



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



Kazuaki Matsumura^a, Naoki Nakajima^b, Hajime Sugai^b, Suong-Hyu Hyon^{c,*}

^a School of Materials Science, Japan Advanced Institute of Science and Technology, 1-1 Asahidai, Nomi, Ishikawa 923-1292, Japan

^b BMG Incorporated, 45 Minamimatsunoki-cho, Higashikujo, Minami-ku, Kyoto 601-8023, Japan

^c Center for Fiber and Textile Science, Kyoto Institute of Technology, Matsugasaki, Kyoto 606-8585, Japan

ARTICLE INFO

Article history:

Received 3 April 2014

Received in revised form 30 May 2014

Accepted 29 June 2014

Available online 6 July 2014

Keywords:

Biodegradation

Bioadhesive

Hydrogel

Dextran

Poly-L-lysine

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.

© 2014 Elsevier Ltd. All rights reserved.

1. 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 (Araki et al., 2009; Hyon, Nakajima, Sugai, & Matsumura, 2014; Naitoh et al., 2013; Takagi 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 (Czeck et al., 2013). In a previous study, we did not focus on the

* Corresponding author. Tel.: +81 75 748 1468; fax: +81 75 748 1468 1.
E-mail address: ma37ha73@kcn.jp (S.-H. Hyon).

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*-hydroxysuccinimide (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.

2. Materials and methods

2.1. 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.

2.2. 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 et al., 2014). The aldehyde content of the dextran was evaluated by simple iodometry.

2.3. 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 our previous report (Hyon et al., 2014).

2.4. Rheological measurements on hydrogels

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

2.5. In vitro gel degradation

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

added, and the tube was sealed. The degradation was observed for a given period at 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 ($n = 3$).

2.6. 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, Davey, & 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% CO₂ 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 °C, 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 °C 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 (IC₅₀).

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 °C 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.

2.7. 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 ($n = 3$).

2.8. Statistical analysis

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

3. Results and discussion

3.1. 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 Fig. 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 et al., 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.

3.2. Rheological measurements

The hydrogel strengths were investigated by performing rheological tests on the various hydrogels. Fig. 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 Fig. 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. Fig. 3(C) shows the effect of the acylation degree of 15.1%OxDex–PLAA hydrogels. Similar to the results shown in Fig. 3(A), a higher acylation ratio resulted in lower dynamic moduli.

Fig. 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.

3.3. 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 Fig. 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 PLAA1037 wt% or PLSA33 wt% 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 (Huang, Soliman, Rosen, & Ho, 1987; Shen, Tseng, & Wu, 2007). 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% and 22.6%OxDex–PLAA20% were selected.

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

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

Fig. 4(C) shows the effects of dextran oxidation and aldehyde introduction on gel degradation; 11.6–39.9%OxDex–PLAA25% hydrogels were used. When dextran with the lowest oxidation

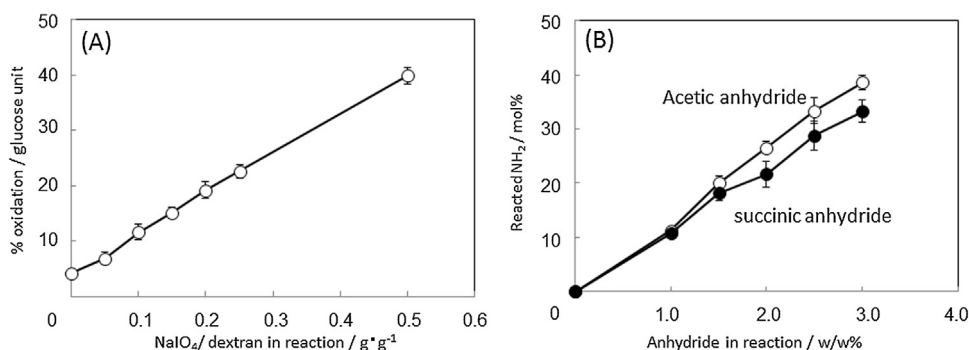


Fig. 1. Effect of periodate concentration on dextran oxidation (A) (a 20 g sample of dextran in 80 mL of water and 0–10 g of sodium periodate in 40 mL of water were mixed, and the reaction was allowed to proceed at 50 °C for 1 h), and the effect of anhydride concentration on PL acylation (B) (10 wt%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

degree (11.6%) was mixed with the PLAA25%, the hydrogel almost disappeared within 4 d under rotation. An increase in the remaining fraction was associated with an increase in the extent of oxidation, and a slower degradation was associated with a higher extent of dextran oxidation.

The effects of AA concentration on acylation and subsequent gel degradation were also investigated [data shown in Fig. 4(D)]; 22.6%OxDex–PLAA10–39% hydrogels were used. For

the 22.6%OxDex–PLAA10% hydrogel, approximately 80% of the hydrogel remained after 8 d. In contrast, 96% of the hydrogel was degraded within 4 d when the 22.6%OxDex–PLAA39% hydrogel was used. Although the degradation profiles were almost the same as those shown in Fig. 4(C), a significantly narrower range of anhydride concentrations was required for a wide range of degradation; this might be ascribed not only to the differences in acylation and the decrease in the amino group content of the PL, but also to

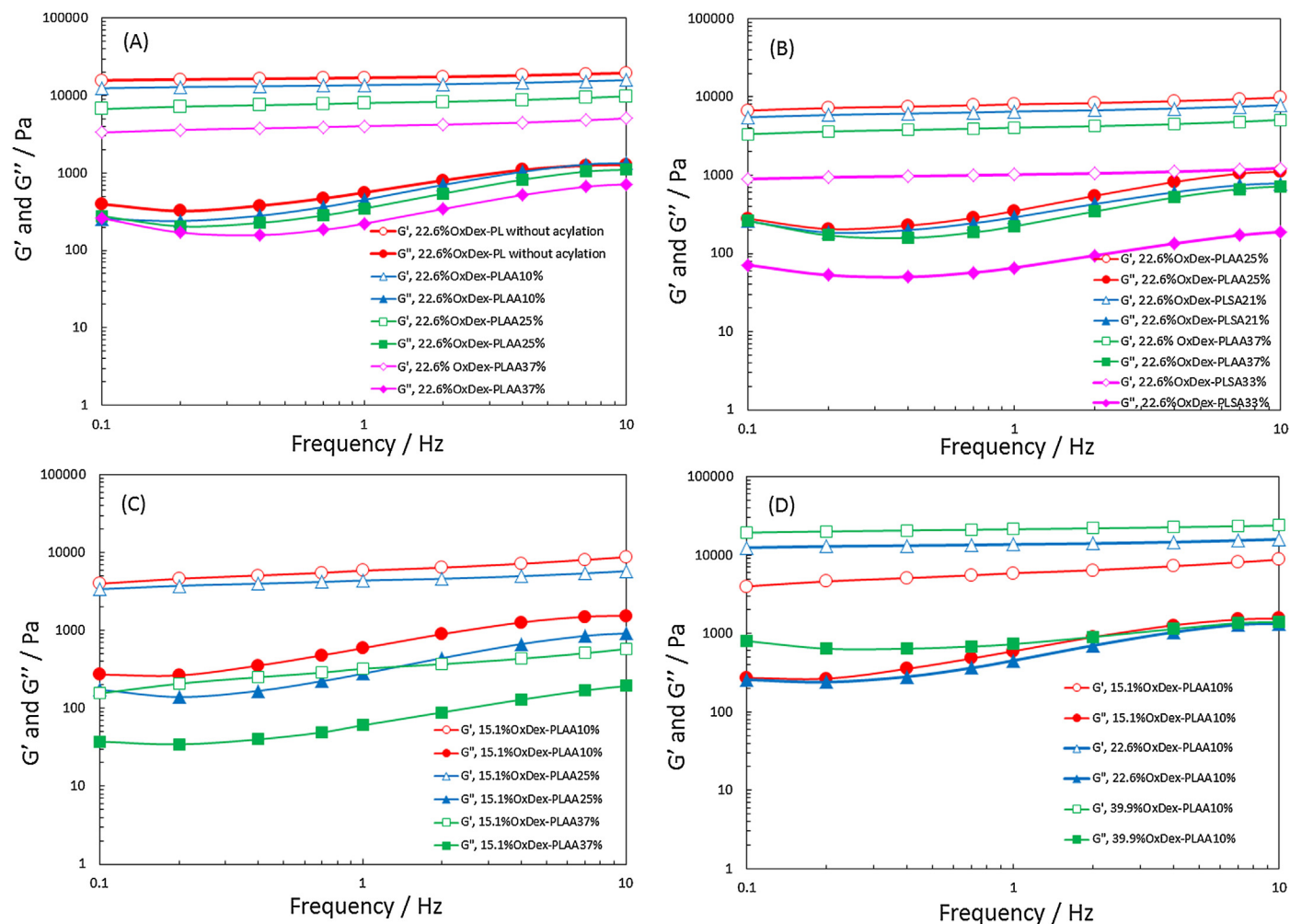


Fig. 2. Dynamic moduli of various dextran–PL hydrogels: G' and G'' of (A) 22.6%OxDex and various AA-substituted PL hydrogels; (B) 22.6%OxDex and PLAA25%, PLAA37%, and PLSA33% hydrogels; (C) 15%OxDex and various AA-substituted PL hydrogels; and (D) various OxDex percentages and PLAA10% hydrogels.

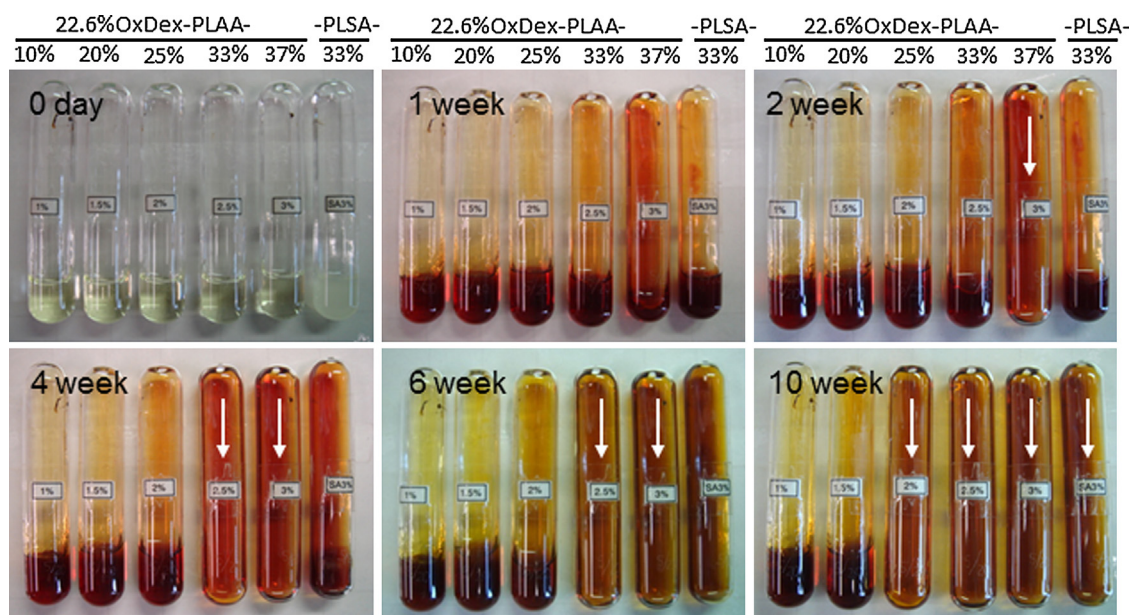


Fig. 3. Degradation of dextran–PL hydrogel at 37 °C in PBS. One milliliter of 20 wt% aqueous aldehyde dextran (22.6%OxDex) was mixed with 1 mL of 10 wt% PLAA10–37% or PLSA33%. Arrows mark the completion of degradation.

the increase in amino group dissociation induced by acetic acid, a byproduct of the acylation, which suppresses the crosslinking reaction. This analysis can also be used to explain the mechanical properties of these hydrogels (Fig. 2).

3.4. Cytotoxicity

The cytotoxicity of the dextran–PL gel degradation products was also evaluated, and the results are given in Table 1. A non-gelating

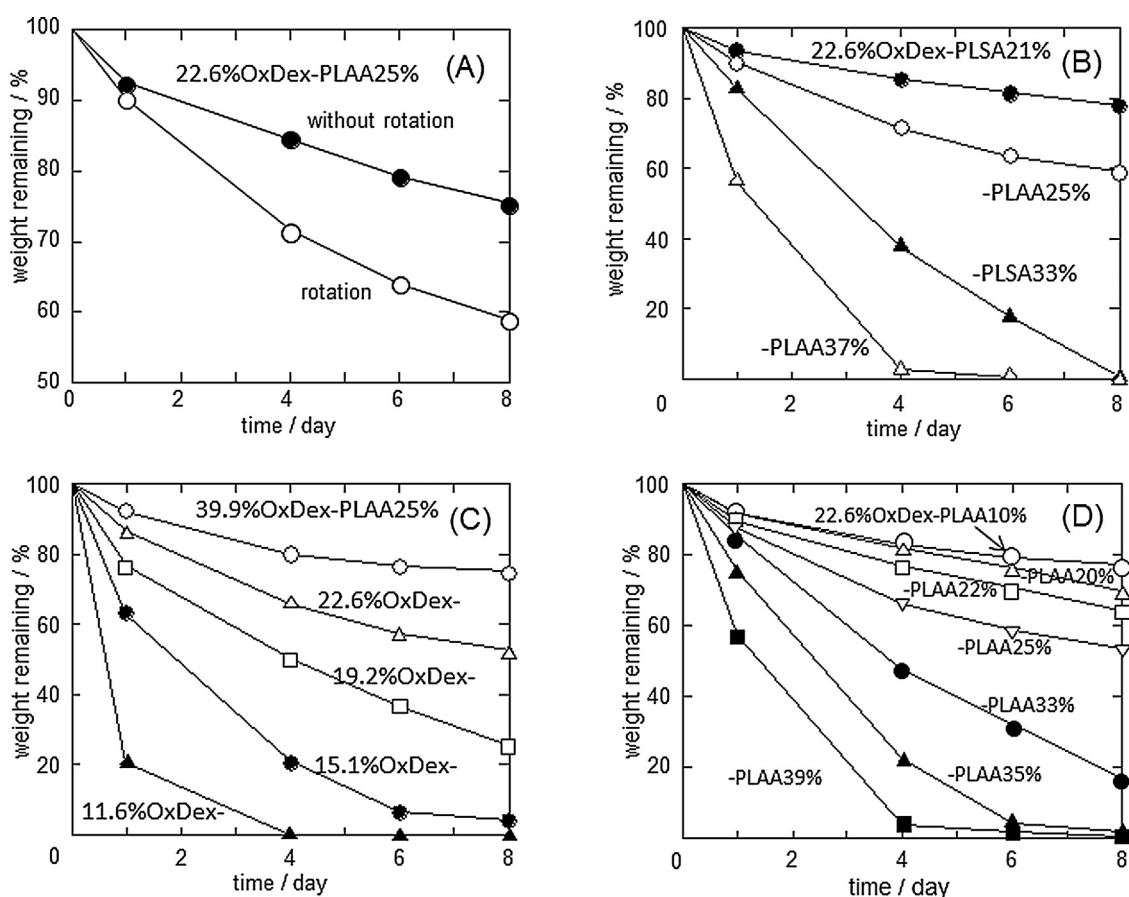


Fig. 4. Quantitative dextran–PL gel degradation at 37 °C in PBS: (A) effect of rotating incubation on degradation speed; (B) comparison of effects of different anhydride species used in PL acylation on gel degradation; (C) effect of dextran oxidation on gel degradation; and (D) effect of AA concentration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

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

Material	IC ₅₀ (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 ($n = 8 \times 8$).

^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.

mixture consisting of 22.6%OxDex and PLAA25% was also investigated. The IC₅₀ of the gel degradation products was 9000 ppm; this value was almost in the same order of those of aldehyde dextran (22.6%OxDex; 5000) and PLAA25% (9200), indicating that the degradation products also showed very low cytotoxicity. Because the mobilities of the aldehyde dextran and PL molecules were considerably suppressed in the hydrogels, it is likely that the amounts of aldehyde and amino groups remaining in the hydrogels were higher than those in the non-gelating mixture. However, almost the same IC₅₀ values were found, regardless of gelation, suggesting again that the aldehyde groups in the dextran and the amino groups in the PL have low cytotoxicities.

3.5. Stabilities of aldehyde dextran and PL in aqueous media

Aldehyde dextran and PL were dissolved in water, and their stabilities were evaluated based on the gelation time change during storage. The gelation point can often be determined by the crossing point of G' and G'' , but, in our case, because some hydrogels formed within 1 min of mixing the oxidized dextran and acylated PL, we chose a conventional stirring method (see Section 2.7) instead of rheological measurements. The results are shown in Fig. 5. At 25 °C, the gelation time gradually increased with storage time, and a 15.5 s delay was observed after 12 months (from 11.8 to 27.3 s). In contrast, 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 PLAA21% 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

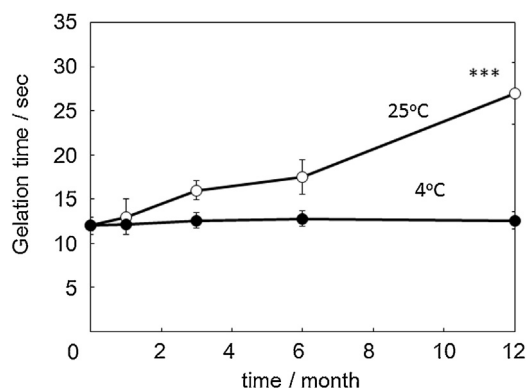


Fig. 5. Gelation time change over long storage times at 4 and 25 °C. Separately stored 20 w/w% aldehyde dextran (22.6% oxidation) and 10 w/w% PLAA21% were mixed and the gelation time was measured at 37 °C. *** $P < 0.001$.

the concentration of anhydride species in PL acylation, as shown in Fig. 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, 2009; Tsujita et al., 2012), prevention of alveolar air leakage in lung surgery (Araki et al., 2007), and tissue regeneration in orthopedic (Kazusa et al., 2013; Yamamoto et al., 2008) 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.

Development of the hydrogel from clear to brown, shown in Fig. 3, indicates that the reaction between the aldehydes of the dextran and the amino groups of the PL might be based on a Maillard reaction via Schiff base formation, similar to the reaction between glutaraldehyde and food proteins (Gerrard, Brown, & Fayle, 2003). Schiff base formation is generally reversible under acidic or basic conditions, but the colorization continued even after gel degradation and decolorization was no longer observed, as indicated in Fig. 3. It is therefore likely that the degradation reaction of the hydrogel is independent of gelation, the crosslinking reaction, and color development.

The aqueous aldehyde dextran and PL solutions were quite stable for at least 12 months at 4 °C (Fig. 5). Usually, the amide bonds in peptide bonds in proteins are very stable at neutral pH and 37 °C, and the high thermal stability of PL has been reported previously (Hiraki, 1995); these facts suggest that degradation of PL in the hydrogel was unlikely to proceed in PBS at 37 °C. In addition, the browning reaction was sustained even after gel degradation occurred, as mentioned above. These findings suggest that gel degradation could be ascribed to degradation of the aldehyde dextran in the hydrogel, although the aldehyde dextran itself was relatively stable in water. The degradation profiles of the hydrogels after storage for 12 months at 4 °C were quite similar to those for the degradation of the fresh hydrogels (data not shown). The degradation of aldehyde dextran, therefore, might begin with the reaction between the aldehyde groups in the dextran and the amino groups in 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.

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. *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. *Artificial 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.
- Canonica, S. (2003). The use of human fibrin glue in the surgical operations. *Acta BioMedica*, 74(Suppl 2), 21–25.
- Czeck, Z., Wilpiszewskab, K., Tyliczszak, B., Jiangd, X., Baid, Y., & 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, A*, <http://dx.doi.org/10.1002/jbm.a.34923> (in press).
- 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. *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 biogel. *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 viability 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, H. N., Jr., 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 chemically defined 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). Aldehyde 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.