Carbohydrate Polymers 87 (2012) 1749–1755

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

# Preparation and characterization of polygalacturonic acid/rosmarinic acid membrane crosslinked by short chain hyaluronate for preventing postoperative abdominal adhesion

Ming-Wei Lee[a,b,*], Tzi-Peng Yang[a,b], Hsiu-Hui Peng[c], Jia-Wei Chen[c]

[a] School of Medical Laboratory and Biotechnology, Chung Shan Medical University, Taichung, Taiwan
[b] Department of Clinical Laboratory, Chung Shan Medical University Hospital, Taichung, Taiwan
[c] Institute of Biomedical Engineering, National Yang Ming University, Taipei, Taiwan

ARTICLE INFO

*Article history:*
Received 25 August 2011
Received in revised form
28 September 2011
Accepted 28 September 2011
Available online 5 October 2011

*Keywords:*
Polygalacturonic acid
Hyaluronate
Rosmarinic acid
Korsmeyer–Peppas model
Anti-adhesion

ABSTRACT

The most used materials for anti tissue adhesion after surgery are polymeric membranes. In this study, a short chain hyaluronate (sHA)-crosslinked polygalacturonic acid (PGA) membrane containing rosmarinic acid (PGA/RA membrane) was prepared and characterized. The *in vitro* dynamic release behavior of RA from the PGA membranes was analyzed. The profiles showed 70% of the total RA released from the membrane after 5 days. The best-fitting mathematical model for RA release from the PGA membrane was the Korsmeyer–Peppas model ($r^2 = 0.996$). *In vivo* evaluation of anti-postoperative tissue adhesion showed that use of a PGA/RA membrane reduced adhesion incidence by 90%. Anti-inflammatory analysis results showed that on the third day following surgery, the number of neutrophils and macrophages in the PGA-treated group (without RA) was 2.1 and 2.4 times that of the PGA/RA-treated group, respectively. Thus, the PGA/RA membrane developed in this study has anti-postoperative tissue adhesion and anti-inflammatory capabilities.



## 1. Introduction

Post-surgical tissue adhesion is one of the most urgent problems to be overcome in the effort to improve surgical techniques. One of the most commonly used anti-adhesion devices is a polymeric membrane that separates and isolates the wounded tissue after surgery (Yang, Chen, Xiong, Xiong, & Wang, 2009; Zhang et al., 2011). In order to develop novel anti-adhesion membranes with good mechanical properties and biocompatibility that are also cheap and easy to apply, we have developed a polygalacturonic acid (PGA)-based anti-adhesion membrane via a crosslinking reaction and found that the resultant membrane is slow to biodegrade and more effective than other commercial products in preventing post-surgical tissue adhesion (Lee, Hung, Cheng, & Wang, 2005). Unfortunately, our previous studies have shown that PGA membranes induce acute inflammation after surgical implantation into Sprague-Dawley rats. Strategies to improve the biocompatibility of PGA membranes in the present work involve preparing PGA membranes that contain rosmarinic acid (RA) for use as post-operative anti-adhesion barriers.

Rosmarinic acid (RA), $C_{18}H_{16}O_8$, is a natural phenol antioxidant carboxylic acid found in many *Lamiaceae* herbs. RA has a number of interesting biological activities, such as antiviral, antibacterial, anti-inflammatory and antioxidant activities (Wendy, Ronald, Laima, & Mark, 2010). Previous studies have reported that an anti-inflammatory mechanism of RA is mediated via the inhibition of the expression of proinflammatory chemokine and cytokines such as IL-1 (Interleukin-1), KC (keratinocyte-derived chemokine), MIP-1 (macrophage inflammatory protein 1), and MCP-1 (macrophage chemotactic protein 1) (Chiaki et al., 2003). Kim et al. reported that RA downregulates the expression of *CCL11* and *CCR3* genes (Lee et al., 2006). The expression of the *CCL11* and *CCR3* proteins are chemokines and are potent chemoattractants and activators of eosinophils, basophils and Th2 lymphocytes. RA has also been reported to inhibit complement *in vivo* as well as *in vitro*. Pangburn et al. reported that this inhibitory effect was due to the inhibition of C3/C5 convertase and showed evidence for the fact that RA inhibits complement activation by covalently binding to the activated thioester in C3b, thus blocking C3b attachment to complement-activating surfaces (Sahu, Rawal, & Pangburn, 1999). RA also inhibits the expression of the proinflammatory gene cyclooxygenase-2 (COX-2). Romagnolo Donato et al. reported that RA may exert anti-inflammatory and anticarcinogenic effects by

* Corresponding author at: School of Medical Laboratory and Biotechnology, Chung Shan Medical University, Taichung, Taiwan. Tel.: +886 4 24730022; fax: +886 4 23248171.
*E-mail address:* d880430@csmu.edu.tw (M.-W. Lee).

0144-8617/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2011.09.088

antagonizing AP-1-dependent activation of COX-2 gene expression (Scheckel, Degner, & Romagnolo, 2008).

In this study, we used RA as an anti-inflammatory drug and inserted it into the PGA membrane. PGA is an anionic plant polysaccharide extensively used in food production industries and as a drug carrier; for example, it is used as a carrier for ophthalmic drugs (Saettone, Monti, Torracca, & Chetoni, 1994) and in anti-allergy medications (Sawabe, Nakagomi, Iwagami, Suzuki, & Nakazawa, 1992). Our previous studies have shown that PGA hydrogels can be made insoluble via a short chain hyaluronate (sHA)-mediated crosslinking reaction and shown evidence that PGA-based membranes have great potential as anti-adhesion barriers. In the present work, a PGA membrane containing RA was prepared and characterized. The dynamic release behavior of RA from the PGA membrane *in vitro* was analyzed. The anti-inflammatory capability of the PGA/RA membrane and its potential ability to prevent adhesion was evaluated *in vivo*.

## 2. Materials and Methods

Polygalacturonic acid sodium salt (PGA, average molecular weight ranging from 80,000-150,000 Da), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), rosmarinic acid (RA), and trichloroacetaldehyde hydrate were purchased from Sigma–Aldrich. 1,1′-carbonyldiimidazole (CDI) and adipic dihydrazide (ADH) were purchased from Aldrich and Merck, respectively. Short chain hyaluronic acid (sHA) with a molecular weight of 4700 Da was purchased from LifeCore Biomedical, Inc. Dulbecco's modified Eagle's medium (DMEM) with low glucose was obtained from GIBCO Life Technologies. Turk's solution was purchased from Kanto Chemical Co., Inc. All chemicals used in this study were of reagent grade.

### 2.1. Synthesis of PGA-ADH

0.20 g of PGA was dissolved in 200 mL deionized water, and then, 1.62 g of ADH (the molar ratio of the carboxyl groups of PGA to ADH was set at 1:10) was added. This solution was maintained at 25 °C (pH 4.7) for 30 min, after which a coupling reaction was initiated by adding 0.89 g of EDC (the molar ratio of PGA acid to EDC was 1:5), and the solution was maintained at 25 °C for 3 h. At the end of the reaction, the pH was adjusted to neutral (7.0), and the solution was dialyzed using dialysis tubing (Spectra/Por membrane MWCO 12–14 kDa) to remove the residual ADH and EDC. The dialyzed PGA-ADH solution was freeze-dried, analyzed by $^1$H NMR (500 MHz, Bruker Advance DRX500), and stored as powder.

### 2.2. Synthesis of sHA-CDI

1 g of sHA was dissolved in 50 mL of formamide, after which 1.62 g of CDI was added (the molar ratio of the carboxyl groups of hyaluronate to CDI was 1:4). After 2 h of reaction at 25 °C, the sHA-CDI product was precipitated by acetone. The precipitate was washed 3 times with an acetone/water (90:10) solution to remove residual CDI and formamide. Finally, the sHA-CDI precipitate was dissolved in 5 mL of deionized water and freeze-dried to obtain sHA-CDI powder. The purified sHA-CDI was analyzed by $^1$H NMR (500 MHz, Bruker Advance DRX500).

### 2.3. Preparation of the PGA/RA membrane

The PGA/RA hydrogel was fabricated by mixing 0.5 mL of PGA-ADH (1%) aqueous solution, 0.5 mL of sHA-CDI (5%) aqueous solution and 0.0047 mg of RA in a Petri dish (with a diameter of 3.5 cm) to form a cylindrical gel. A rotational viscometer (Brookfield, RVDV-III + CP) was used to detect gel point at 37 °C at a rotational speed of 150 rpm. The hydrogel was then cast into a film at 43 °C for 2 days. This PGA/RA membrane was cut into a circular shape with a diameter of 1 cm, and the film thickness was measured using an electrical thickness tester (Mitutoyo, MDC-25 SB).

### 2.4. In vitro release studies

The *in vitro* drug release studies were performed in 1.5 ml micro test tubes. The PGA/RA membranes were placed into the tubes and immersed in 1 ml of phosphate buffer (0.02 M, pH 7.2). Samples ($n = 4$) were incubated at 37 °C with shaking for 5 days. At defined time points, 1 ml of the release buffer was withdrawn and replaced with fresh buffer. The RA content was determined spectrophotometrically at 325 nm. The kinetics of RA release from PGA membranes was determined by finding the best fit of the dissolution data to four distinct models, zero-order (1), first-order (2), Higuchi (3) and Korsmeyer–Peppas (4), as follows (Aguilar-de-Leyva, Sharkawi, Bataille, Baylac, & Caraballo, 2010; Vueba, Batista de Carvalho, Veiga, Sousa, & Pina, 2004):

$$Q_t = Q_0 + k_0 t \tag{1}$$

$$\log\ Q_t = \log\ Q_0 - \frac{k_1 t}{2.303} \tag{2}$$

$$Q_t = k_H t^{1/2} \tag{3}$$

$$\frac{Q_t}{Q_\infty} = k t^n \tag{4}$$

where $Q_t$ is the amount of drug released at time $t$, $Q_0$ the amount of drug in the solution at $t = 0$ (usually, $Q_0 = 0$), $Q_\infty$ the total amount of drug in the matrix, and $Q_t/Q_\infty$ the fraction of drug released at time t. In these equations, $k_0$ is the zero-order release constant, $k_1$ is the first-order kinetic constant, $k_H$ represents the Huguchi rate constant, $k$ is the Korsmeyer–Peppas dissolution rate constant and the '$n$' is the release exponent.

### 2.5. Animal implant study

A total of 20 Sprague-Dawley rats (200–250 g) were used for this study and were operated on in a surgical research laboratory. Aseptic midline laparotomies were conducted while the animals were anesthetized with 4% trichloroacetaldehyde monohydrate (1 mL/100 g). The distal 3 cm of the cecum and opposing abdominal wall were scraped with a scalpel blade until the serosal surface was disrupted and hemorrhaged but not perforated. The denuded peritoneal wall was then covered with a PGA or PGA/RA membrane (diameter 1.0 cm). Rats of the control group were not given any anti-adhesion membrane but were treated with RA powder. Contact between the cecum and the opposing peritoneal wall was maintained in all animal groups with non-occluding loops of 4/0 polypropylene suture placed 2 cm apart. After completion of the procedure, the abdomen was closed in a double layer using 4/0 polypropylene sutures in a continuous fashion. The experimental rats were sacrificed on the 3rd and 7th days after surgery to examine the process of adhesion formation at the injured site. The abdominal wall of the injured site was removed and fixed in 10% formalin solution. The tissues were processed by the standard procedure for histological examinations, and thin sections were examined after staining with hematoxylin–eosin (Lee et al., 2005). The animal experiments in this study were approved by the Chung-Shan Medical University Experimental Animal Center.

### 2.6. Anti-inflammatory analysis

Peritoneal fluids were collected before the post-operated animals were sacrificed on the 3rd and 7th days after surgery. The

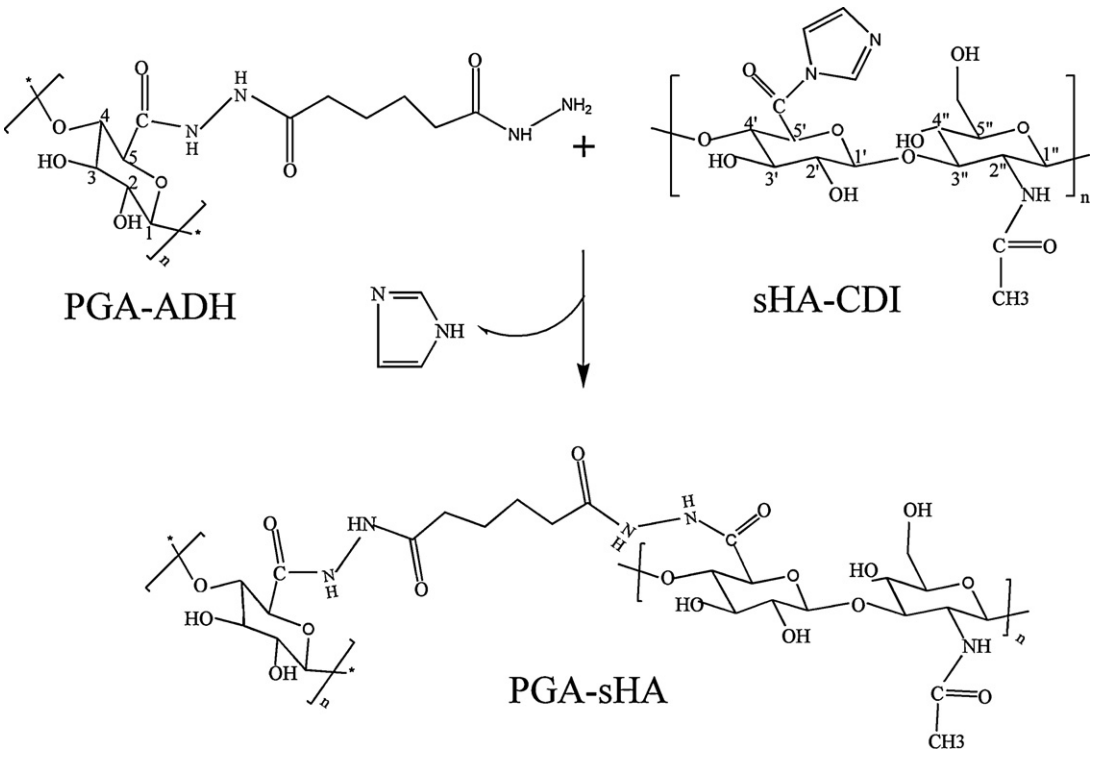


**Fig. 1.** The crosslinking mechanism of polygalacturonic acid film.

peritoneal fluid was aspirated through a pipette with a bulb tip after 3 mL of Dulbecco's modified Eagle medium containing heparin was injected into the peritoneal cavity. Turk's solution (0.01% Giemsa stain, 3% acetic acid) was used to stain the white blood cells, and the numbers of neutrophils and macrophages in the collected fluid were determined by cell counting using a hemocytometer (Kolaczkowska, Koziol, Plytycz, & Arnold, 2010).

### 2.7. Statistical analysis

Each of the experiments was repeated at least four times, and the values were expressed as means ± standard deviations. For comparison between two groups of data, the Student's *t*-test was performed. Differences were considered to be statistically significant at $P < 0.05$.

## 3. Results and discussion

### 3.1. Synthesis and characterization of PGA hydrogel

We have developed a method to crosslink plant polysaccharide PGA using short chain hyaluronate (sHA) as the crosslinker. PGA was grafted with adipic acid dihydrazide (ADH) to yield PGA-ADH, and sHA was grafted with 1,1′-carbonyldiimidazole (CDI) to yield sHA-CDI. At room temperature without adding catalyst, sHA-CDI can react with PGA-ADH to form amide bonds, resulting in the formation of a PGA hydrogel (Xuejun, Netti, Ambrosio, Nicolais, & Sannino, 2004; Yeo et al., 2006). The reaction mechanism is depicted in Fig. 1.

The $^1$H NMR spectrum of PGA-ADH presented the following signals: $\delta$ 1.7 and 2.4 ppm (methylenes of ADH) (Tan et al., 2009), $\delta$ 3.9 ppm (H2 of galacturonic acid), $\delta$ 4.2 ppm (H3 of galacturonic acid), $\delta$ 4.5–4.8 ppm (H4/H5 of galacturonic acid), and $\delta$ 5.2 ppm (H1 of galacturonic acid) (Parisot, Ghochikyan, Langlois, Sakanyan, & Rabiller, 2002). PGA-ADH had a 82% degree of substitution. The $^1$H NMR spectrum of the sHA-CDI presented the following signals: $\delta$ 8.6 ppm (–CH=N of imidazole), $\delta$ 7.5 ppm (–CH=C of imidazole) (Alila, Ferraria, Botelho do Rego, & Boufi, 2009), $\delta$ 4.6 ppm (H1′ of N-acetylglucosamine, GlcNAc), $\delta$ 4.5 ppm (H1″ of Glucurnic acid, GluA), $\delta$ 3.9 ppm (H2′ of GlcNAc), $\delta$ 3.6–3.8 ppm (H3′, H6′ of GlcNAc and H4″, H5″ of GluA), $\delta$ 3.5 ppm (H4′, H5′ of GlcNAc and H3″ of GluA), and $\delta$ 3.4 ppm (H2″ of GluA). sHA-CDI had a 65% degree of substitution. ADH is extensively used as a bis-hydrazide linker for derivatization, particularly with regards to carboxyl groups in polysaccharides used in biomedical applications. ADH has very low acute toxicity in rats, with an LD50 > 5000 mg/kg (Kennedy, 2002). On the other hand, CDI is commonly used for the activation of aliphatic carboxylic acids in peptides to form imidazole carboxylic esters, and the CDI-mediated reaction does not release toxic substances (Hearn, 1987).

### 3.2. Fabrication of the PGA/RA membrane

The CDI groups of sHA can interact with the primary amines of PGA-ADH to form a PGA hydrogel. The gelation time, determined using a rotational viscometer, was 18.8 ± 2.1 min. The relative viscosity versus time profiles for the gelation of PGA was shown in Fig. 2(a). The viscosity reached a constant value, indicating that the gelation of PGA was complete (Katayama, Nakauma, Todoriki, Phillips, & Tada, 2006). The morphology of the hydrogel was shown in Fig. 2(b). When 0.047 mg of RA was dissolved in a 1% PGA-ADH solution and then reacted with sHA-CDI, a PGA/RA hydrogel was formed. In this system, RA enters into the PGA hydrogel, and the drug loading efficiency can reach 100%. After evaporation of the PGA/RA hydrogel at 43 °C for 2 days, a membrane was fabricated with a thickness of 81.3 ± 7.5 μm.

### 3.3. In vitro release studies

The *in vitro* release studies for PGA containing RA were performed using 0.02 M PBS (pH 7.2, 37 °C) as a representative medium. Using a spectrophotometer, the amount of RA released

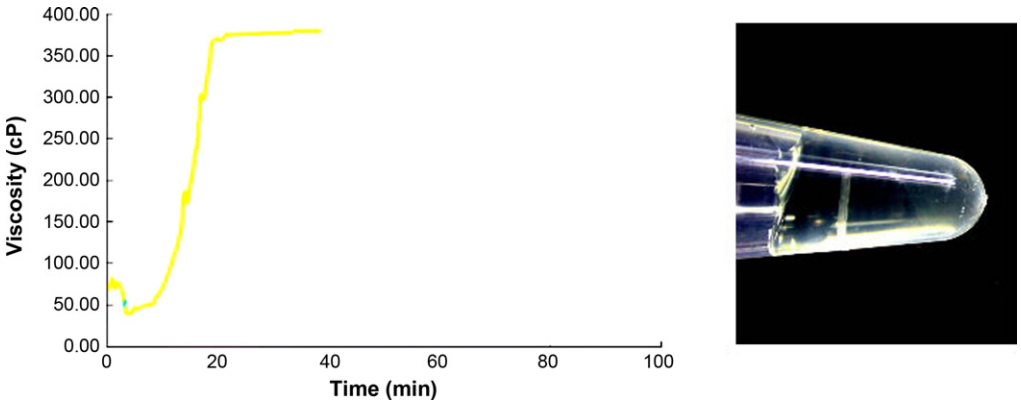


**Fig. 2.** (a) The relative viscosity versus time profiles for the gelation of PGA. (b) The morphology of the PGA hydrogel.

was measured by absorbance at 325 nm. Fig. 3 plots the percentage release of RA from PGA membranes versus time. The experimental results show that the amount of RA released increases with the release time. The release kinetics curve of RA has a steep slope between 0 and 6 h, which contributes to the initial burst of elution. This may be due to the drug being released quickly from the outermost edges of the PGA membrane. The experiment lasted 24 h, and the amount of RA released was 45%. After 24 h, the release rate of RA stabilized, and the percentage of RA released increased by approximately 10% every other day. After the release experiment had lasted 120 h, the amount of RA released was 70% (Debrassi et al., 2011). The RA in this study was used as an anti-inflammatory agent to suppress acute inflammation after abdominal surgery. Acute inflammation usually occurs 1–3 days after an operation, indicating that the observed release rate of RA from PGA meets the requirements of clinical applications.

Drug release modeling and determination of the critical parameters of carrier systems are important for understanding and elucidating mechanical and drug transport properties. This system uses PGA as a carrier of RA; PGA is a biodegradable polysaccharide polymer with water-absorbing and swelling properties. Diffusion, swelling and erosion mechanisms are the most important rate-controlling mechanisms of controlled-release products. In order to better characterize drug release from the polymeric systems studied, four common pharmacokinetics models is used to analyze the release kinetics of RA from the PGA membrane. The model that best fits the RA release data is selected based on the correlation coefficient ($r^2$) value of various models. Zero order kinetic describe systems where the drug release rate is independent of the concentration of the dissolved substance (Ochoa et al., 2011). The amount of RA released versus release time is shown in Fig. 4a. A poor linear relationship was evident ($r^2 = 0.7522$), due to the obvious burst

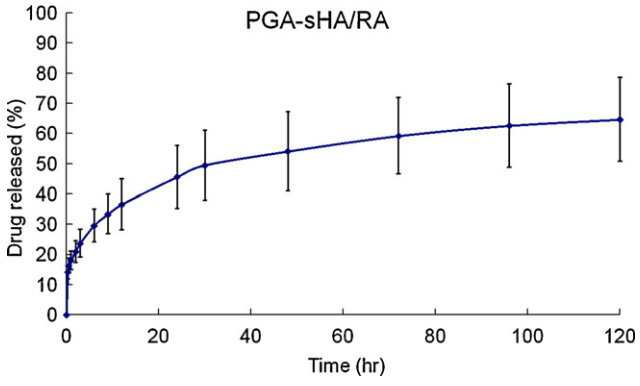


**Fig. 3.** *In vitro* RA release profile of the drug-loaded PGA membrane in PBS.

effect. The release behavior shown by the RA could be suitable for delivering a high dose initially, followed by a sustained release. The first-order model fits the release data (Fig. 4b) with low correlation coefficients ($r^2 = 0.680$). First order equation can be used to describe concentration gradients between a static liquid layer and a solid surface or bulk liquid. When the concentration gradient is constant, the surface area of the PGA membrane remains constant during the dissolution process. However, for a biodegradable polymeric matrix, disintegration occurs during the dissolution process, and the surface area generated therefore varies with time (Grambow & Müller, 2001). The first mathematical model used to describe drug release from a matrix system was the Higuchi model. The Higuchi model considers drug release as a diffusion process based on the following hypotheses: the initial drug concentration in the matrix is much higher than drug solubility; drug diffusion takes place only in one dimension; drug particles are much smaller than drug delivery system thickness; matrix swelling and dissolution are negligible; drug diffusivity is constant; and perfect sink conditions are always attained in the release environment. The release data were analyzed on the basis of the Higuchi model, and the resulting plot described a straight line with a $r^2$-value of 0.9285 (Fig. 4c), suggesting that the release of RA from PGA membranes occurs as a process dependent on the square root of time instead based on Fickian diffusion. There remains a curve deviation, which is due to the burst release of RA from the PGA membrane. This initial burst release of the drug is a result of a combination of diffusion and erosion phenomena that result in the elution of approximately 70% of the drug loaded in the matrix within the first 5 days. The PGA membrane is also capable of swelling, although drug release mechanisms from swellable matrices are not yet completely characterized. These processes may be classified as either purely diffusion- or erosion-controlled, while most systems exhibit a combination of these mechanisms (Raval, Parikh, & Engineer, 2010). The Korsmeyer–Peppas model also suggests that Fickian diffusion plays an important role in drug release. In order to understand the balance between these mechanisms, the Korsmeyer–Peppas equation is used, with the $n$-value (release exponent) indicating different release mechanisms. If the n value is 0.5, the release mechanism follows Fickian diffusion; $0.5 < n < 1$, the value indicates anomalous transport mechanism; $n = 1$ is used for case-II transport; and $n > 1$ is used for super case-II transport. The Korsmeyer–Peppas model fits the RA release data (Fig. 4d) with high correlation coefficients ($r^2 = 0.996$), but $n$ was observed to be <0.5, indicating that diffusion is not the only mechanism by which drug release can be explained. Therefore, the results demonstrate that, along with diffusion, erosion and degradation of PGA also influence RA release kinetics from the biodegradable drug delivery system (Sahoo, Sasmal, Nanda, Phani, & Nayak, 2010; Schliecker et al., 2004).

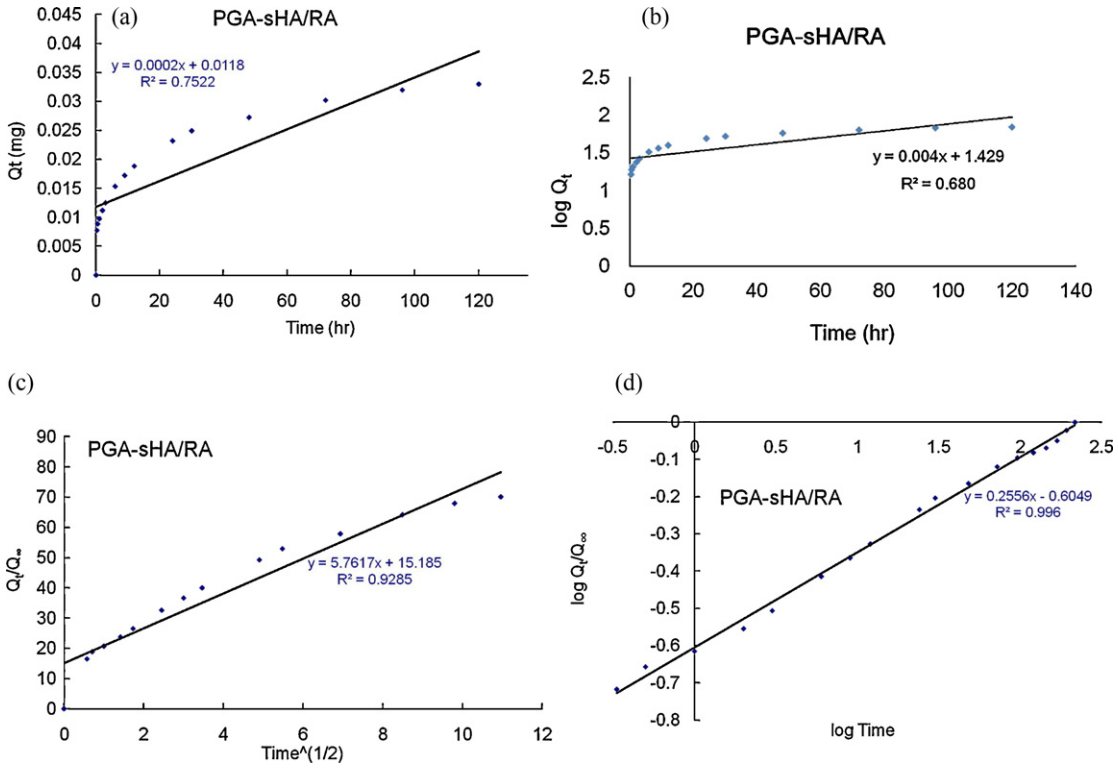


**Fig. 4.** Kinetic models applied to the release of RA from the RA-loaded PGA membranes: (a) zero order, (b) first order, (c) Higuchi, (d) Korsmeyer–Peppas.

### 3.4. *In vivo evaluation of PGA/RA for the prevention of postoperative adhesion*

The PGA/RA membrane was tested as a physical barrier to prevent tissue adhesion following abdominal surgery. The occurrence of tissue adhesion between the cecum and the peritoneum was examined on the 3rd and 7th days after surgery (Fig. 5). In experimental group 1, the PGA membrane reduced adhesion incidences by 100%. In experimental group 2, the PGA/RA membrane reduced adhesion incidences by 90%. In the control group (without a membrane but with RA), adhesion was reduced by 50%. The superior anti-adhesion capability of the PGA and PGA/RA membranes was demonstrated throughout the observation period when compared to the control group (Table 1).

Tissues surrounding the injured sites were dissected and examined. Photomicrographs of the sectioned, H&E stained tissues on the 7th day of the repairing process are shown in Fig. 6 In the control group, newly formed dense adhesive tissue was found between the peritoneal wall and the mucosa of the cecum. With PGA-treated and PGA/RA-treated, on the 3rd day after surgery, the surgical lesions had not completely healed and did not form adhesive tissue between the peritoneal wall and the mucosa of the cecum. On the 7th day after surgery, the tissues around the surgical lesions had completely healed. Histological observation showed that on the 3rd and 7th days following surgery, inflammatory cells were found around the surgical lesions in all groups.

**Table 1**
Formation of post-surgical tissue adhesion of the rats operated.

| | 3 days | 7 days | Combined data |
|---|---|---|---|
| Without membrane | 3/5[a] | 2/5 | 5/10 |
| PGA | 0/5 | 0/5 | 0/10 |
| PGA/RA | 0/5 | 1/5 | 1/10 |

[a] Number of rats formed tissue adhesion/number of rats operated.

Quantitation of the inflammatory cells was performed by a cell-counting method.

The use of physical barriers to prevent the formation of post-operative adhesion has been demonstrated in many experimental models. However, the molecular mechanism by which the physical barriers caused significant reductions in adhesion formation is not clear. PGA and PGA/RA membranes have superior anti-adhesion capabilities that may be attributable to their negatively charged surfaces, but the biological effects of the gel might also play an important role in mediating adhesion formation.

### 3.5. *Quantitative analysis of the inflammatory cells*

Peritoneal fluid from the surgical lesions was collected, and Turk's solution was used to stain the white blood cells. A hemocytometer was used to calculate the number of neutrophils and macrophages in the peritoneal fluid. The results showed that on the 3rd day following surgery, the number of neutrophils in the PGA-treated group was 2.1 times (*P*-value 0.031) that of the PGA/RA-treated group, and the number of macrophages in the PGA-treated group was 2.4 (*P*-value 0.004) times that of the PGA/RA-treated group. On the 7th day following surgery, the numbers of neutrophils and macrophages in the PGA-treated group were 1.4 (*P*-value 0.19) and 2.4 (*P*-value 0.013) times those of the PGA/RA-treated group, respectively. Neutrophils are the predominant inflammatory cell type found in a wound during the first 2–3 days after injury. Macrophage populations then become the predominant inflammatory cell type present until the end of the inflammatory process in the wound (Verstraelen et al., 2008). Our results demonstrated that a PGA/RA membrane could effectively inhibit acute and chronic inflammation in rats. Both PGA and PGA/RA membranes demonstrate anti-adhesion capabilities, but PGA/RA is the better choice for clinical applications.

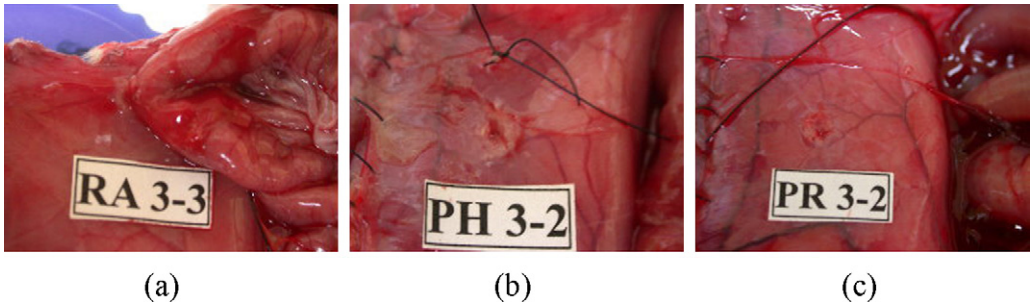


**Fig. 5.** Repair of the injured sites between the cecum and the peritoneal wall in the operated rats 3 days after surgery adhesion formation: (a) control group: without a membrane but with RA and (b) treated with PGA membrane. (c) Treated with PGA/RA membrane.

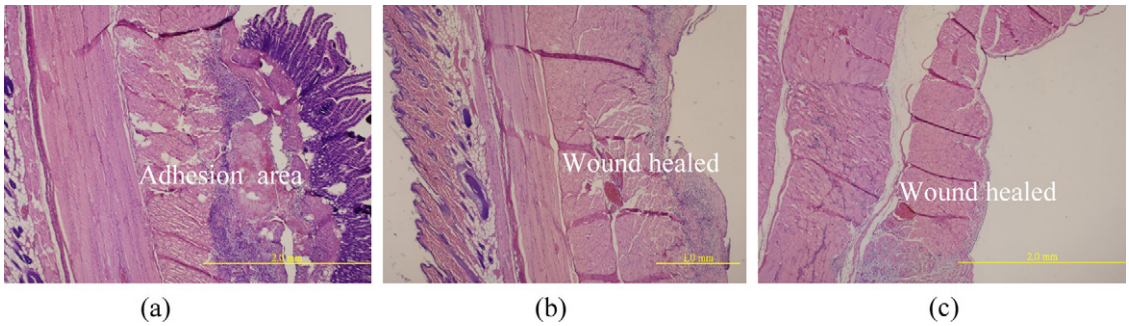


**Fig. 6.** Histological observation of the wound site in the rats 3 days after surgery, (a) control group: without a membrane but with RA and (b) treated with PGA membrane. (c) Treated with PGA/RA membrane.

## 4. Conclusion

A novel, biodegradable, PGA-based membrane for preventing postsurgical adhesion has been developed. PGA plays a role not only as a physical barrier for the separation of wounded tissues after surgery but also as an anti-inflammatory drug carrier. In this study, the release mechanism of RA from the PGA membrane was determined *in vitro*. The anti-adhesion and anti-inflammatory capabilities of the PGA/RA membrane were also demonstrated *in vivo*. The present results provide useful information on the types of polysaccharides and additives that can be employed in the formulation of membranes for use in biological applications.

## Acknowledgement

This research was supported in part by a grant from National Science Council, Republic of China (NSC 98-2221-E-040-007-MY3).

## References

Aguilar-de-Leyva, A., Sharkawi, T., Bataille, B., Baylac, G. & Caraballo, I. (2010). Release behaviour of clozapine matrix pellets based on percolation theory. *International Journal of Pharmaceutics*, *404*(1-2), 133–141.

Alila, S., Ferraria, A. M., Botelho do Rego, A. M. & Boufi, S. (2009). Controlled surface modification of cellulose fibers by amino derivatives using N,N′-carbonyldiimidazole as activator. *Carbohydrate Polymers*, *77*(3), 553–562.

Chiaki, S., Hirohisa, T., Naomi, O., Naoko, S., Midori, N., Rie, Y., Ken-ichiro, I., Yoji, K., Toshihiko, O. & Toshikazu, Y. (2003). Rosmarinic acid inhibits lung injury induced by diesel exhaust particles. *Free Radical Biology & Medicine*, *34*(8), 1060–1069.

Debrassi, A., Bürger, C., Rodrigues, C. A., Nedelko, N., Slawska-Waniewska, A., Dłużewski, P., Sobczak, K. & Greneche, J. M. (2011). Synthesis, characterization and in vitro drug release of magnetic N-benzyl-O-carboxymethylchitosan nanoparticles loaded with indomethacin. *Acta Biomaterialia*, *7*(8), 3078–3085.

Grambow, B. & Müller, R. (2001). First-order dissolution rate law and the role of surface layers in glass performance assessment. *Journal of Nuclear Materials*, *298*(1-2), 112–124.

Hearn, M. T. (1987). 1,1′-Carbonyldiimidazole-mediated immobilization of enzymes and affinity ligands. *Methods in Enzymology*, *135*, 102–117.

Katayama, T., Nakauma, M., Todoriki, S., Phillips, G. O. & Tada, M. (2006). Radiation-induced polymerization of gum arabic (*Acacia senegal*) in aqueous solution. *Food Hydrocolloids*, *20*(7), 983–989.

Kennedy, G. L. (2002). Toxicity of adipic acid. *Drug and Chemical Toxicology*, *25*(2), 191–202.

Kolaczkowska, E., Koziol, A., Plytycz, B. & Arnold, B. (2010). Inflammatory macrophages, and not only neutrophils, die by apoptosis during acute peritonitis. *Immunobiology*, *215*(6), 492–504.

Lee, J., Jung, E., Kim, Y., Park, J., Hong, S., Hyun, C. G., Park, D. & Kim, Y. S. (2006). Rosmarinic acid as a downstream inhibitor of IKK-β in TNF-α-induced upregulation of CCL11 and CCR3. *British Journal of Pharmacology*, *148*(3), 366–375.

Lee, M. W., Hung, C. L., Cheng, J. C. & Wang, Y. J. (2005). A new anti-adhesion film synthesized from polygalacturonic acid with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide crosslinker. *Biomaterials*, *26*(18), 3793–3799.

Ochoa, L., Igartua, M., Hernández, R. M., Gascón, A. R., Solinis, M. A. & Pedraz, J. L. (2011). Novel extended-release formulation of lovastatin by one-step melt granulation: in vitro and in vivo evaluation. *European Journal of Pharmaceutics and Biopharmaceutics*, *77*(2), 306–312.

Parisot, J., Ghochikyan, A., Langlois, V., Sakanyan, V. & Rabiller, C. (2002). Exopolygalacturonate lyase from *Thermotoga maritima*: cloning, characterization and organic synthesis application. *Carbohydrate Research*, *337*(16), 1427–1433.

Raval, A., Parikh, J. & Engineer, C. (2010). Dexamethasone eluting biodegradable polymeric matrix coated stent for intravascular drug delivery. *Chemical Engineering Research and Design*, *88*(11), 1479–1484.

Sahoo, S., Sasmal, A., Nanda, R., Phani, A. R. & Nayak, P. L. (2010). Synthesis of chitosan-polycaprolactone blend for control delivery of ofloxacin drug. *Carbohydrate Polymers*, *79*(1), 106–113.

Saettone, M. F., Monti, D., Torracca, M. T. & Chetoni, P. (1994). Mucoadhesive ophthalmic vehicles: evaluation of polymeric low-viscosity formulations. *Journal of Ocular Pharmacology*, *10*(1), 83–92.

Sahu, A., Rawal, N. & Pangburn, M. K. (1999). Inhibition of complement by covalent attachment of rosmarinic acid to activated C3b. *Biochemical Pharmacology*, *57*(12), 1439–1446.

Sawabe, Y., Nakagomi, K., Iwagami, S., Suzuki, S. & Nakazawa, H. (1992). Inhibitory effects of pectic substances on activated hyaluronidase and histamine release from mast cells. *Biochimica et Biophysica Acta*, *1137*(3), 274–278.

Scheckel, K. A., Degner, S. C. & Romagnolo, D. F. (2008). Rosmarinic acid antagonizes activator protein-1-dependent activation of cyclooxygenase-2 expression in human cancer and nonmalignant cell lines. *Journal of Nutrition*, *138*(11), 2098–2105.

Schliecker, G., Schmidt, C., Fuchs, S., Ehinger, A., Sandow, J. & Kissel, T. (2004). In vitro and in vivo correlation of buserelin release from biodegradable implants using statistical moment analysis. *Journal of Controlled Release*, *94*(1), 25–37.

Tan, H., Ramirez, C. M., Miljkovic, N., Li, H., Rubin, J. P. & Marra, K. G. (2009). Thermosensitive injectable hyaluronic acid hydrogel for adipose tissue engineering. *Biomaterials*, *30*(36), 6844–6853.

Verstraelen, S., Bloemen, K., Nelissen, I., Witters, H., Schoeters, G. & Van Den Heuvel, R. (2008). Cell types involved in allergic asthma and their use in in vitro models to assess respiratory sensitization. *Toxicology in Vitro*, *22*(6), 1419–1431.

Vueba, M. L., Batista de Carvalho, L. A. E., Veiga, F., Sousa, J. J. & Pina, M. E. (2004). Influence of cellulose ether polymers on ketoprofen release from hydrophilic matrix tablets. *European Journal of Pharmaceutics and Biopharmaceutics*, *58*(1), 51–59.

Wendy, P., Ronald, S. F., Laima, S. K. & Mark, B. H. (2010). Protection against LPS-induced cartilage inflammation and degradation provided by a biological extract of *Mentha spicata*. *BMC Complementary and Alternative Medicine*, *10*, 10–19.

Xuejun, X., Netti, P. A., Ambrosio, L., Nicolais, L. & Sannino, A. (2004). Preparation and characterization of a hydrogel from low-molecular weight hyaluronic acid. *Journal of Bioactive and Compatible Polymers*, *19*(1), 5–15.

Yang, D. J., Chen, F., Xiong, Z. C., Xiong, C. D. & Wang, Y. Z. (2009). Tissue anti-adhesion potential of biodegradable PELA electrospun membranes. *Acta Biomaterialia*, *5*(7), 2467–2474.

Yeo, Y., Highley, C. B., Bellas, E., Ito, T., Marini, R., Langer, R. & Kohane, D. S. (2006). In situ cross-linkable hyaluronic acid hydrogels prevent post-operative abdominal adhesions in a rabbit model. *Biomaterials*, *27*(27), 4698–4705.

Zhang, Z., Ni, J., Chen, L., Yu, L., Xu, J. & Ding, J. (2011). Biodegradable and thermoreversible PCLA–PEG–PCLA hydrogel as a barrier for prevention of post-operative adhesion. *Biomaterials*, *32*(21), 4725–4736.