



## Full length article

# Antiadhesion effect of the C17 glycerin ester of isoprenoid-type lipid forming a nonlamellar liquid crystal



Takahide Murakami<sup>a,b</sup>, Ichiro Hijikuro<sup>c</sup>, Kota Yamashita<sup>a,b</sup>, Shigeru Tsunoda<sup>a</sup>, Kenjiro Hirai<sup>a</sup>, Takahisa Suzuki<sup>a,b</sup>, Yoshiharu Sakai<sup>a</sup>, Yasuhiko Tabata<sup>b,\*</sup>

<sup>a</sup> Department of Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan

<sup>b</sup> Laboratory of Biomaterials, Institute for Frontier Life and Medical Sciences, Kyoto University, Kyoto, Japan

<sup>c</sup> Farnex Incorporated, Tokyo Institute of Technology Yokohama Venture Plaza, 4259 – 3, Nagatsuta-cho, Midori-ku, Yokohama, Kanagawa, Japan

## ARTICLE INFO

## Article history:

Received 11 July 2018

Received in revised form 20 November 2018

Accepted 4 December 2018

Available online 6 December 2018

## Keywords:

Antiadhesion agent

Isoprenoid-type lipid

Nonlamellar liquid crystal

Inverted hexagonal phase

Hexosomes

## ABSTRACT

Postoperative adhesion is a relevant clinical problem that causes a variety of clinical complications after abdominal surgery. The objective of this study is to develop a liquid-type antiadhesion agent and evaluate its efficacy in preventing tissue adhesion in a rat peritoneal adhesion model. The liquid-type agent was prepared by submicron-sized emulsification of C17 glycerin ester (C17GE), squalene, pluronic F127, ethanol, and water with a high-pressure homogenizer. The primary component was C17GE, which is an amphiphilic lipid of one isoprenoid-type hydrophobic chain and can form two phases of self-assembly nonlamellar liquid crystals. The C17GE agent consisted of nanoparticles with an internal inverted hexagonal phase when evaluated by small-angle X-ray scattering (SAXS) and cryo-transmission electron microscopy (cryo-TEM). Upon contact with the biological tissue, this agent formed a thin membrane with a bioadhesive property. After this agent was applied to a sidewall injury of rats, it showed a percentage average of adhesion significantly less than that obtained with the Seprafilm® anti-adhesion membrane in a rat model. Additionally, the retention of the agent prolonged at the applied site in the peritoneal cavity of rats. In conclusion, the C17GE agent is promising as an antiadhesion material.

## Statement of Significance

Postoperative adhesion remains a common adverse effect. Although various materials have been investigated, there are few products commercially available to prevent adhesion. For the sheet-type agent, it is inconvenient to be applied through small laparotomy, especially in laparoscopic surgery. Additionally, the liquid-type agent currently used requires a complicated procedure to spray at the targeted site.

Our liquid-type antiadhesion agent can form liquid crystals and act as a thin membrane-like physical barrier between the peritoneum and tissues to prevent adhesion. Indeed, the antiadhesion agent used in our present study significantly prevents adhesion compared with the antiadhesion membrane most used clinically. Moreover, our agent is highly stable by itself and easy to use in laparoscopic surgery, thus leading to a promising new candidate as an antiadhesion material.

© 2018 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.

## 1. Introduction

Postoperative adhesion remains a major adverse effect in most abdominal surgeries and occurs in more than 90% of patients after laparotomy [1,2]. Postoperative adhesion can cause serious complications such as small intestinal obstruction, difficulty in unex-

pected subsequent operation, female infertility, and chronic abdominal pain, which often lead to low quality of life (QOL) for patients [3,4]. Various biomaterials have been investigated to prevent or minimize the formation of peritoneal adhesion. On the one hand, there are sheet-type physical barriers of natural materials such as hyaluronic acid and its derivatives [5,6] and gelatin [7–9] and synthetic polymers such as poly-L-lactic acid [10,11] and polylactic acid [12,13]; on the other hand, there are the liquid-type physical barriers of natural polymer hydrogels such as hyaluronic acid and its derivatives [14–16], chitosan and its derivatives [17–

\* Corresponding author at: 53 Kawaharacho, Shogoin, Sakyo-ku Kyoto 606-8507, Japan.

E-mail address: [yasuhiko@infront.kyoto-u.ac.jp](mailto:yasuhiko@infront.kyoto-u.ac.jp) (Y. Tabata).

19], trehalose [20], and pullulan [21] and synthetic polymer hydrogels [22–24]. Among these materials, there are various types of materials with regard to properties, such as thermosensitive hydrogel, which rapidly forms a viscous gel at the body temperature [18,19,22,23]; hydrogel initiated by contact with body fluids containing glucose [14]; and nonreactive gel, which only rests in place because of its immanent viscosity [15–17,21,24]. However, most of the materials have not yet been clinically available to use. Presently, there are mainly three products (Seprafilm®, Interceed®, and AdSpray®) commercially available for the indication to reduce postoperative adhesion, including two sheet-type and one spray-type antiadhesion barrier systems. For the sheet-type barrier systems, although their efficacy has been clinically demonstrated for intraperitoneal physical barriers [25–28], their clinical usage is sometimes difficult when applied through small incisions in laparotomy, especially during laparoscopic surgery. On the other hand, although the spray-type barrier system enables unlimited use for various types of surgical procedures including laparoscopic surgery, it requires intricate procedures including dissolution and spray using a double-lumen syringe [29].

Herein, a liquid-type antiadhesion thin membrane, which was prepared by the self-assembly nonlamellar liquid crystal formation of an amphiphilic lipid of an isoprenoid-type hydrophobic chain, was designed to overcome the drawbacks of other antiadhesion barrier systems. Liquid crystal is a substance that flows like a liquid but maintains some of the structural characteristics of crystalline solids. It can be classified into two categories, namely, lyotropic liquid crystal normally comprising amphiphilic substances and water and thermotropic liquid crystal comprising a rigid component with one or two flexible aliphatic chains [30]. The lyotropic liquid crystal can be classified as lamellar and nonlamellar structures such as hexagonal and bicontinuous cubic phases. In addition, aqueous nanodispersions of the nonlamellar liquid crystalline phases are known as ISAsomes (internally self-assembled “somes” or nanoparticles). In the case of hexagonal and cubic phases, these nanoparticles have been termed hexosomes and cubosomes [31,32]. In this study, to obtain liquid-type barrier-forming liquid crystals, a submicron-sized emulsion was prepared from different component percentages of C17 glycerin ester (C17GE), which is an amphiphilic lipid of one isoprenoid-type hydrophobic chain; squalene; pluronic F127 as a stabilizer; ethanol; and water. An amphiphilic lipid of an isoprenoid-type hydrophobic chain, derived from five-carbon isoprene units, can form two phases of nonlamellar liquid crystals, namely, the inverted hexagonal and the inverted bicontinuous cubic phase, in excess water [33] (Fig. 1). The nanodispersion of C17GE itself formed the inverted bicontinuous cubic

phase. The addition of an oil, squalene, induces a structural transition from the inverted bicontinuous cubic phase to the inverted hexagonal phase, thereby enabling to confer homogeneous dispersion stability to the C17GE agent. In addition, it is known that the isoprenoid-type lipid that forms the nonlamellar liquid crystals exhibits distinctive physical properties, for instance, considerably lower solute permeability, higher salt tolerance of the bilayer membranes [34,35], and a temperature stability range from 0 °C to at least 65 °C [36] than the conventional amphiphilic lipids.

To date, nonlamellar liquid crystal systems have been extensively paid much attention for their applications as drug vehicles, which offer two distinct promising strategies: the formation of ISAsomes and the bulk of liquid crystal [30–32,37,38]. Various lipids that form liquid crystals have been evaluated to be applicable to various routes of administration, as they are able to provide a matrix with sustained drug release that can also protect nucleic acids and other biological materials against both physical and chemical degradation. Indeed, the bulk of C17GE has been developed for transdermal drug delivery to improve skin permeation of various drugs [39,40]. In this study, we focus on the feature that liquid crystal formation generates a thin membrane, and in addition, the surface of the liquid crystal formed may have a property of bioadhesion. Once the nonlamellar liquid crystal is in contact with the surface of biological tissues, it adheres to the tissue surface while acting as a thin membrane to prevent tissue adhesion, which seems to be a new application.

In this study, a liquid-type antiadhesion agent was designed from the amphiphilic lipid with an isoprenoid-type hydrophobic chain, which can form a nonlamellar liquid crystal. When this agent was applied to a sidewall injury on the peritoneal membrane of rats, the efficacy of the liquid-type agent in preventing postoperative adhesion and the retention of this agent in the peritoneal cavity of rats were evaluated.

## 2. Materials and methods

### 2.1. Materials

Propionic acid, trimethyl orthoacetate, and glycerol were purchased from Tokyo Chemical Industry Co., Ltd (Tokyo, Japan). Tetrahydronerolidol was obtained from Kuraray Co., Ltd (Tokyo, Japan). Squalene, potassium carbonate, and N,N-dimethylformamide were purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan). Surfactant, pluronic F127 (polyoxyethylene polyoxypropylene (200EO) (70PO)), was purchased

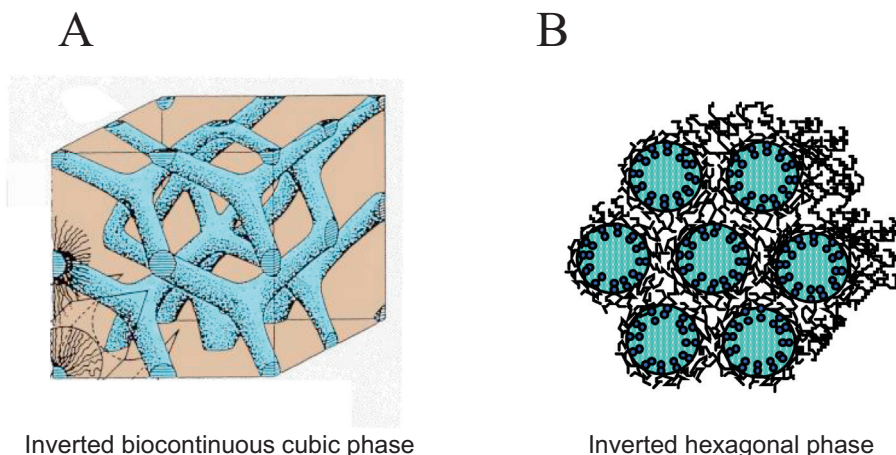


Fig. 1. Two representative phases of nonlamellar liquid crystals.

from NOF Co., Ltd (Tokyo, Japan). All other reagents were used without further purification.

## 2.2. Animals

Wistar rats (female; 130–150 g of body weight; 8 weeks old) were purchased from Shimizu Laboratory Supplies Co., Ltd (Kyoto, Japan) and maintained under a pathogen-free environment. All interventions were performed during the light or dark cycle. All animal experiments were conducted in accordance with the Helsinki Convention, and our institutional guidelines were approved by Kyoto University Animal Care Committee.

## 2.3. Synthesis of mono-O-(5,9,13-trimethyltetradec-4-enoyl) glycerol (C17 glycerin ester)

The chemical synthesis of C17GE is shown in Fig. 2. A solution containing a mixture of 3.9 mmol propionic acid and 7.8 mmol trimethyl orthoacetate was slowly added dropwise to a solution of 39 mmol tetrahydronerolidol that was dissolved in 86 mmol trimethyl orthoacetate at 140 °C, followed by stirring for 18 h at the same temperature. Another solution containing a mixture of 1.3 mmol propionic acid and 2.3 mmol trimethyl orthoacetate was added, with additional stirring for 2 h. The samples were subjected to simple distillation (external temperature of 140 °C, vacuum degree: 15 kPa) to exclude components with low boiling points, and the resulting residue was purified by silica gel column chromatography (ethyl acetate/hexane mixture) to obtain methyl 5,9,13-trimethyltetradec-4-enoate. The methyl 5,9,13-trimethyltetradec-4-enoate (1.0 g) solution was slowly added dropwise to a solution containing 7.1 mmol glycerol and 4.3 mmol potassium carbonate in dry N,N-dimethylformamide at 80 °C. The resulting solution was stirred at 100 °C for 18 h, and then, 1 M hydrochloric acid was added. The resulting solution was extracted with ether; the extract was washed with saturated sodium bicarbonate aqueous solution and saturated brine successively and then dried over anhydrous sodium sulfate. After filtration, the filtrate obtained was concentrated, followed by purification by silica gel column chromatography (ethyl acetate/hexane mixture) to obtain Mono-O-(5,9,13-trimethyltetradec-4-enoyl)glycerol, which had lipid with hydrophobic chain lengths of 17 carbon atoms, referred to as C17 glycerin ester (C17GE). The purity of C17GE obtained was 98.3%.

## 2.4. Preparation of the C17GE agent

Pluronic F127 was dissolved in a solution containing a mixture of C17GE, squalene, and ethanol in a water bath at 50 °C at different percentages of lipid composition (Table 1). After the addition of distilled water, the mixture was stirred with a spatula for a few minutes. The resulting coarse suspension was homogenized using a high-pressure homogenizer (Star Burst minimo, Sugino Machine Ltd, Uozu, Japan) at a pressure of 150 MPa to form C17GE agents as a white emulsion (Fig. 3A). The temporal stability of C17GE agents obtained was evaluated at room temperature and 4 °C.

## 2.5. Characterization of the C17GE agent

### 2.5.1. Measurement of particle size and zeta potential

The particle size and zeta potential of C17GE agents with different percentages of lipid composition were measured on a dynamic light scattering Nano-ZS ZEN3600 Zetasizer (Malvern Instruments Ltd, Worcestershire, UK). Each sample was diluted in water and shaken using a vortex mixer before measurement. The measurements were performed independently three times for every sample.

### 2.5.2. Measurement of viscosity

The viscosity of C17GE agents prepared at different lipid compositions was measured using a Gemini II rheometer (Malvern Instruments Ltd., Worcestershire, UK) with a cone plate  $\phi$  of 40 mm and cone angle at 25 °C. The dispersions were subjected to increasing and decreasing shear rates for 30 min in 60 steps from  $1 \text{ s}^{-1}$  to  $10000 \text{ s}^{-1}$ . The measurements were performed independently three times for every sample.

### 2.5.3. Measurement of the bioadhesive property

Peritoneal segments of Wistar rats ( $1.5 \text{ cm}^2$ ) were resected and fixed on the plate, and 1 ml of phosphate-buffered saline (PBS) was applied on the peritoneum for 5 min. After draining the PBS, the C17GE agent was applied on the peritoneum, followed by addition of 1 ml of PBS to form a liquid crystal. The peritoneal segment was placed into a tube containing 5 ml of PBS, followed by gentle stirring for 1 h at 37 °C. After removal of the peritoneal segment, 2.5 ml of ethyl acetate was added to the remaining aqueous solution to extract the lipid in the aqueous solution. After the extract was dried over sodium sulfate and filtered, the resulting filtrate was concentrated at reduced pressure, followed by dilution with 0.1 ml of ethyl acetate. Thin-layer chromatography (TLC) of the solution was performed to detect the lipid in the aqueous solution.

## 2.6. Confirmation of liquid crystal structure

### 2.6.1. Small-angle X-ray scattering (SAXS) measurement

The structural arrangements of the liquid crystal dispersion were analyzed by small-angle X-ray scattering (SAXS) using the NANO-Viewer (Rigaku, Tokyo, Japan) equipped with a Pilatus 100 K/RL 2D detector, which was performed by X-ray irradiation at wavelength  $\lambda = 0.1542 \text{ nm}$  (Cu-K $\alpha$ ), 40 kV, and 50 mA. Diffraction analyses were performed using a vacuum-resistant glass capillary cell at 25 °C. The obtained SAXS pattern was plotted as a function of the modulus of the scattering vector,  $q = (4\pi/\lambda) \sin(\theta/2)$ , where  $\theta$  is the scattering angle. The scattering intensities of all samples were measured in a relative unit, but a quantitative contrast of the measurements was standardized under the same experimental conditions.

### 2.6.2. Observation by cryo-transmission electron microscopy (cryo-TEM)

The prepared liquid crystal dispersion was observed by cryo-transmission electron microscopy (cryo-TEM) (JEM-2200FS, JEOL

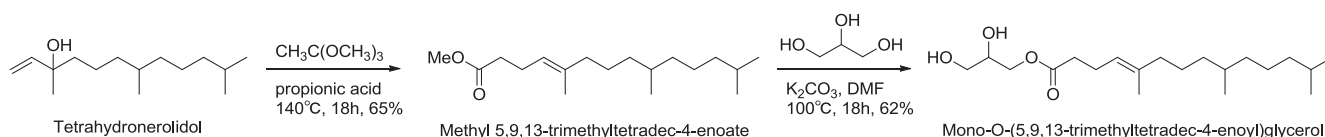
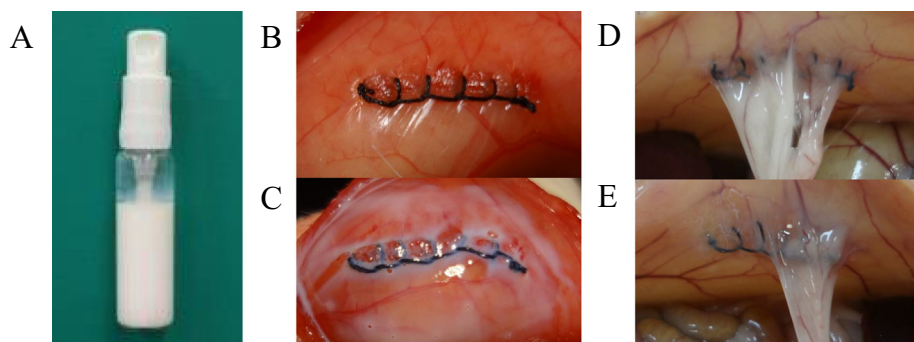


Fig. 2. Chemical structure and preparation scheme of Mono-O-(5,9,13-trimethyltetradec-4-enoyl)-glycerol (C17 glycerin ester) (C17GE).

**Table 1**

Composition percentage of C17GE agents used.

| Component composition (wt%) |                  |          |         |       |
|-----------------------------|------------------|----------|---------|-------|
| Lipid <sup>a</sup>          | Oil <sup>b</sup> | Pluronic | Ethanol | Water |
| 15                          | 1.5              | 3.75     | 2       | 77.75 |
| 18                          | 1.8              | 4.5      | 2       | 73.70 |
| 21                          | 2.1              | 5.25     | 2       | 69.65 |
| 25                          | 2.5              | 6.25     | 2       | 64.25 |

<sup>a</sup> Lipid: C17GE.<sup>b</sup> Oil: squalene.

**Fig. 3.** (A) Appearance of the C17GE agent used (lipid composition = 25 wt%) (B) Tissue appearance of sidewall injury closed with six continuous sutures. (C) Tissue appearance of the sutured injury after application of the C17GE agent. (D) Appearance of the range 100% of peritoneal adhesion. (E) Appearance of the range 60% of peritoneal adhesion.

Ltd, Tokyo, Japan). Briefly, 2.5  $\mu$ l of the sample was placed on a glow-discharged carbon support TEM grid (Quantifoil), blotted, and rapidly plunged into liquid ethane using Vitrobot (FEI, Oregon, USA). The ice-embedded sample obtained was transferred into a JEM-2200FS microscope. Images were recorded with a Tietz F415 CCD camera and a DE20 direct detection camera. The defocus varied from 2 to 4  $\mu$ m accordingly.

### 2.7. Evaluation of in vivo antiadhesion effect in a rat peritoneal adhesion model

A rat model of sidewall injury was used to analyze the antiadhesion efficacy of C17GE agents. Wistar rats were intraperitoneally anesthetized with 15 wt% pentobarbital and underwent laparotomy with a 5 cm long midline incision. Then, a 20 mm longitudinal peritoneal incision was made on the bilateral abdominal wall and closed with six continuous 5-0 silk sutures (Nescosuture<sup>®</sup>; Alfresa, Japan). The sutures were placed equidistantly over the defect, with the first stitch at the proximal end and the last stitch at the distal end of the wound (Fig. 3B). After hemostasis was completely confirmed, 72  $\mu$ l of C17GE agents with various lipid compositions was instilled into the right wound (Fig. 3C), and the left side was left untreated as the control. After the application of the sample, the midline wound was closed with one-layer continuous 4-0 nylon sutures (Bear Medic Co., Tokyo, Japan). For the Septrafilm<sup>®</sup> (Glenzeme, Cambridge, USA), which is composed of sodium hyaluronate and sodium carboxymethyl cellulose, group (n = 10), the right wound was covered with a 2  $\times$  3 cm<sup>2</sup> Septrafilm<sup>®</sup>, and the left injured site was not covered. At 1 week after surgery, the animals were sacrificed and the abdominal wall was opened to assess the strength and range of tissue adhesion. The strength of adhesion was scored for each abdominal sidewall as follows: score 0, no adhesion; score 1, peelable adhesion with mild traction; score 2, peelable adhesion with strong traction; and score 3, adhesion involved tissue damage by peeling. The range of adhesion was calculated for each abdominal sidewall as a fraction of tissue adhesion

from 0% to 100% (given as a percentage of 20 mm longitudinal peritoneal incision).

### 2.8. Evaluation of in vivo retention of the C17GE agent in the peritoneal cavity

The time profile of the C17GE agent retention in the peritoneal cavity was assessed using IVIS (IVIS Spectrum, SPC, Japan). To prepare a C17GE agent containing 1,1'-diiodo-3,3'-bis(4-methyl-5-sulfinylphenyl)-4,4'-bipyridine (DiR) of a lipophilic, near-infrared fluorescent cyanine dye (Biotium, Fremont, USA) (0.2 mM), 10 mM DiR ethanol solution was added instead of ethanol in preparation of the C17GE agent with a lipid composition of 25 wt% as described above. DiR ethanol solution (10 mM) was added to water to prepare the DiR aqueous solution (0.2 mM).

Wistar rats were anesthetized and underwent laparotomy by the same procedure as the rat peritoneal adhesion model. The right sidewall peritoneum was incised and then sutured with 5-0 silk continuously. The C17GE agent containing DiR (0.2 mM) was applied to the right lateral wall similar to the procedure performed for the evaluation of antiadhesion efficacy. The DiR aqueous solution (0.2 mM) was used as the control group. After sample application, the midline wound was sutured in a similar manner as mentioned above. Rats were imaged at different time points of 0, 1, 3, 6, and 12 h or 1, 3, 7, and 14 days using the IVIS apparatus. The excitation and emission filters were set at 745 and 800 nm, respectively.

### 2.9. Statistical analysis

Statistical data were expressed as the mean  $\pm$  standard deviation (SD). Student's *t*-test was used to compare mean values between the two groups (two-tailed). A difference of *p* < 0.05 was considered to be statistically significant. All statistical analyses were performed using JMP version 12 (SAS Institute, Cary, NC, USA).

### 3. Results

#### 3.1. Characterization of C17GE agents

Table 2 shows the particle size, zeta potential, and viscosity of C17GE agents prepared at different lipid compositions. The particle size of C17GE agents tended to gradually increase as the lipid composition increased. The zeta potentials of C17GE agents were negative, irrespective of the lipid composition. The viscosity of C17GE agents depended on the lipid composition. The agent was a free-flowing liquid at low lipid composition percentages (less than 18 wt%). However, it showed the behavior of a viscid liquid at higher percent lipid compositions. The viscosity of the antiadhesion agent should be optimal to allow stable coating on complicated wound surfaces without flowing down. On the basis of the requirement of agent properties, the C17GE agent with a lipid composition of 25 wt% was used for the following experiment. In addition, Fig. 4 shows the hysteresis loop, where the shear viscosity of the C17GE agent was reduced upon application at a finite shear, but the viscosity tended to recover to the original value when the shear was discontinued. It is practically easy to apply the agent to various shapes of targets. This is because the viscosity of the agent decreased when shaken or sprayed and rapidly increased to the original after application over the target. The phase separation of lipid in C17GE agents was not observed at room temperature and 4 °C for at least 1 year. Moreover, the particle size and SAXS diffraction pattern of C17GE agents remained unchanged at the two temperatures (Table S1 and Fig. S1), which shows the temporal stability of C17GE agents. TLC analyses demonstrated that lipids in the aqueous solution were not substantially detected after stirring for 1 h at 37 °C in the aqueous solution. Further, the lipids on the peritoneum segments were visibly observed at the applied area and were found to be same as those applied. This indicates the bioadhesive property of C17GE agents.

#### 3.2. Structure of the C17GE agent

Fig. 5A shows the X-ray profile of the C17GE agent without squalene in the nanodispersion state. Five peaks were observed nearly at  $\sqrt{2}$ ,  $\sqrt{3}$ ,  $\sqrt{4}$ ,  $\sqrt{6}$ , and  $\sqrt{8}$ , which typically correspond to the inverted bicontinuous cubic Pn3m phase. Fig. 5B shows the X-ray profile of the C17GE agent with a lipid composition of 25 wt% in the nanodispersion state. Three peaks were observed nearly at 1,  $\sqrt{3}$ , and  $\sqrt{4}$ , which typically correspond to the inverted hexagonal phase. All of the C17GE agents prepared at different lipid compositions represented the hexosomes, irrespective of the lipid composition, as shown in Table 1. The lattice parameter ( $a$ ) of the hexagonal phases was determined using the relation  $d^{-1} = (a^{-1})(4/3)^{1/2} (h^2 + hk + k^2)^{1/2}$  from linear fits of the plots of  $d^{-1}$  versus  $(h^2 + hk + k^2)^{1/2}$ , where  $d$  is the measured peak position and  $h$  and  $k$  are the Miller indices. The calculated lattice parameter of the inverted hexagonal phase of the C17GE agent with a lipid composition of 25 wt% was 50.4 Å. This value was almost equivalent to that of liquid crystals comprising glyceryl monooleate (GMO), which is the most thoroughly studied and well-characterized lipid

with a tendency to form nonlamellar liquid crystals in excess water [41]. Fig. 6A and 6B show cryo-TEM microphotographs of the C17GE agent. This clearly indicated that the agent had the inverted hexagonal phase in nanoparticles. Fourier transform results of the magnified areas showed the structural periodicity of nanoparticles, which is consistent with the phase structure (Fig. 6C).

#### 3.3. In vivo antiadhesion effect

The antiadhesion efficacy of C17GE agents was evaluated in a rat model of sidewall injury. As shown in Fig. 7A, the nontreatment side in most rats showed the range of adhesion to be 69% of an extensive adhesion. For the side applied with the C17GE agent, the range of adhesion (15%) decreased to a significantly strong extent compared with that of the nontreatment side (adhesion reduction rate; 77.6%,  $p = 0.002$ ). Interestingly, no peritoneal adhesion was observed in 8 of 11 rats. On the contrary, there was no significant difference in the strength and range of adhesion between the Septrafilm® and nontreatment groups (range; 59% vs. 90%, adhesion reduction rate; 35.0%,  $p = 0.11$ ) (Fig. 7B). Moreover, the C17GE agent treatment reduced the range of peritoneal adhesion significantly compared with that of the Septrafilm® group (adhesion reduction rate; 77.6% vs. 35.0%,  $p = 0.014$ ). Fig. 7C shows the liquid composition effect on the adhesion prevention efficacy of the C17GE agent. The C17GE agents with lipid compositions of 21 and 25 wt% groups showed significantly smaller percent average ranges of adhesion than the nontreatment group. There was no significant difference in the adhesion reduction rate between the Septrafilm® group and the group with C17GE agents with lipid compositions of less than 18 wt%.

#### 3.4. In vivo retention of the C17GE agent in the peritoneal cavity

Fig. 8 shows the in vivo retention of the DiR-labeled C17GE agent of 25 wt% lipid composition in the peritoneal cavity. The fluorescence signal of the DiR-labeled C17GE agent was visualized intensely around the applied area immediately after the application. The signal intensity decreased gradually with time but remained even 7 days later, and it disappeared within 14 days. On the contrary, the fluorescence signal of the DiR aqueous solution was detected immediately after the application at the left paracolic sulcus because the liquid solution was found to flow to the left paracolic sulcus because of its viscosity. However, it disappeared within 6 h. The visible fluorescent signal in the midline incision could be an artifact remaining at the nonmetabolized site.

### 4. Discussion

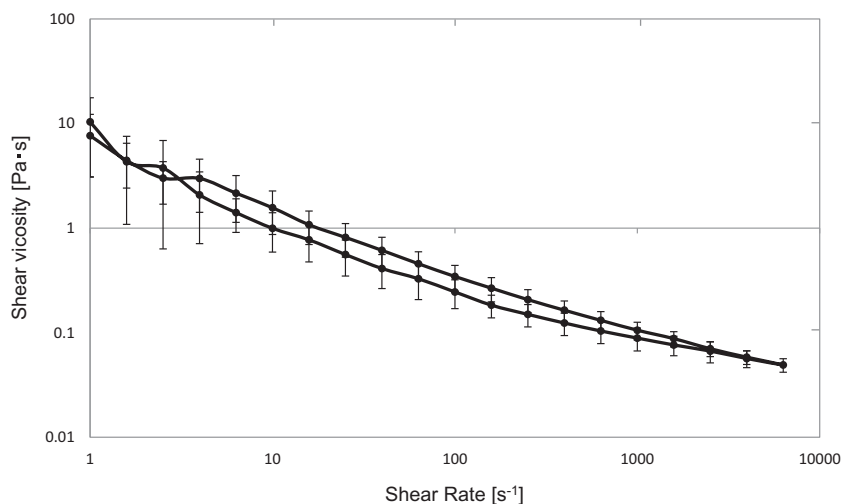
Postoperative adhesion is a common problem after abdominal surgery, while diverse antiadhesion drugs and devices have been developed to prevent the postoperative adhesion. However, unfortunately, only few drugs and devices have been proven to be effective and safe in clinical trials [25–29]. The present study demonstrates that the liquid-type antiadhesion agent of an amphiphilic lipid with an isoprenoid-type hydrophobic chain is promis-

**Table 2**  
Characterization of C17GE agents used.

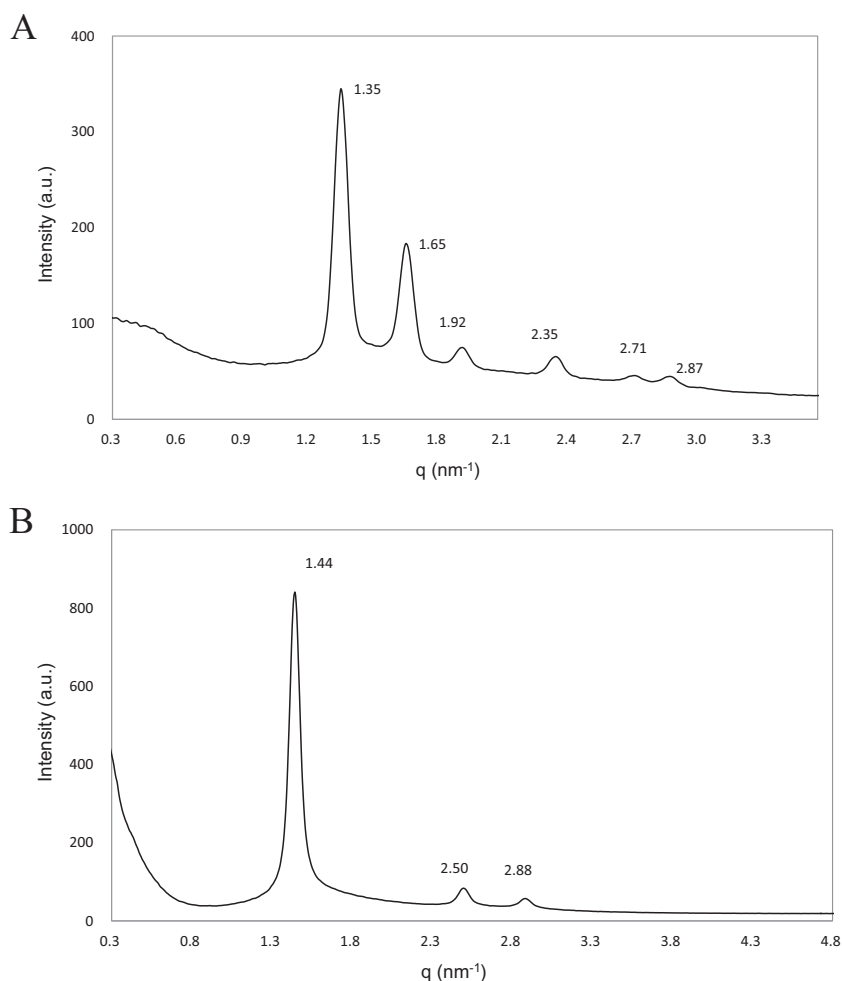
| Lipid percentage | Particle size (nm) | PdI   | Zeta potential (mV) | Viscosity (Pa·s)  |                     |
|------------------|--------------------|-------|---------------------|-------------------|---------------------|
| 15               | 122.5 ± 1.4        | 0.072 | −35.6 ± 1.4         | 0.40 <sup>a</sup> | 0.0045 <sup>b</sup> |
| 18               | 124.9 ± 1.4        | 0.066 | −17.0 ± 1.9         | 0.37 <sup>a</sup> | 0.0065 <sup>b</sup> |
| 21               | 168.7 ± 1.4        | 0.058 | −25.0 ± 0.1         | 0.46 <sup>a</sup> | 0.0144 <sup>b</sup> |
| 25               | 187.9 ± 2.3        | 0.104 | −24.8 ± 0.2         | 0.44 <sup>a</sup> | 0.0329 <sup>b</sup> |

<sup>a</sup> Shear rate 100 s<sup>−1</sup>.

<sup>b</sup> Shear rate 10000 s<sup>−1</sup>.



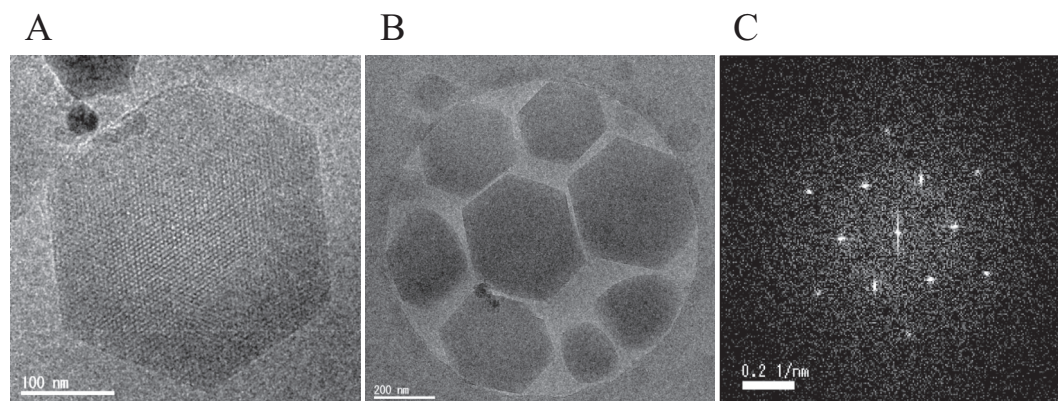
**Fig. 4.** A representative shear viscosity relationship of the C17GE agent with a lipid composition of 25 wt%.



**Fig. 5.** (A) SAXS diffraction pattern of the C17GE agent without squalene. (B) SAXS diffraction pattern of the C17GE agent with a lipid composition of 25 wt% in the presence of squalene.

ing to prevent tissue adhesion, which seems to be a new application. The amphiphilic lipid has an inherent ability to self-assemble in excess water, thereby leading to the formation of agents with internal inverted nonlamellar liquid crystalline phases. SAXS measurement results revealed that the agent can form two

phases of the nonlamellar liquid crystals: the inverted hexagonal and the inverted bicontinuous cubic phase (Fig. 5). The agent was easy to use by emulsifying several materials including liquid crystal-forming lipids with a high-pressure homogenizer. The efficacy of the agent in preventing peritoneal adhesion was found to



**Fig. 6.** Representative Cryo-TEM images of the C17GE agent with a lipid composition of 25 wt%: (A) Magnification  $\times 30000$ , scale bar: 100 nm and (B) magnification  $\times 15000$ , scale bar: 200 nm, (C) Fourier transform pattern of the C17GE agent.

be significant compared with that of the Septrafilm® antiadhesion membrane clinically used in a rat peritoneal adhesion model (Fig. 7). In addition, the retention of the agent applied in the peritoneal cavity was prolonged. It is likely that the agent forms a liquid crystal between the peritoneum and tissues, thus leading to an enhanced retention in the body.

It is reported that various biomaterials such as biological scaffolds, membranes, and drugs were designed to suppress the formation of peritoneal adhesion [42–44]. Among them, many hydrogels, which are a kind of water-containing polymer material with a three-dimensional network structure, have been developed for the application of nonsheet-type antiadhesion. The mechanical properties can be tuned by adjusting the concentrations of materials, which is necessary for their use as an injected or spray-type agent in laparoscopic surgery. The low tensile strength of hydrogels limits their use in load-bearing relevance and can result in the premature dissolution or flow away of hydrogel from the targeted site [44]. On the contrary, the C17GE agent is a suitable hydrogel in terms of the adjustable mechanical properties. Indeed, the particle size and the viscosity of C17GE agents could be readily arranged according to the optimal use by changing the lipid compositions (Table 2).

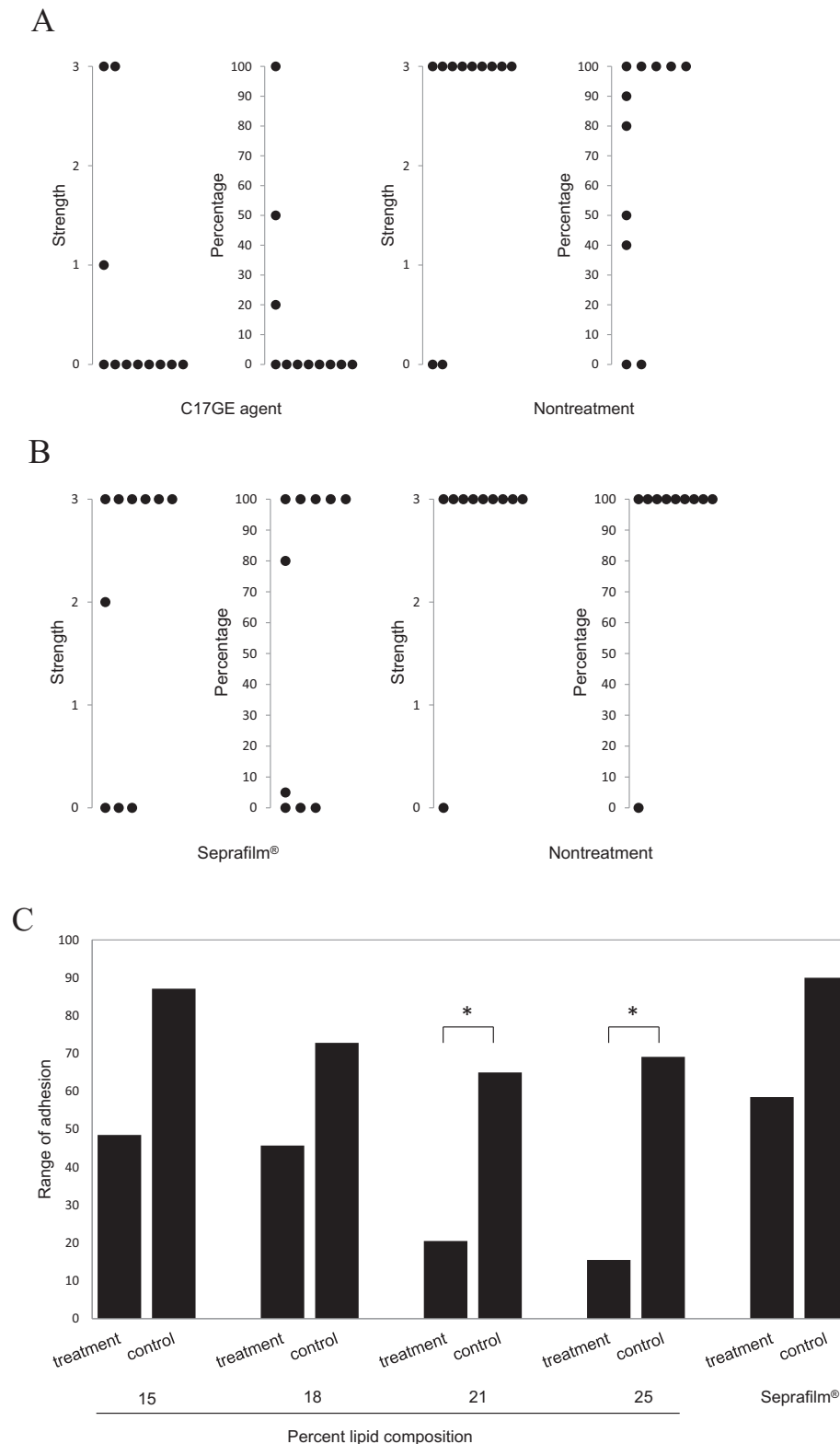
Recently, AdSpray® (Terumo Corporation, Tokyo, Japan), which is composed of N-hydroxysuccinimide (NHS)-modified carboxymethyl dextrin and sodium carbonate/sodium hydrogen carbonate, has been clinically used as a spray-type bio-absorbable antiadhesion system [29]. In this system, two powders are dissolved in distilled water for injection and loaded into a double syringe and mixed with compressed medical air. This preparation procedure is complicated intraoperatively. However, the C17GE agent is extremely stable by itself and ready for use without mixing two or more materials, which may be superior for laparoscopic surgery.

The SAXS measurement and cryo-TEM micrographs of the C17GE agent represented hexosomes (Figs. 5 and 6). Conversely, the nanodispersion of the C17GE agent without squalene represented cubosomes and showed a moderate adhesion prevention effect. However, the low dispersion stability and high viscosity of the agent were not ideal for the purpose of antiadhesion. In the case of C17GE agents without squalene, it is only possible to prepare the agents with up to 18–20 wt% lipid composition because of the low dispersion stability. Simple mixing of C17GE with squalene could induce a phase transition from cubosomes to hexosomes and enable to confer the agent with dispersion stability and lower viscosity and prepare C17GE agents with a higher lipid composition (up to 30 wt%). In this study, the C17GE agent with a lipid composition of 25 wt% was used for the experiment because

the agent with a lipid composition of 30 wt% could not be sprayed with high viscosity. Moreover, highly negative zeta potentials for the prepared nanodispersions were obtained, although the C17GE agent exerted electrical neutrality. This can be explained by the preferential adsorption of hydroxyl ions at the liquid–water interface [45] or the presence of charged fatty acid impurities in lipid [46]. Thus, the stabilization of these colloidal nanoparticles is most likely achieved through a combination of steric and electrostatic effects in the presence of oil and the highly negative zeta potential, respectively.

The C17GE agent significantly decreased the extent of peritoneal adhesions compared with that of nontreatment and Septrafilm® groups (Fig. 7A and B). Septrafilm® could not effectively decrease the extent of peritoneal adhesion in our rat model, although it is a standard antiadhesion product. This is probably caused by a rat model for inducing experimental peritoneal adhesion. The rat cecal ablation model without sutures was mainly used to assess the antiadhesion effect of Septrafilm® [47]. On the contrary, our rat model showed a higher probability to induce tissue adhesion, that is, traumatization and sutures have the potential to promote tissue adhesion at higher probability in a rat model [48]. Additionally, there was a difference in percent average range of adhesions at the untreated sites between the C17GE agent and Septrafilm® group in the present rat peritoneal adhesion model as shown in Fig. 7C. It is probable that the liquid-type agent flows down slightly to the opposite side despite the high viscosity or the agent attached to the intestinal tract tends to contact with the opposite side. Even then, the adhesion reduction rate of the C17GE agent was significantly higher than that of Septrafilm®.

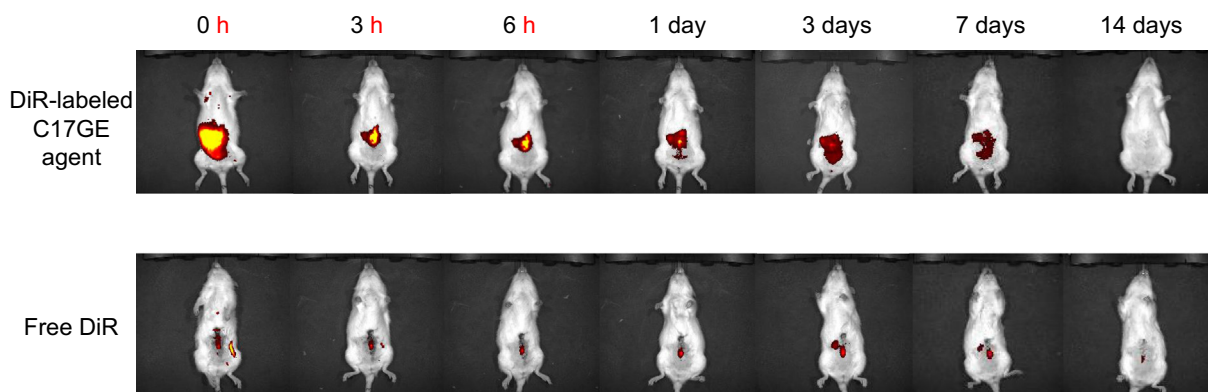
The C17GE agent remained in the peritoneal cavity for a longer time period (Fig. 8). We can say with certainty that a liquid crystal structure is present at the affected area and remained in the body for more than 1 week. In addition, the liquid crystal-forming C17GE agent applied on the peritoneal segment mostly remained even after stirring in aqueous solution in vitro. These may be explained in terms of the unique property. It is likely that the bioadhesion of inverted hexagonal phase is achieved on the peritoneum because of the hydrophobic chain around the liquid crystal. In addition, the self-assembly structure of the liquid crystal would act as a physical barrier to prevent the peritoneal adhesion. Moreover, the efficacy in preventing tissue adhesion enhanced with an increase in the lipid compositions of C17GE agents (Fig. 7C). Taken together, it is hypothesized that a liquid crystal structure is overlain to each other on the surface of the tissues after application of C17GE agents. This would result in more potency of the agent as a physical barrier.



**Fig. 7.** In vivo antiadhesion effects of the C17GE agent and Seprafil®. (A) Percent average range of adhesion of the C17GE agent was  $15 \pm 32\%$  (non-treatment group:  $69 \pm 40\%$ ). (B) Percent average range of adhesion of Seprafil® was  $59 \pm 50\%$  (non-treatment group:  $90 \pm 32\%$ ). (C) Percent adhesion range of C17GE agents at different lipid compositions and Seprafil® in a rat peritoneal adhesion model. The reduction rates of the adhesion range were 44.3% (15 wt% lipid), 37.2% (18 wt% lipid), 64.7% (21 wt% lipid), 77.6% (25 wt% lipid), and 35.0% (Seprafil®), respectively. \* $p < 0.05$  indicates significant difference between the two groups. The error bars were very big to be applicable because the percent adhesion ranged from 0 to 100%.

For better clinical application, it is important to provide unlimited coverage of the target peritoneum in both open and laparoscopic surgery. Additionally, it should be biodegradable and

biocompatible [49,50]. The antiadhesion agent used in the present study was prepared by emulsifying the mixture of C17GE/squalene/pluronic F127/ethanol and water. Although biological



**Fig. 8.** Retention profile of the DiR-labeled C17GE agent with a lipid composition of 25 wt% after its application in the peritoneal cavity of rats. Color scale: Min =  $1.6 \times 10^8$ , Max =  $9.2 \times 10^8$ .

activities of C17GE itself are still indistinct, other related compounds with isoprenoid-type hydrophobic chains, for example, Gefarnate (geranyl farnesylacetate) and Teprenone, anti-ulcer drugs, are metabolized by the liver and excreted by the kidney and intestinal tract. Therefore, it is potentially predictable that the C17GE agent follows similar metabolic pathways. Presently, hydrophobic additives such as vitamin E and oleic acid are mixed to liquid crystals, and the addition of oleic acid induces a structural transition from cubosomes to hexosomes [51,52]. Indeed, we also confirmed the addition of oils such as squalene, squalane, and isopropyl myristate led to a structural transition from cubosomes to hexosomes. Among them, squalene is a naturally occurring oil produced in plants and also abundant in human beings and has been used in the development of vaccine adjuvants [50]. Based on that, we chose squalene as a hydrophobic additive for the following experiment. Further, the C17GE agent forming the inverted hexagonal phase has hydrophilic residues, which are wrapped inside its structure. The high water content properties of the agent resemble those of biological tissues, which may act in favorable biocompatibility. In addition, Pluronic F127 is prevalent in a variety of pharmaceutical additives and widely used for the stabilization of ISAsomes. Indeed, we confirmed the addition of stabilizers such as Pluronic F127 and Polysorbate 80 (P80) [53,54]. Pluronic F127 could confer the highest dispersion stability and temporal stability to the agent at room temperature and  $4^\circ\text{C}$ . Based on that, Pluronic F127 was used as a stabilizer for the experiment. Furthermore, it is reported that ISAsomes often exhibit poor hemocompatibility and may induce cellular toxicity, which limits their application for intravenous administration [55,56]. Moreover, pluronic F127 activates the human complement system in the blood [57,58]. Recent studies introduce citrem as an alternative stabilizer, which is an anionic citric acid ester of monoglycerides [59,60]. Citrem can modulate the internal nanostructures of ISAsomes and abolish surfactant-mediated hemolysis, as well as complement activation. Hence, citrem might be an attractive agent for the clinical application of the C17GE agent as a stabilizer.

To date, the hexosomes composed of glycerate-based surfactants such as GMO and phytanyl glycerate have shown great potential in drug delivery [61,62]. Hydrophilic drugs are entrapped in the internal water domain, whereas lipophilic drugs are located within the lipid domain and amphiphilic drugs in the interface. Actually, the C17GE agent containing DiR remained detectable in the peritoneal cavity for more than 1 week (Fig. 8). This suggests that the C17GE agent has an ability to sustain the lipophilic substance for the drug delivery system. Therefore, if other lipophilic substances are incorporated into the lipid domain of the C17GE agent, it may be possible to enhance the antiadhesion effect further.

There are several limitations of this study. The first limitation is the toxicity of C17GE. Fibrosis, inflammation, and vascularization in the wound applied with the C17GE agent, as well as Septrafilm<sup>®</sup>, were microscopically observed to some extent. This might be biologically explained in terms of the influence of the wound healing and the foreign body reaction of silk thread. Actually, there was no practical problem that could affect the process of wound healing in a rat adhesion model. Furthermore, cytotoxicity test, skin sensitization test, intradermal reaction, and muscle implantation of the C17GE agent were already conducted to experimentally confirm biocompatibility (data not shown). However, further studies are needed to be performed for better understanding of systematic toxicity. Second, the retention time in the peritoneal cavity was assessed only using a fluorescent substance. More detailed evaluation should be performed. Additionally, the metabolism of the C17GE agent is not yet fully understood. Further studies with a radioisotope-labeled C17GE agent is required to evaluate the degradability and metabolizing profiles.

In summary, it should be noted in this study that there was a significant antiadhesion effect of the C17GE agent over an antiadhesion membrane clinically used. It is concluded that the C17GE agent is one of the important options to prevent postoperative adhesion.

## 5. Conclusions

In this study, we developed a liquid-type antiadhesion agent composed of C17GE, which is an amphipathic lipid of one isoprenoid-type hydrophobic chain; squalene; pluronic F127; ethanol; and water. In the nanodispersion state, the self-assembly of C17GE resulted in the formation of agents with an internal inverted hexagonal phase (hexosomes). The agent formed a thin membrane with a bioadhesive property and reduced the percentage range of tissue adhesion significantly compared with that of Septrafilm<sup>®</sup> of an antiadhesion membrane clinically used in a rat peritoneal adhesion model. These findings indicate that the C17GE agent is a promising new candidate as an antiadhesion material.

## Conflict of interest

The authors declare no potential conflicts of interest.

## Funding

This study was financially supported by The Small and Medium Enterprise Agency. This work was partially supported by the Adaptable and Seamless Technology Transfer Program through

target-driven R&D from Japan Science and Technology Agency (JST), Japan, and the grant ID is AS2321324F. This study is partially based on results obtained from a project subsidized by the New Energy and Technology Development Organization (NEDO).

## Contributors

All authors conceived the study concept and study design. TM, KY, KH, YT, and YS designed the research studies. TM, KY, KH, TS, and IH performed experiments. TM, IH, and ST conducted experiments and analyzed data. IH assisted with the design of experiments. TM and ST wrote the manuscript. YS and YT supervised and edited the manuscript.

## Acknowledgments

This study was financially supported by The Small and Medium Enterprise Agency. This work was partially supported by the Adaptable and Seamless Technology Transfer Program through target-driven R&D from Japan Science and Technology Agency. This study is partially based on results obtained from a project subsidized by the New Energy and Technology Development Organization (NEDO).

We gratefully thank Dr. Yoko Kayama, Terrabase Co., Ltd, for technical assistance with the observation by Cryo-TEM.

We gratefully thank Kyoto University Radioisotope Research Center for the use of facilities.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.actbio.2018.12.009>.

## References

- [1] D. Menzies, H. Ellis, Intestinal obstruction from adhesions—how big is the problem?, *Ann R. Coll. Surg. Engl.* 72 (1) (1990) 60–63.
- [2] K. Okabayashi, H. Ashrafi, E. Zacharakis, H. Hasegawa, Y. Kitagawa, T. Athanasiou, A. Darzi, Adhesions after abdominal surgery: a systematic review of the incidence, distribution and severity, *Surg. Today* 44 (3) (2014) 405–420.
- [3] H. van Goor, Consequences and complications of peritoneal adhesions, *Colorectal Dis.* 9 (Suppl. 2) (2007) 25–34.
- [4] M. Ouaissi, S. Gaujoux, N. Veyrie, E. Denève, C. Brigand, B. Castel, J.J. Duron, A. Rault, K. Slim, D. Nocca, Post-operative adhesions after digestive surgery: their incidence and prevention: review of the literature, *J. Visc. Surg.* 149 (2) (2012) e104–e114.
- [5] G.Y. Ji, C.H. Oh, B.G. Moon, S. Yi, I.B. Han, D.H. Heo, K.T. Kim, D.A. Shin, K.N. Kim, Efficacy and safety of sodium hyaluronate with 1,4-butanediol diglycidyl ether compared to sodium carboxymethylcellulose in preventing adhesion formation after lumbar discectomy, *Korean J. Spine* 12 (2) (2015) 41–47.
- [6] S. Hayashi, M. Fujii, T. Takayama, Prevention of postoperative small bowel obstruction in gastric cancer, *Surg. Today* 45 (11) (2015) 1352–1359.
- [7] F. Tian, C. Dou, S. Qi, L. Zhao, B. Chen, H. Yan, L. Zhang, Preventive effect of dexamethasone gelatin sponge on the lumbosacral epidural adhesion, *Int. J. Clin. Exp. Med.* 8 (4) (2015) 5478–5484.
- [8] H. Tsujimoto, H. Takamori, M. Tsuji, M. Hayashi, J. Ikeda, T. Orikasa, H. Torii, Y. Ozamoto, S. Suzuki, S. Morita, Y. Ikeda, A. Hagiwara, Development of gelatin flakes, a new type of anti-adhesive material: a preliminary study of in vivo rat adhesion models, *Surg. Today* 44 (2) (2014) 391–394.
- [9] Y. Hu, K. Yamashita, N. Tabayashi, T. Abe, Y. Hayata, T. Hirose, S. Hiraga, T. Tojo, S. Suzuki, Y. Ikeda, S. Taniguchi, Gelatin sealing sheet for arterial hemostasis and anti-adhesion in vascular surgery: a dog model study, *Biomed. Mater. Eng.* 25 (2) (2015) 157–168.
- [10] K. Fujino, M. Kinoshita, A. Saitoh, H. Yano, K. Nishikawa, T. Fujie, K. Iwaya, M. Kakiyama, S. Takeoka, D. Saitoh, Y. Tanaka, Novel technique of overlaying a poly-L-lactic acid nanosheet for adhesion prophylaxis and fixation of intraperitoneal onlay polypropylene mesh in a rabbit model, *Surg. Endosc.* 25 (10) (2011) 3428–3436.
- [11] S. Liu, M. Qin, C. Hu, F. Wu, W. Cui, T. Jin, C. Fan, Tendon healing and anti-adhesion properties of electrospun fibrous membranes containing bFGF loaded nanoparticles, *Biomaterials* 34 (19) (2013) 4690–4701.
- [12] S. Avital, T.J. Bollinger, J.D. Wilkinson, F. Marchetti, M.D. Hellinger, L.R. Sands, Preventing intra-abdominal adhesions with polylactic acid film: an animal study, *Dis. Colon Rectum* 48 (1) (2005) 153–157.
- [13] A. Hinoki, A. Saito, M. Kinoshita, J. Yamamoto, D. Saitoh, S. Takeoka, Polylactic acid nanosheets in prevention of postoperative intestinal adhesion and their effects on bacterial propagation in an experimental model, *Br. J. Surg.* 103 (6) (2016) 692–700.
- [14] S. Sakai, K. Ueda, M. Taya, Peritoneal adhesion prevention by a biodegradable hyaluronic acid-based hydrogel formed in situ through a cascade enzyme reaction initiated by contact with body fluid on tissue surfaces, *Acta Biomater.* 24 (2015) 152–158.
- [15] H.K. Sheldon, M.L. Gainsbury, M.R. Cassidy, D.I. Chu, A.F. Stucchi, J.M. Becker, A sprayable hyaluronate/carboxymethylcellulose adhesion barrier exhibits regional adhesion reduction efficacy and does not impair intestinal healing, *J. Gastrointest. Surg.* 16 (2) (2012) 325–333.
- [16] T. Ito, Y. Yeo, C.B. Highley, E. Bellas, C.A. Benitez, D.S. Kohane, The prevention of peritoneal adhesions by in situ cross-linking hydrogels of hyaluronic acid and cellulose derivatives, *Biomaterials* 28 (6) (2007) 975–983.
- [17] C.I. Lauder, G. Garcea, A. Strickland, G.J. Maddern, Use of a modified chitosan-dextran gel to prevent peritoneal adhesions in a rat model, *J. Surg. Res.* 171 (2) (2011) 877–882.
- [18] J.Y. Fang, J.P. Chen, Y.L. Leu, J.W. Hu, Temperature-sensitive hydrogels composed of chitosan and hyaluronic acid as injectable carriers for drug delivery, *Eur. J. Pharm. Biopharm.* 68 (3) (2008) 626–636.
- [19] C.Z. Wei, C.L. Hou, Q.S. Gu, L.X. Jiang, B. Zhu, A.L. Sheng, A thermosensitive chitosan-based hydrogel barrier for post-operative adhesions' prevention, *Biomaterials* 30 (29) (2009) 5534–5540.
- [20] A. Ohata, N. Tamura, K. Iwata, N. Abe, K. Doi, Y. Saito, M. Katoh, H. Nojima, Trehalose solution protects mesothelium and reduces bowel adhesions, *J. Surg. Res.* 191 (1) (2014) 224–230.
- [21] S. Bang, E. Lee, Y.G. Ko, W.I. Kim, O.H. Kwon, Injectable pullulan hydrogel for the prevention of postoperative tissue adhesion, *Int. J. Biol. Macromol.* 87 (2016) 155–162.
- [22] Q. Wu, L. Li, N. Wang, X. Gao, B. Wang, X. Liu, Z. Qian, Y. Wei, C. Gong, Biodegradable and thermosensitive micelles inhibit ischemia-induced postoperative peritoneal adhesion, *Int. J. Nanomed.* 9 (2014) 727–734.
- [23] Z. Zhang, J. Ni, L. Chen, L. Yu, J. Xu, J. Ding, Biodegradable and thermoreversible PCL-PEG-PCL hydrogel as a barrier for prevention of post-operative adhesion, *Biomaterials* 32 (21) (2011) 4725–4736.
- [24] S.H. Bae, S.R. Son, S. Kumar Sakar, T.H. Nguyen, S.W. Kim, Y.K. Min, B.T. Lee, Evaluation of the potential anti-adhesion effect of the PVA/Gelatin membrane, *J. Biomed. Mater. Res. B Appl. Biomater.* 102 (4) (2014) 840–849.
- [25] Prevention of postsurgical adhesions by INTERCEED(TC7), an absorbable adhesion barrier: a prospective randomized multicenter clinical study. INTERCEED(TC7) Adhesion Barrier Study Group, *Fertil Steril* 51(6), 1989, 933–8.
- [26] R. Azziz, Microsurgery alone or with INTERCEED Absorbable Adhesion Barrier for pelvic sidewall adhesion re-formation. The INTERCEED (TC7) Adhesion Barrier Study Group II, *Surg. Gynecol. Obstet.* 177 (2) (1993) 135–139.
- [27] M.P. Diamond, Reduction of adhesions after uterine myomectomy by Seprafilm membrane (HAL-F): a blinded, prospective, randomized, multicenter clinical study. Seprafilm Adhesion Study Group, *Fertil. Steril.* 66 (6) (1996) 904–910.
- [28] J.M. Becker, M.T. Dayton, V.W. Fazio, D.E. Beck, S.J. Stryker, S.D. Wexner, B.G. Wolff, P.L. Roberts, L.E. Smith, S.A. Sweeney, M. Moore, Prevention of postoperative abdominal adhesions by a sodium hyaluronate-based bioresorbable membrane: a prospective, randomized, double-blind multicenter study, *J. Am. Coll. Surg.* 183 (4) (1996) 297–306.
- [29] T. Suto, M. Watanabe, T. Endo, K. Komori, M. Ohue, Y. Kanemitsu, M. Itou, Y. Takii, T. Yatsuoka, M. Shiozawa, T. Kinugasa, H. Ueno, T. Takayama, T. Masaki, H. Masuko, H. Horie, M. Inomata, The primary result of prospective randomized multicenter trial of new spray-type bio-absorbable adhesion barrier system (TCD-11091) against postoperative adhesion formation, *J. Gastrointest. Surg.* (2017).
- [30] R. Rajababala, M.N. Musa, N. Kifli, S.R. David, Oral and transdermal drug delivery systems: role of lipid-based lyotropic liquid crystals, *Drug Des. Dev. Ther.* 11 (2017) 393–406.
- [31] R. Hirlekar, S. Jain, M. Patel, H. Garse, V. Kadam, Hexosomes: a novel drug delivery system, *Curr. Drug Deliv.* 7 (1) (2010) 28–35.
- [32] I.D. Azmi, S.M. Moghimi, A. Yaghmur, Cubosomes and hexosomes as versatile platforms for drug delivery, *Ther. Deliv.* 6 (12) (2015) 1347–1364.
- [33] J. Yamashita, M. Shiono, M. Hato, New lipid family that forms inverted cubic phases in equilibrium with excess water: molecular structure–aqueous phase structure relationship for lipids with 5,9,13,17-tetramethyloctadecyl and 5,9,13,17-tetramethyloctadecanoyl chains, *J. Phys. Chem. B* 112 (39) (2008) 12286–12296.
- [34] K. Yamauchi, K. Doi, M. Kinoshita, F. Kii, H. Fukuda, Archaeobacterial lipid models: highly salt-tolerant membranes from 1,2-diphytanylglycerol-3-phosphocholine, *Biochim. Biophys. Acta* 1110 (2) (1992) 171–177.
- [35] K. Shinoda, W. Shinoda, M. Mikami, Molecular dynamics simulation of an archaeal lipid bilayer with sodium chloride, *Phys. Chem. Chem. Phys.* 9 (5) (2007) 643–650.
- [36] M. Hato, J. Yamashita, M. Shiono, Aqueous phase behavior of lipids with isoprenoid type hydrophobic chains, *J. Phys. Chem. B* 113 (30) (2009) 10196–10209.
- [37] A. Angelova, V.M. Garamus, B. Angelov, Z. Tian, Y. Li, A. Zou, Advances in structural design of lipid-based nanoparticle carriers for delivery of macromolecular drugs, phytochemicals and anti-tumor agents, *Adv. Colloid Interface Sci.* 249 (2017) 331–345.

- [38] S. Akbar, A. Anwar, A. Ayish, J.M. Elliott, A.M. Squires, Phytantriol based smart nano-carriers for drug delivery applications, *Eur. J. Pharm. Sci.* 101 (2017) 31–42.
- [39] W.R. Kadhum, T. Oshizaka, H. Ichiro, H. Todo, K. Sugibayashi, Usefulness of liquid-crystal oral formulations to enhance the bioavailability and skin tissue targeting of p-amino benzoic acid as a model compound, *Eur. J. Pharm. Sci.* 88 (2016) 282–290.
- [40] W.R. Kadhum, S. Sekiguchi, I. Hijikuro, H. Todo, K. Sugibayashi, A Novel chemical enhancer approach for transdermal drug delivery with C17-mono glycerol ester liquid crystal-forming lipid, *J. Oleo Sci.* 66 (5) (2017) 443–454.
- [41] H. Qiu, M. Caffrey, The phase diagram of the monoolein/water system: metastability and equilibrium aspects, *Biomaterials* 21 (3) (2000) 223–234.
- [42] C. Brochhausen, V.H. Schmitt, C.N. Planck, T.K. Rajab, D. Hollemann, C. Tappich, B. Krämer, C. Wallwiener, H. Hierlemann, R. Zehbe, H. Planck, C.J. Kirkpatrick, Current strategies and future perspectives for intraperitoneal adhesion prevention, *J. Gastrointest. Surg.* 16 (6) (2012) 1256–1274.
- [43] B.C. Ward, A. Panitch, Abdominal adhesions: current and novel therapies, *J. Surg. Res.* 165 (1) (2011) 91–111.
- [44] W. Wu, R. Cheng, J. das Neves, J. Tang, J. Xiao, Q. Ni, X. Liu, G. Pan, D. Li, W. Cui, B. Sarmento, Advances in biomaterials for preventing tissue adhesion, *J. Control. Release* 261 (2017) 318–336.
- [45] W.R. Kadhum, T. Hada, I. Hijikuro, H. Todo, K. Sugibayashi, Development and optimization of orally and topically applied liquid crystal drug formulations, *J. Oleo Sci.* 66 (9) (2017) 939–950.
- [46] A. Yagmur, S. Al-Hosayni, H. Amenitsch, S. Salentinig, Structural Investigation of bulk and dispersed inverse lyotropic hexagonal liquid crystalline phases of eicosapentaenoic acid monoglyceride, *Langmuir* 33 (49) (2017) 14045–14057.
- [47] J.W. Burns, M.J. Colt, L.S. Burgees, K.C. Skinner, Preclinical evaluation of Seprafilim bioresorbable membrane, *Eur. J. Surg. Suppl.* 577 (1997) 40–48.
- [48] B. Kraemer, C. Wallwiener, T.K. Rajab, C. Brochhausen, M. Wallwiener, R. Rothmund, Standardised models for inducing experimental peritoneal adhesions in female rats, *Biomed. Res. Int.* 2014 (2014) 435056.
- [49] Y. Yeo, D.S. Kohane, Polymers in the prevention of peritoneal adhesions, *Eur. J. Pharm. Biopharm.* 68 (1) (2008) 57–66.
- [50] G. Del Giudice, E. Fragapane, R. Bugarini, M. Hora, T. Henriksson, E. Palla, D. O'hagan, J. Donnelly, R. Rappuoli, A. Podda, Vaccines with the MF59 adjuvant do not stimulate antibody responses against squalene, *Clin. Vacc. Immunol.* 13 (9) (2006) 1010–1013.
- [51] A. Yagmur, L. de Campo, L. Sagalowicz, M.E. Leser, O. Glatter, Emulsified microemulsions and oil-containing liquid crystalline phases, *Langmuir* 21 (2) (2005) 569–577.
- [52] I.D.M. Azmi, J. Østergaard, S. Stürup, B. Gammelgaard, A. Urtti, S.M. Moghimi, A. Yagmur, Cisplatin encapsulation generates morphologically different multicompartments in the internal nanostructures of nonlamellar liquid-crystalline self-assemblies, *Langmuir* 34 (22) (2018) 6570–6581.
- [53] J. Barauskas, M. Johnsson, F. Tiberg, Self-assembled lipid superstructures: beyond vesicles and liposomes, *Nano Lett.* 5 (8) (2005) 1615–1619.
- [54] H. Azhari, M. Strauss, S. Hook, B.J. Boyd, S.B. Rizwan, Stabilising cubosomes with Tween 80 as a step towards targeting lipid nanocarriers to the blood-brain barrier, *Eur. J. Pharm. Biopharm.* 104 (2016) 148–155.
- [55] I.D. Azmi, L. Wu, P.P. Wibroe, C. Nilsson, J. Østergaard, S. Stürup, B. Gammelgaard, A. Urtti, S.M. Moghimi, A. Yagmur, Modulatory effect of human plasma on the internal nanostructure and size characteristics of liquid-crystalline nanocarriers, *Langmuir* 31 (18) (2015) 5042–5049.
- [56] T. Abraham, M. Hato, M. Hirai, Polymer-dispersed bicontinuous cubic glycolipid nanoparticles, *Biotechnol. Prog.* 21 (1) (2005) 255–262.
- [57] S.M. Moghimi, A.J. Andersen, D. Ahmadvand, P.P. Wibroe, T.L. Andresen, A.C. Hunter, Material properties in complement activation, *Adv. Drug Deliv. Rev.* 63 (12) (2011) 1000–1007.
- [58] I. Hamad, A.C. Hunter, S.M. Moghimi, Complement monitoring of Pluronic 127 gel and micelles: suppression of copolymer-mediated complement activation by elevated serum levels of HDL, LDL, and apolipoproteins AI and B-100, *J. Control. Release* 170 (2) (2013) 167–174.
- [59] P.P. Wibroe, I.D. Mat Azmi, C. Nilsson, A. Yagmur, S.M. Moghimi, Citrem modulates internal nanostructure of glyceryl monooleate dispersions and bypasses complement activation: towards development of safe tunable intravenous lipid nanocarriers, *Nanomedicine* 11 (8) (2015) 1909–1914.
- [60] I.D. Azmi, P.P. Wibroe, L.P. Wu, A.I. Kazem, H. Amenitsch, S.M. Moghimi, A. Yagmur, A structurally diverse library of safe-by-design citrem-phospholipid lamellar and non-lamellar liquid crystalline nano-assemblies, *J. Control. Release* 239 (2016) 1–9.
- [61] B.J. Boyd, D.V. Whittaker, S.M. Khoo, G. Davey, Hexosomes formed from glycerate surfactants—formulation as a colloidal carrier for irinotecan, *Int. J. Pharm.* 318 (1–2) (2006) 154–162.
- [62] B.J. Boyd, D.V. Whittaker, S.M. Khoo, G. Davey, Lyotropic liquid crystalline phases formed from glycerate surfactants as sustained release drug delivery systems, *Int. J. Pharm.* 309 (1–2) (2006) 218–226.