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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.

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

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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.

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% 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 °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 (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 °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%