General Thoracic and Cardiovascular Surgery (2020) 68:793–800
https://doi.org/10.1007/s11748-020-01297-3

ORIGINAL ARTICLE

Management of retrosternal adhesion after median sternotomy by controlling degradation speed of a dextran and ε-poly (L-lysine)-based biocompatible glue

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Received: 6 September 2019 / Accepted: 14 January 2020 / Published online: 24 January 2020

Abstract

Objective Retrosternal adhesion after median sternotomy possibly raises the risk of cardiac injury at resternotomy. A biodegradable glue "Lydex" is composed of food additives, dextran and ε-poly (L-lysine), and the degradation speed can be controlled by the composition. In the present study, we evaluated the preventative effect of Lydex on retrosternal adhesion and the relationship between degradation speed and the progression of retrosternal fibrosis.

Methods Japanese white rabbits are subjected to median sternotomy. Lydex 1, 2 and 3 were loaded at the retrosternal space of rabbits in allocated groups before sternal closure, respectively ($n=11$ for each group). Retrosternal adhesion was macroscopically evaluated after surgery. Retainment of Lydex, retrosternal fibrosis and the infiltration of macrophages are histologically evaluated, respectively.

Results All Lydex groups exhibited less retrosternal adhesion at 4 weeks after loading compared to unloaded control. The degradation speed of Lydex varied according to the compositions. Lydex with faster degradation (Lydex 2 or Lydex 3) showed lower progression of retrosternal fibrosis compared to that with slower degradation (Lydex 1) [fibrosis ratio: control vs Lydex 1 vs Lydex 2 vs Lydex 3: 0.60 ± 0.15 vs 0.18 ± 0.17 vs 0.00 ± 0.00 vs 0.00 ± 0.00, $P=0.0005$ (Lydex 1 vs Lydex 2), $P=0.0005$ (Lydex 1 vs Lydex 3)]. Retrosternal infiltrations of macrophages in Lydex 1 and Lydex 3 groups are not higher compared to that in unloaded control.

Conclusions The degradation speed of Lydex could be controlled according to the compositions. The degradation speed affected the progression of retrosternal fibrosis.

Keywords Cardiac surgery · Median sternotomy · Retrosternal adhesion · Biomaterials

Introduction

Retrosternal adhesion after cardiac or vascular surgery accompanying median sternotomy raises the operative risk of re-surgery due to possible injury of heart or surrounding organs [1], or increased demand for blood transfusion [2]. To prevent the progression of retrosternal adhesion, biological barrier films or synthetic materials can be placed at the timing of initial sternotomy [3]. However, the clinical advantages are reported to be marginal [4] and inflammatory processes due to the implants may result in localized constrictive pericarditis [5]. To overcome the situation, highly biocompatible materials are anticipated for the purpose of adhesion control.

We have previously reported a novel biocompatible glue named "Lydex" [6–8]. Lydex is a biodegradable glue derived from dextran and ε-poly (L-lysine), food additives commonly used in our daily life without healthcare problems and are approved by the US Food and Drug Administration. In our rabbit median sternotomy model, Lydex could efficiently reduce retrosternal adhesion through the attenuation

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of the infiltration of macrophages and progression of fibrosis compared to animals implanted with expanded polytetrafluoroethylene (ePTFE), indicating an excellent biocompatibility of Lydex [6, 9].

Lydex holds a unique property that the degradation speed can be controlled according to the compositions and/or modifications of aldehyde dextran and ε-poly (L-lysine) [10]. Faster degradation after surgery results in less retainment of Lydex which may be advantageous for the attenuation of foreign body-mediated inflammatory processes but disadvantageous for the sustained effect of Lydex to prevent retrosternal adhesion. It remains unclear how long Lydex should be retained at the retrosternal space to ideally prevent retrosternal adhesion in vivo.

In the present study, we examined whether the compositional change of Lydex affects the degradation speed and retrosternal retainment of Lydex using our rabbit median sternotomy model loaded with multiple types of Lydex, and investigated whether the degradation speed of Lydex affects the extent of retrosternal fibrosis and adhesion.

Methods

The study protocol was approved by the Kyoto University Ethics Committee for Animal Research. All animals received humane care in compliance with the guidelines prescribed in the Principles of Laboratory Animal Care, formulated by the National Society for Medical Research, and the Guide for the Care and Use of Laboratory Animals, created by the Institute of Laboratory Animal Resources of the National Research Council and published by the National Academy Press (revised 2011).

Biocompatible glue, Lydex

The biocompatible glue "Lydex" was provided by BMG Inc, Kyoto, Japan. Lydex is composed of a Schiff base formulation of aldehyde dextran and ε-poly (L-lysine). In the present study, we used three different classes of Lydex: Lydex 1, Lydex 2 and Lydex 3 with different compositions of aldehyde dextran and ε-poly (L-lysine). Lydex 1 was designed for slow degradation, and Lydex 3 for fast degradation (Lydex 2 for intermediate degradation).

For preparation of Lydex 1 and 2, 20 g of dextran-70 (MW: 70 kDa; Meiyo Sangyo Co., Ltd., Aichi, Japan) was dissolved in 80 ml of distilled water, then 3.0 g of sodium periodate dissolved in 40 ml of water was added to the dextran. For preparation of Lydex 3, dextran-40 (MW: 40 kDa) was used instead of dextran-70. For Lydex 2 and Lydex 3, 2.5 g of sodium periodate dissolved in 40 ml water was added to the dextran. The oxidation reaction proceeded at 50 °C for 1 h followed by dialysis, air-drying and crushing into fine powder composed of granules < 0.1 mm. Next, 0.5 g of succinic anhydride was added to 20 g of aqueous ε-poly (L-lysine) (25 w/w%, 4 kDa; jNC Corp., Tokyo, Japan) followed by stirring at 50 °C for 1 h to induce acylation. In the process of air-drying and crushing into fine powder composed of granules < 0.1 mm, ε-poly (L-lysine) was recovered with 12 mol% of its amino groups acylated. For Lydex 1, 10 g of aldehyde dextran (–CHO content 0.30/sugar unit) and 2.5 g of ε-poly (L-lysine) powder were mixed and then sterilized by γ-ray irradiation. For Lydex 2 and Lydex 3, aldehyde dextran (–CHO content 0.27/sugar unit) was used instead of aldehyde dextran (–CHO content 0.30/sugar unit). The powder could be immediately gelled into glue through the addition of water (Fig. 1).

Experimental animal model

We used 41 male Japanese white rabbits weighting 2.8–3.5 kg. Those rabbits were randomly divided into 4 groups (control group $n = 8$, Lydex 1 group $n = 11$, Lydex 2 group $n = 11$, Lydex 3 group $n = 11$). All rabbits were sedated by intravenous injection of ketamine hydrochloride (5 mg/kg) and sodium thiopental (15 mg/kg). Immediately after the sedation, rabbits were intubated and mechanically ventilated (tidal volume; 10 ml/kg, respiratory rate; 30 times/min). General anesthesia was maintained with isoflurane (1.0—3.0%). Intravenous antibiotics (0.5 g of cefazolin for each) were administered 30 min before the skin incision. The chest hair was shaved, and the skin was disinfected with iodine-alcohol solution. Each rabbit was placed on the operation table in the supine position. A midline skin

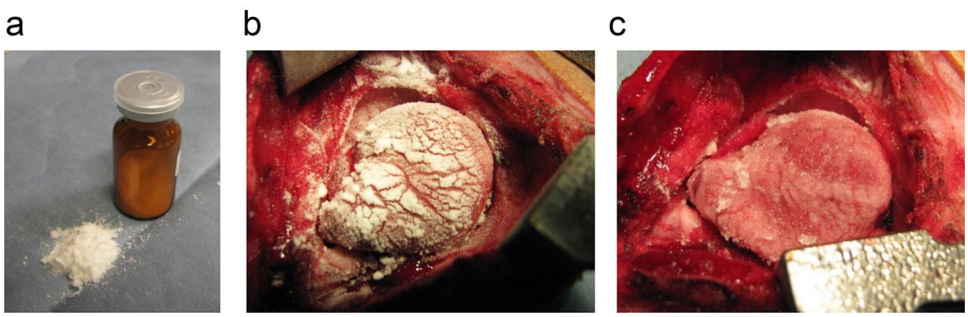

Fig. 1 Gross appearance of Lydex. **a** Lydex powder preserved in a vial. **b** Lydex applied onto a surface of a rabbit heart. **c** After application of water. Lydex turned into a gel form

incision was performed from the neck to the epigastric part, then a median sternotomy was conducted with surgical scissors. A sternal retractor was placed, and the pericardium was resected as large area as possible. In control group, no material was applied to the pericardial defect. In Lydex 1, 2 and 3 groups, 3 types of Lydex were loaded to the site of pericardial defect, respectively. We used 0.5–1.3 g of Lydex for each animal. The sternum was closed with interrupted Nylon sutures. The muscle was closed with interrupted polyglycolic sutures and the skin was closed with continuous Nylon sutures.

Macroscopic evaluations

Four weeks after the initial operation, five rabbits in each group underwent re-sternotomy under general anesthesia. We evaluated the adhesion in the retrosternal space macroscopically and scored the adhesion score which was established by Heydorn and colleagues [11]. The adhesion score was classified from 0 to 4 as mentioned below; 0 = no adhesion, 1 = trivial adhesion that could be readily separated by finger dissection, 2 = mild adhesion requiring blunt dissection, 3 = severe adhesion requiring sharp dissection, and 4 = not dissectable without heart injury.

Histological evaluations

Four weeks after the initial operation, three rabbits in each group were sacrificed for microscopic evaluation, respectively. The entire thorax, including thoracic wall, heart and bilateral lungs, was removed en bloc through a midline skin incision after the perfusion fixation with 10% formaldehyde. After the removal, samples were soaked into 10% formaldehyde for 1 day followed by soaked into 10% of ethylenediaminetetraacetic acid aqueous solution for about 10 days for the purpose of decalcification, then embedded in paraffin. The sections were subjected to hematoxylin and eosin staining and Sirius red staining, respectively. We used mouse monoclonal anti-rabbit macrophage antibody RAM11 (Dako, Glostrup, Denmark; catalog No. M0633) for the immunostaining of macrophages. The fibrotic area was automatically measured with a computer-based method. Five high-magnification (3200×) views from retrosternal space of Sirius red staining sections per animal were randomly selected and subjected to automatic measurement of red fibrotic area using BZ-9000 Software (Keyence Corp., Osaka, Japan), and the fibrotic area ratio was calculated as the average of the values of the five views. We counted the number of macrophages of randomized five views with a magnification of 3200× for each animal with RAM11 immunostaining samples. To evaluate the retainment of Lydex, animals at 1 week, 2 weeks and 3 weeks after the initial operation (1 animal for each timing and each Lydex composition) were killed and subjected to hematoxylin and eosin staining, respectively. Hematoxylin and eosin staining samples from three rabbits killed at 4 weeks after the initial operation were also used to evaluate the retainment of Lydex.

Statistics

All data are described as mean ± standard deviation. All data were analyzed by one-way analysis of variance with Tukey's test as post hoc. The statistical analysis was performed using a computer software (JMP Pro 14.0.0, SAS Institute Inc., Cary, USA). P value < 0.05 was considered statistically significant.

Results

Macroscopic findings

All rabbits survived during the observation period up to 4 weeks. Rabbits in each group were macroscopically examined for retrosternal adhesion at 4 weeks after surgery, respectively (Fig. 2a). There was no sign of pericarditis in all rabbits macroscopically. Whereas rabbits of control group exhibited severe retrosternal adhesion, those of Lydex groups presented macroscopically less adhesion. The adhesion scores were 3.20 ± 0.84 in control group, 1.80 ± 0.45 in Lydex 1 group, 1.20 ± 0.45 in Lydex 2 group and 1.40 ± 0.89 in Lydex 3 group, respectively. Control group showed significantly higher adhesion score compared to those of Lydex groups, respectively [P = 0.025 (vs Lydex 1), 0.0016 (vs Lydex 2), 0.0039 (vs Lydex 3)]. There was no significant difference among three Lydex groups (Fig. 2b).

Degradation of Lydex

The extent of retained Lydex was histologically evaluated at 1, 2, 3 and 4 weeks after loading, respectively (Fig. 3). All rabbits loaded with Lydex 1, Lydex 2 or Lydex 3 exhibited large masses of retained Lydex at 1 week after loading. At 2 weeks after loading, rabbit from Lydex 1 group showed multiple masses of Lydex, whereas small fragments of Lydex were observed in Lydex 2-loaded rabbit and no retained Lydex in Lydex 3-loaded rabbit. At 3 weeks after loading, we could not find retained Lydex from all samples. However, a part of samples from Lydex 1 group exhibited retained Lydex at 4 weeks after loading. No retained Lydex was found from samples of Lydex 2 or Lydex 3 at 4 weeks after loading. Animals loaded with Lydex showed very few cellular infiltrations in retrosternal space at 4 weeks after loading, whereas control animal without loading of Lydex exhibited prominent cellular infiltration.

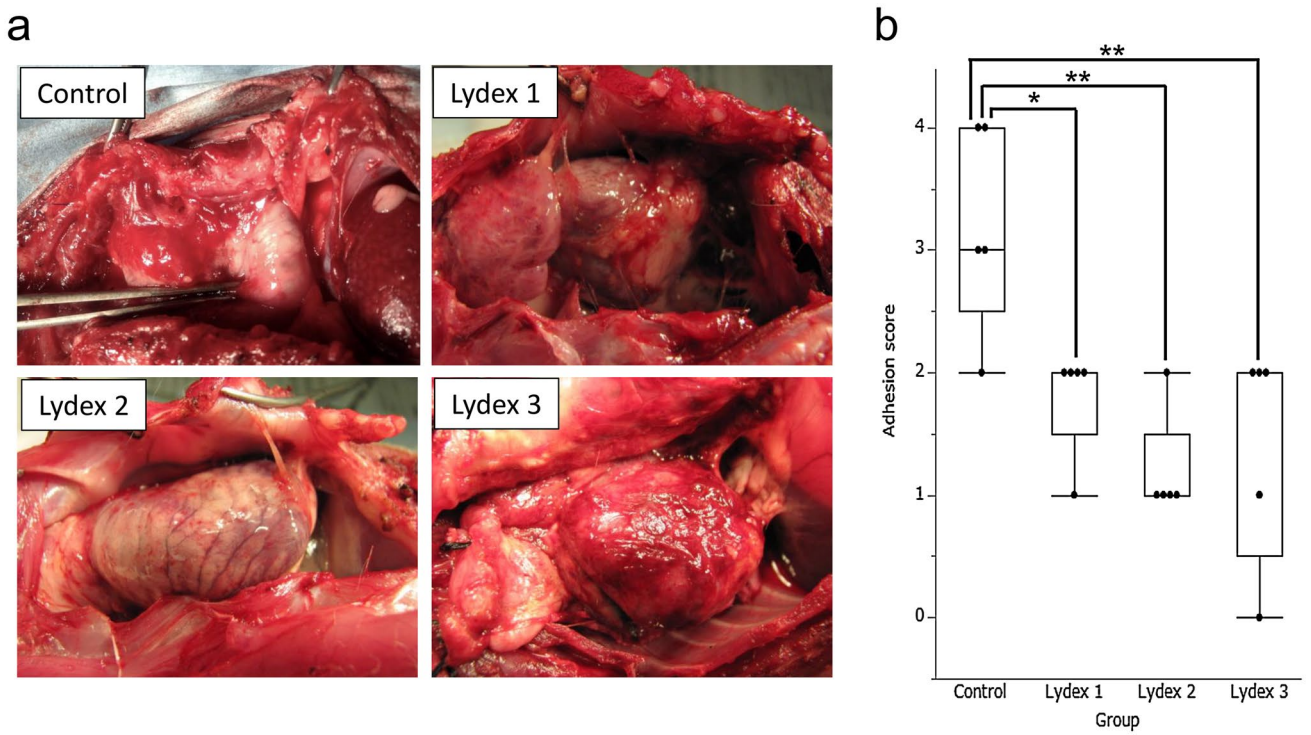

Fig. 2 Macroscopic findings after loading of Lydex. **a** Representative macroscopic view at the evaluation of retrosternal adhesion at 4 weeks after the initial operation. **b** Adhesion score. $^{*}P<0.05$, $^{**}P<0.01$

Progression of fibrosis at the retrosternal space

The extent of retrosternal fibrosis was histologically evaluated at 4 weeks after surgery (Fig. 4). Rabbits of control group showed dense fibrosis at retrosternal space, whereas few or no deposition of fibrotic tissue was observed from rabbits loaded with Lydex (Fig. 4a). The ratio of fibrotic area of control group was significantly higher compared to those of other groups [control vs Lydex 1 vs Lydex 2 vs Lydex 3: 0.60 ± 0.15 vs 0.18 ± 0.17 vs 0.00 ± 0.00 vs 0.00 ± 0.00, $P<0.0001$ (control vs Lydex 1), <0.0001 (control vs Lydex 2), <0.0001 (control vs Lydex 3)]. Rabbits from Lydex 1 group showed higher fibrosis ratio compared to those from Lydex 2 and Lydex 3 [$P=0.0005$ (Lydex 1 vs Lydex 2), $P=0.0005$ (Lydex 1 vs Lydex 3)] (Fig. 4b).

Infiltration of macrophages at the retrosternal space

Infiltration of macrophages was evaluated by immunostaining for RAM11, a macrophage marker (Fig. 5). The numbers of macrophages in each group per field were as follows; 0.33 ± 0.62 in control, 1.33 ± 1.95 in Lydex 1, 1.87 ± 1.60 in Lydex 2 and 0.87 ± 0.74 in Lydex 3. The macrophage number in Lydex 2 was significantly higher compared to that in control ($P=0.015$). There was no significant difference in macrophage numbers between control and Lydex 1, and between control and Lydex 3 [$P=0.19$ (control vs Lydex 1), 0.70 (control vs Lydex 3)] (Fig. 5b). There were no significant differences among the 3 Lydex groups as well [$P=0.70$ (Lydex 1 vs Lydex 2), 0.19 (Lydex 2 vs Lydex 3, $P=0.78$ (Lydex 1 vs Lydex 3)].

Discussion

In the present study, we have validated the potential of Lydex for the prevention of retrosternal adhesion. We have also confirmed that the degradation speed of Lydex varied according to the compositions of Lydex in vivo. Compositions with faster degradation (Lydex 2 or Lydex 3) showed lower progression of retrosternal fibrosis compared to that with slower degradation (Lydex 1). Retrosternal infiltrations of macrophages in Lydex 1 and Lydex 3 groups are not accelerated compared to that in unloaded control.

The number of patients undergoing repeated sternotomies for cardiac or vascular reoperation continues to rise [12]. Although the initial sternotomy is not hazardous in general, the retrosternal adhesion formed after the initial sternotomy potentially increases the risk of complications during the adhesion dissection such as massive hemorrhages related to cardiac injury, and prolonged operation times [4].

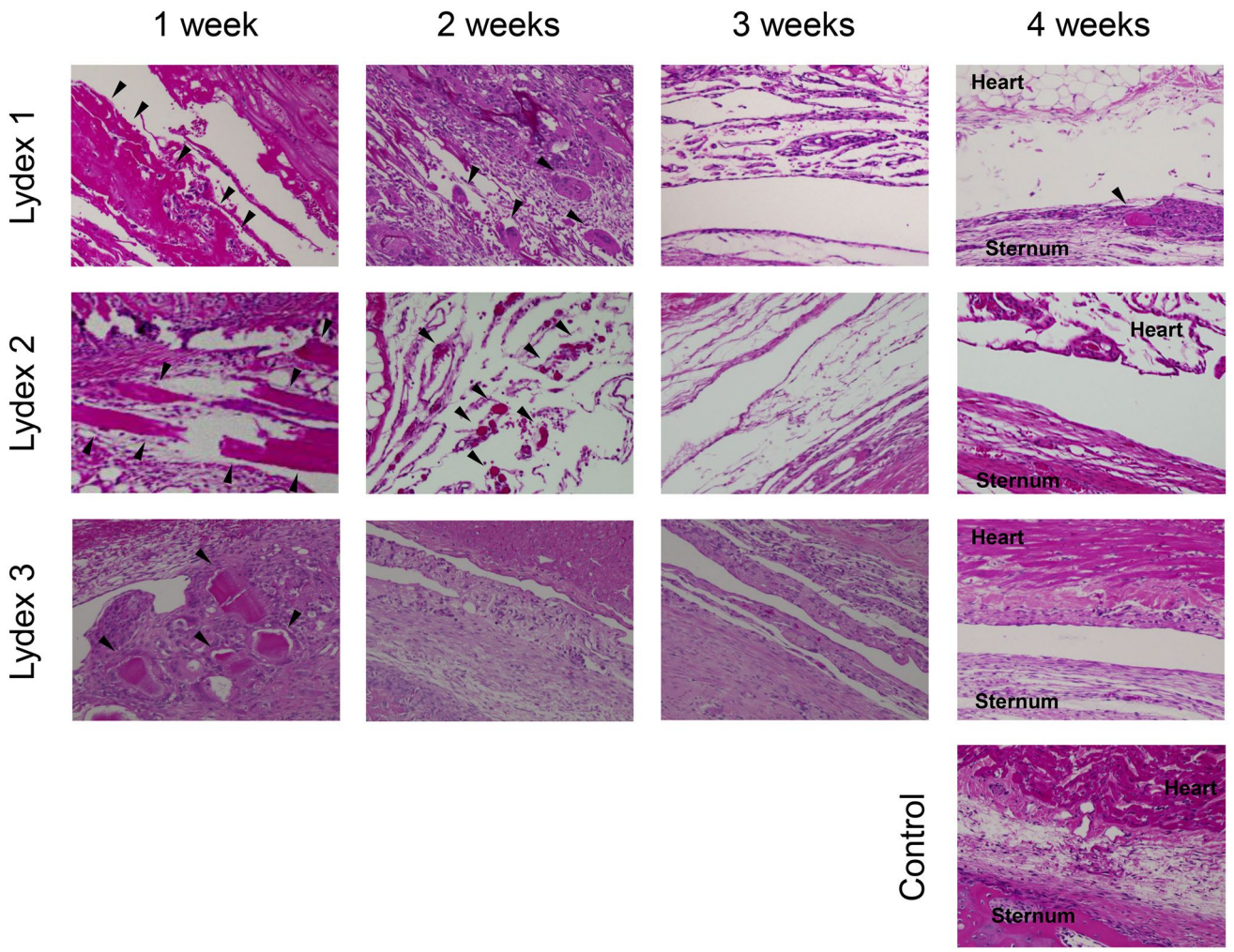

Fig. 3 Retainment of Lydex after loading. Representative Hematoxylin and eosin staining at 1, 2, 3 and 4 weeks after the initial operation, respectively. Magnification = × 100. Arrowheads indicate retained Lydex

Preventative strategies to minimize the risk have been cumulated by surgeons so far including pre-operative imaging with computed tomography [13], establishment of cardiopulmonary bypass before resternotomy via femoral cannulation [12] and specialized devices for the dissection of retrosternal adhesion such as video-assisted retrosternal dissection system using Mayfield resternotomy retractor [14]. Placement of prosthetic materials such as Dacron/ePTFE [15] or bioresorbable barrier films [3] to prevent the progression of retrosternal adhesion is reported to hold negligible clinical benefits [6] whereas a certain risk of foreign body-mediated retrosternal inflammation exists. In the present study, we have reported the potential of a biodegradable glue, Lydex as an alternative to reduce the risk of resternotomy which can be easily loaded at the timing of initial sternal closure.

Lydex is a chemical compound derived from food additives, dextran and ε-poly (L-lysine) [10] which are widely used in clinical practices and in our daily life. Various possibilities in therapeutic application of Lydex have been reported such as for an adhesive to secure hemostasis during surgeries [16], a sealant for air leakage of lung [17] and an attachment medium for sustained release of antibiotics [18] or antiarrhythmic drugs [19]. The Lydex powder immediately gels with water based on Schiff base formation. After that, it solidifies within a short period of time (approximately within 2 min) [8] which allows the material to be kept in the applied site without dislodgement and is advantageous for the broad clinical application of Lydex. As a material for prevention of retrosternal adhesion, in vivo degradation speed would be crucial because the local retainment of the material might hold advantages and disadvantages, that is, pharmacological effects on adhesion prevention as the merits and foreign body-mediated local inflammation as the demerit. The elucidation of the optimal balance would be anticipated for the clinical application, and the unique property of Lydex which allows us to easily control the

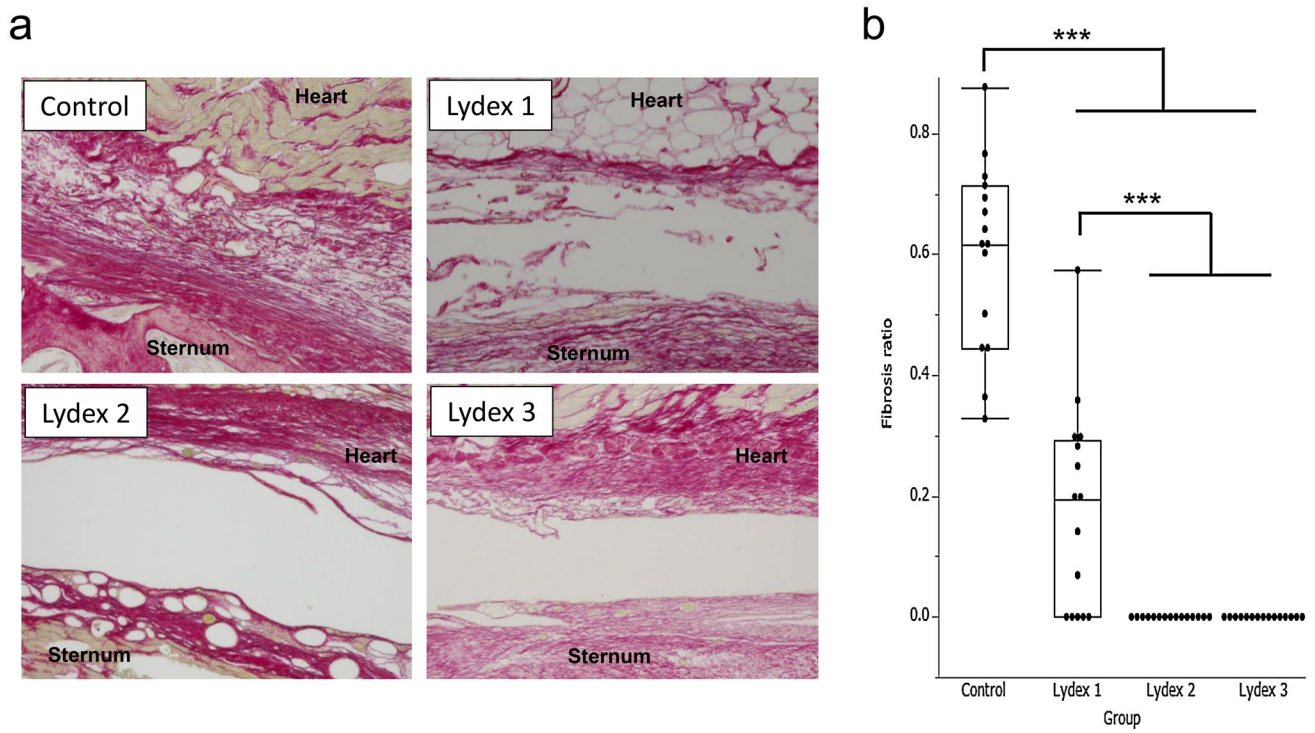

Fig. 4 Retrosternal fibrosis. **a** Representative Sirius red staining at 4 weeks after the initial operation. Magnification = × 100. **b** Fibrosis ratio. ***$P < 0.001$

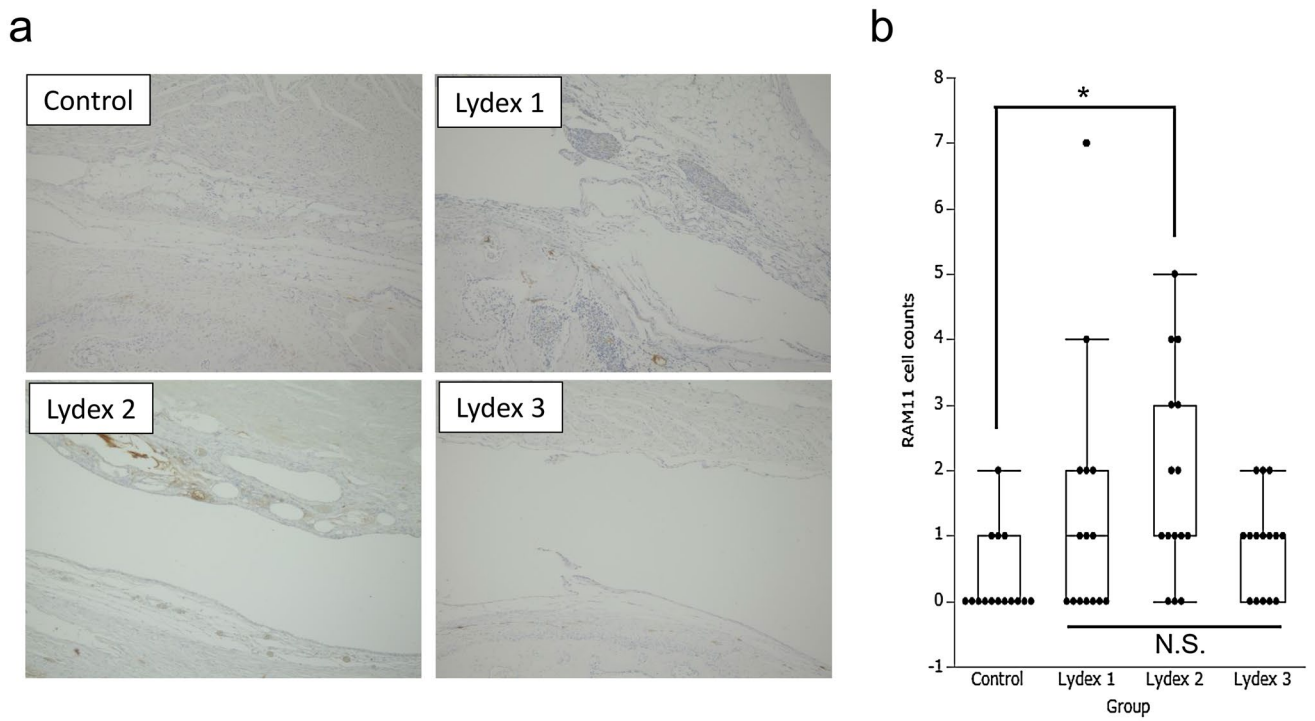

Fig. 5 Retrosternal infiltration of macrophages. **a** Representative RAM11 immunostaining at 4 weeks after the initial operation (brown). Magnification = × 100. **b** RAM11-positive cell counts. *$P < 0.05$. *N.S.* not significant

degradation speed was suitable to investigate the balance. The present study indicated that faster degradation of Lydex correlated to reduced retrosternal adhesion. It should be further investigated whether the result would be also relevant in human cases through clinical studies in which clinically optimal loading dosage should be examined.

In the present study, the extent of retrosternal infiltration of macrophages is not correlated to adhesion progression and degradation speed of Lydex. The infiltration was not activated in Lydex 1 and Lydex 3-loaded animals compared to those in unloaded control, whereas significant infiltration of macrophages was observed in Lydex 2-loaded animals. The result may indicate that the timings when macrophage infiltration and scar formation become maximum would be different considering the progression mechanism of postoperative adhesion in which scar formation comes later than macrophage infiltration [9]. Time-course evaluations for the macrophage infiltration and scar formation in future studies would be required to clarify this point. Another possibility would be the existence of other factors than the retainment of Lydex which can affect the macrophage infiltration, and the factors should be further investigated in our future studies. The results from Lydex 1 and 3 groups were comparable with our previous report in which Lydex was loaded at the retrosternal space of rabbits [6]. In the study, macrophage counts after loading of Lydex was comparable with that of unloaded control, whereas a significant increase of macrophage infiltration was observed in ePTFE-placed animals. In accordance with the previous study, the present study indicated the biocompatibility of Lydex 1 and 3 as biodegradable materials regardless of the difference in compositions. Based on the present study, we should recommend using Lydex 3 because it is designed to be degraded fastest among the 3 classes of Lydex and was enough suppressing the macrophage infiltration and scar formation even though the shortest remaining period at retrosternal region.

Conclusions

We have validated the unique potential of Lydex in which the degradation speed in vivo can be controlled according to the compositions, and that the degradation speed affects the prevention of retrosternal adhesion. The results would contribute to the clinical application of the novel biodegradable glue as an option for the prevention of retrosternal adhesion and the improvement of the surgical outcomes of cardiac and vascular re-surgery requiring resternotomy.

Acknowledgements This work was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture of Japan (to T.T.) (Grant number 24592060). We thank F. Kataoka (Kyoto University) for technical supports. We thank BMG Inc, Kyoto, Japan for providing Lydex.

Compliance with ethical standards

Conflict of interest S.H.H. holds a patent right for the biodegradable glue Lydex and is one of the patent inventors. All other authors have nothing to disclose regarding commercial support.

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