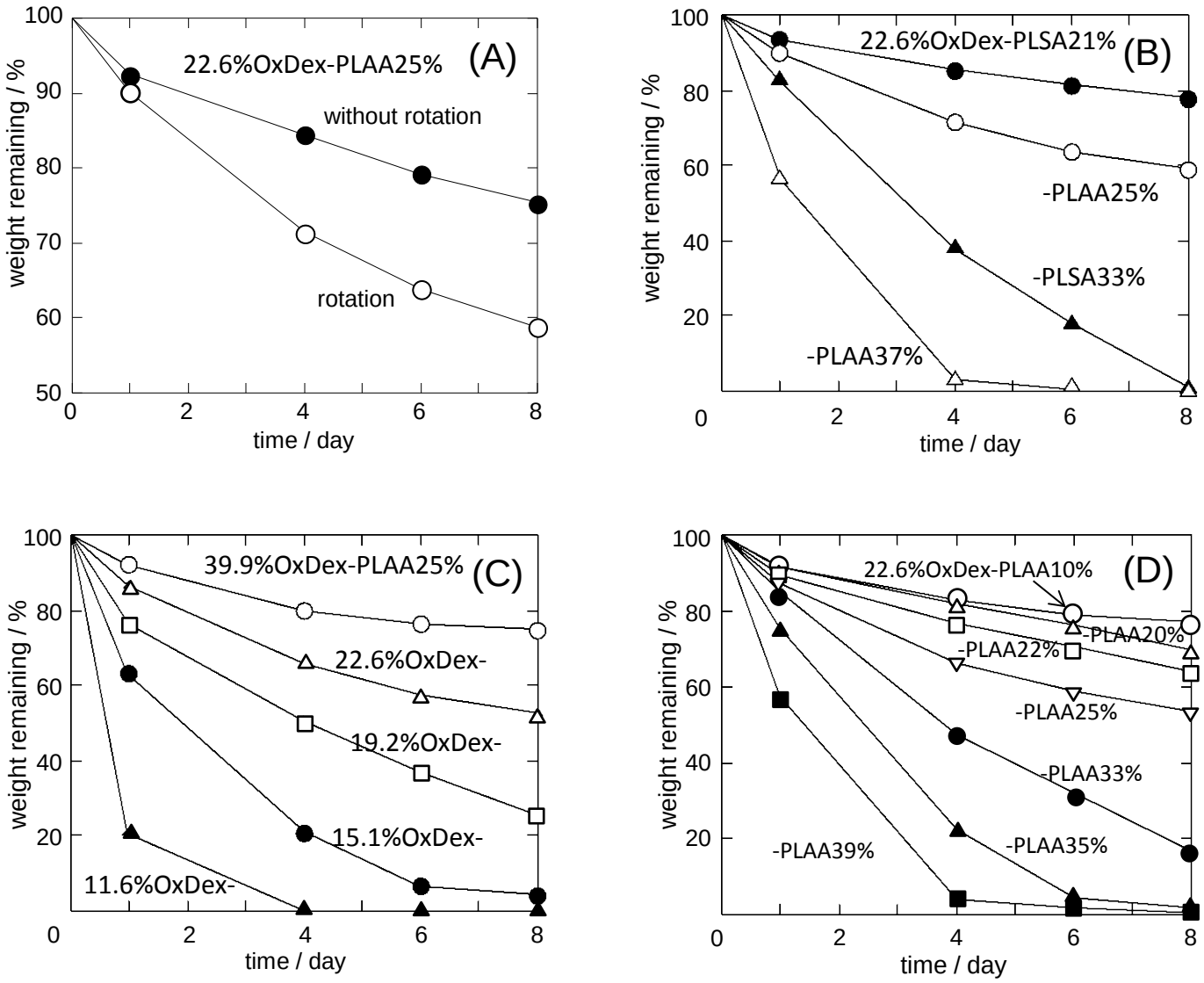

22.6%OxDex-PLAA25%
(A)
without rotation
rotation
weight remaining / %
time / day
22.6%OxDex-PLSA21%
(B)
-PLAA25%
-PLSA33%
-PLAA37%
39.9%OxDex-PLAA25%
(C)
22.6%OxDex-
19.2%OxDex-
15.1%OxDex-
11.6%OxDex-
22.6%OxDex-PLAA10%
(D)
-PLAA20%
-PLAA22%
-PLAA25%
-PLAA33%
-PLAA35%
-PLAA39%

Figure 4

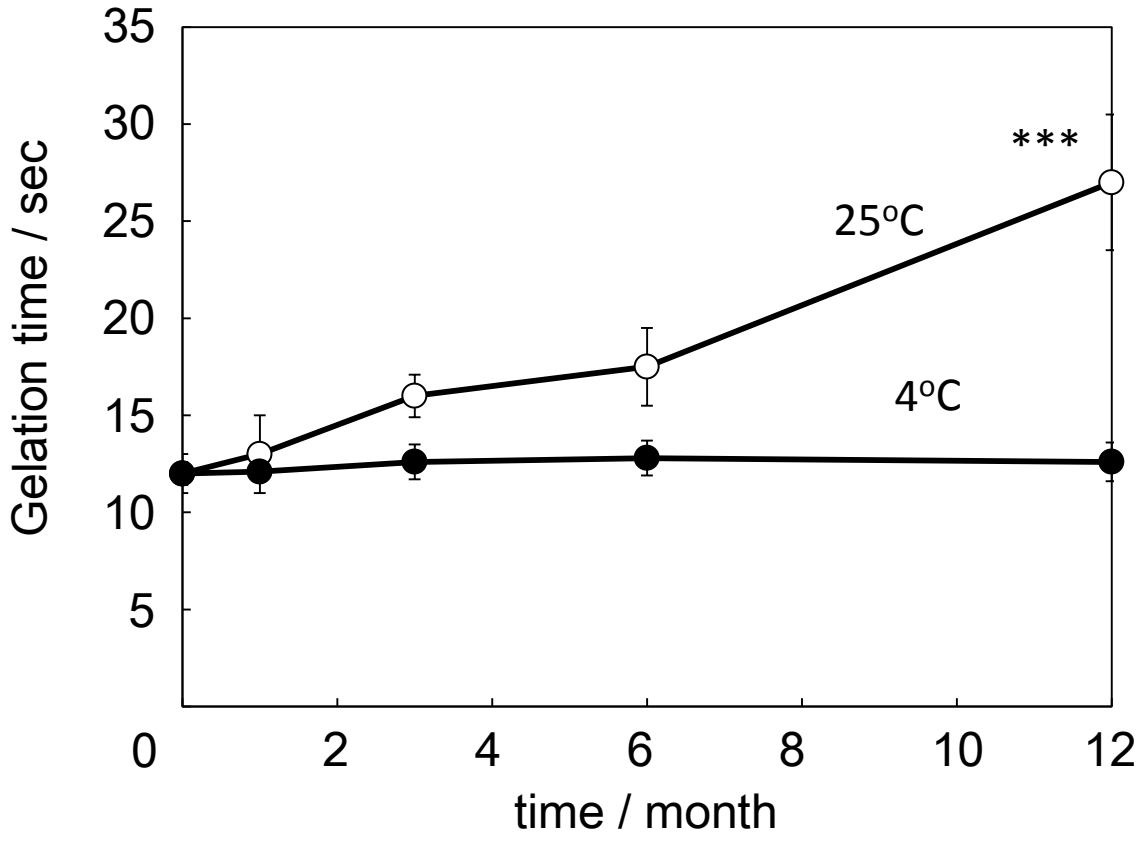

35
30
25
20
15
10
5
0
2
4
6
8
10
12
Gelation time / sec
time / month
***
25°C
4°C

Figure 5