Effect of Molecular Weight of Poly(Ethylene Glycol) Dicarboxylate on the Properties of Cross-Linked Hydrogel Film as an Antiadhesion Barrier

By NUNUK ARIES NURULITA

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Effect of Molecular Weight of Poly(Ethylene Glycol) Dicarboxylate on the Properties of Cross-Linked Hydrogel Film as an Antiadhesion Barrier

Haryanto , N. Aries Nurulita, E. Sundhani, P. Ho Huhc, and S. Cheol Kim

ABSTRACT

Poly(ethylene glycol) dicarboxylate (PEGDC)/poly(ethylene oxide) (PEO) cross-linked hydrogel films were developed as an antiadhesion barrier using an e-beam. The effects of molecular weight of PEGDC on hydrogel properties were investigated. The decrease in molecular weight of PEGDC increased the gel fraction and tissue adhesion, whereas the mechanical strength did not change considerably. On the other hand, the swelling ratio decreased rapidly with decreasing molecular weight of PEGDC. The cytotoxicity of PEGDC (2000 or 3000) was low, whereas that of PEGDC (1000) was higher. In animal studies, all hydrogels showed a better antiadhesive effect compared to the control.

KEYWORDS

Carboxyl content; cross-linked hydrogel; electron beam; molecular weight; poly(ethylene glycol) dicarboxylate; tissue adhesion

Introduction

Adhesion occurs in almost all open abdominal surgical procedures. The most common site for adhesion after pelvic gynecological surgeries is the ovary.[1] Adhesions can result in critical complications, including small bowel obstruction, infertility, problems with next opera-tions, and chronic debilitating pain.[2,3] Interceed, Sepra film, and Adept products have been approved and are now available for clinical use in the United States. Inter-ceed is made of oxidized regenerated cellulose. Sepra film is a blend of modified hyaluronic acid and

carboxy methyl cellulose. Adept is produced from an icodextrin solution that can be dispersed easily throughout the abdominopelvic cavity. All commercial antiadhesion products have different efficacies and do not fully satisfy the requirements for clinical implementation.[4]

A good adhesion-prevention product should be resolvable, nonreactive, easy to apply, and capable of being fixed. A previous study reported that polyethylene glycol dicarboxylate/polyethylene oxide-cross-linked hydrogel film has a good antiadhesive effect compared to Guardix-SG1 in an animal study.[5] The present paper describes the effects of molecular weight of PEGDC on hydrogel films as an antiadhesion barrier.

Hydrogels are three-dimensional network structures that absorb and retain a large amount of water. Cross-links must be present in a hydrogel to prevent dissol-ution of the hydrophilic polymer chains in an aqueous environment.[6] A hydrogel of many synthetic and natural polymers was produced with their end use mainly in pharmaceutical and biomedical fields.[7] Owing to their high water absorption capacity and bio-compatibility, they have been used in many medical applications such as contact lenses,[8] wound dressings,-[9] dental materials,[10] tissue engineering,[11] drug delivery,[12] and antiadhesion barriers.[13,14]

Poly(ethylene oxide) (PEO) has been used in a range of biomedical applications. The material has several advantageous properties as a biomaterials, such as low toxicity, biocompatible, water-soluble polymer, antith-rombogenic polymer, and nonionic polymer, but it lacks contact with the tissue.[15,16] Poly(ethylene glycol) dicarboxylate (PEGDC) contains carboxyl groups that increase the adhesive strength of the film to the tissue.[17,18] A lower molecular weight of PEGDC might improve the mechanical strength and physical proper-ties of hydrogel film due to the higher physical cross-link density. Furthermore, a lower molecular weight of PEGDC is expected to increase the adhesion of a hydro-gel film on the desired site to ensure the prevention of a postoperative adhesion.

The effects of molecular weigth of PEGDC on the gel fraction, swelling ratio, cross linking density, mechanical strength, tissue adhesion and cytotoxicity of

hydrogel films were investigated. The animal study was evaluated to examine the effects of molecular weight of PEGDC on the postoperative adhesion formation in a rat model.

Experimental

Materials

Poly(ethylene oxide) (PEO) (Mn 6 105), poly(ethylene glycol) (PEG) (Mn 3350, 2000, 1000), maleic anhydride (Mw 98.06), Dulbecco's modified Eagle's medium (DMEM), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), fetal bovine serum, and penicillin–streptomycin were purchased from Sigma-Aldrich, St. Louis, USA. Mouse fibroblast cells (NIH3T3) were purchased from ATCC (Manassas, VA, USA) and the cytotoxicity kits were purchased from Life Technology, Invitrogen, USA.

Synthesis of PEGDC

First, 6.7 g sample of PEG 3350 was dissolved in 50 ml toluene in a two-necked flask. The solution was stirred for 30 min.[5] The stirred solution was dried at 130°C by purging nitrogen for 1 h. After cooling, 0.392 g of maleic anhydride was added and the reaction mixture was stir- red for 18 h at 85°C under an N2 atmosphere. The tolu- ene was then removed by vacuum evaporation at 85°C under reduced pressure. The crude product was dis-solved in a small amount of methylene chloride and precipitated thrice into diethyl ether to remove the unreacted maleic anhydride. The precipitate was then dried in an oven at 50°C for 6 h. The same procedure was followed using a different molecular weight of PEG (2000 and 1000) in the same mole composition.-[19,20] For the next PEGDC, PEG 1000, 2000, and 3350 are referred to as PEGDC (1000), (2000), and (3350), respectively. Selected IR data of Poly(ethylene glycol) dicarboxylate were as follows.

PEGDC (1000), IR (KBr): 3,479, 2,883, 1,727, 1,448, 1,371, 1,105 cm−1.
PEGDC (2000), IR (KBr): 3,500, 2,863, 1,720, 1,625, 1,460, 1,359, 1,073 cm−1.
PEGDC (3350), IR (KBr): 3,482, 2,862, 1,728, 1,626, 1,468, 1,385, 1,131 cm−1.

Preparation of the hydrogel films

PEO and PEGDC (with various molecular weight) were used to prepare the hydrogel films. A 5% aqueous sol-ution of PEGDC/PEO (w/w) was prepared by stirring the solution at room temperature for 24 h with a 10/100 (w/w) composition of PEGDC/PEO. The calcu-lated amount of aqueous solution was poured into a petri dish for the formation of a dry PEGDC/PEO film with a thickness of 0.1 mm. The PEGDC/PEO solution was dried in an oven at 55°C for 24 h, and the remaining moisture was then removed from the vacuum oven for 6 h at the same temperature. Dry PEGDC/PEO films were sealed in an evacuated polyethylene bag, followed by irradiation at 300 kGy using an EB with beam currents of 5 and 10 mA and a voltage of 0.7 MeV.

Cytotoxicity test of PEGDC

An MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay was applied to check the metabolic activity of the cells on PEGDC/PEO films and control-2D. The NIH3T3 cells of a mouse fibroblast cell line were grown in DMEM (Invitrogen, Carlsbad, CA) that contained 10% fetal bovine serum (FBS) (Invi-trogen, Carlsbad, CA) and 1% penicillin–streptomycin (Invitrogen, Carlsbad, CA) at 37°C in a humidified atmosphere containing 5% CO2. The cells (1 × 104) were seeded overnight on a 24-well cell culture plate and incubated for an additional 1, 3, 5, and 7 days. Briefly, 500 µL of an MTT solution (5 mg/ml, Sigma Chemicals) was added to each well, which was followed by incubation for 4 h at 37°C. The supernatant was aspi- rated, and the MTT-formazan crystals produced by the metabolically viable cells were dissolved in 1,500 µl of DMSO. The absorbance was measured at a wavelength of 595 nm using an ELISA reader.[21]

Determination of gel fraction

Immediately after irradiation, the sealed film was opened and the weight of the dry film (2 × 2 cm) was measured accurately. The cross-linked hydrogel film was then placed in distilled water at 50°C for 24 h to remove the uncross-linked soluble parts. The obtained insoluble gel was dried in an oven at 50°C for 24 h

and then in a vacuum oven at 50°C for 6 h to obtain a constant dry weight. The gel fraction was determined from the weight ratio between the weight of an insoluble dry gel and the initial weight of a dry film using the following formula:

Gel fraction ¼ ðWc=WoÞ ◆ 100½%◆

where Wc and Wo are the weights of insoluble dry gel and the initial weight of a dry film.[22]

Determination of swelling ratio

The swelling ratio was determined by submerging the hydrogel film (1.5 ◆ 1.5 cm) in a buffered solution at room temperature until the hydrogel reached the equi-librium weight. The weight of the equilibrium swollen gel was measured after removing the excess surface water using filter papers. The equilibrium swelling ratio (q) was calculated using the following equation:

q ¼ ðWt=WoÞ ◆ 100½%◆

where Wt and Wo are the weight of the swollen gel at equilibrium and that of the dry film, respectively.[23]

Mechanical properties

The tensile strength and elongation at break of the cross-linked hydrogel films were measured using a ten-sile test machine (Instron 2710-105, USA) at a constant extension rate of 10 mm/min at room temperature.

Tissue adhesion

A tissue adhesion test was performed using a universal tensile machine. A test strip of hydrogel film, measuring 1 inch in width and 7 inches in length, was prepared, bent back on itself, and joined together with a half-inch strip of masking tape. The specimen formed a tear drop-shaped loop. The taped end of the specimen loop was inserted into the upper grips. The test fixture was placed in the lower grip of the tensile tester. The tensile tester was activated, so that the crosshead moves downward until the specimen loop is in complete contact with

one square inch of moist bovine intestine for 5 min. The maximum force required to remove the specimen loop from the bovine intestine was recorded as a measure of a film's adherence to the intestine.[24]

Animal study

This study was performed with assistance from the LPPT Gadjah Mada University. The animal care and use committee of Gadjah Mada University approved the methods described. Forty Sprague Dawley rats, weighing 240 to 325 g, were used for the study. All rats were housed in a 12-h-day and 12-h-night environment at standard temperature (22°C). They were fed standard laboratory food and tap water ad libitum. All rats were anesthetized with ketamine hydrochloride and xylazine hydrochloride intramuscularly.

A midline laparotomy incision measuring 4 cm was produced and the cecum was identified. The ventral side of the cecum was then abraded by thermal cautery until the burn area reached approximately 2 cm2 (1 ◆ 2 cm). The rats in the first group did not received the film on the burned area. The rats in the second, third, and fourth group received a 6 cm2 (2 ◆ 3 cm) PEGDC/ PEO film barrier with different molecular weights of PEGDC (1,000, 2,000, and 3,350 g/mol), which was placed between the burned area and the peritorium. The laparotomy incision was closed with 4/0 black silk suture. All rats were allowed to receive tap water and food ad libitum 1 day later until the 21st day.

All animals were euthanized by carbon dioxide inha-lation on the 21st day postoperatively and examined for adhesion formation by two surgeons in a double-blinded manner. The adhesion criteria were classified according to Avital et al.'s study (Table 1).[25]

Results and discussion

Cytotoxicity of PEGDC/PEO hydrogel films

In the first treatment, the hydrogel film from PEGDC (1000) showed the significant inhibition of cell viability compared to control-2D. The cell density also appears lower than that without treatment. The cell metabolic

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Table 1. Adhesion severity based on the adhesion grade and percent of injured cecal surface area.

Severity	Grade	% area involved
Absent	0	0
Moderate	1	1 ~ 100
	2	1 ~ 100
	3	<100
Severe	3	100

0 ¼ no adhesions; 1 ¼ thin filamentous, easily separated adhesions; 2 ¼ thick adhesions, difficult to dissect, do not tear organ when separated; 3 ¼ thick adhesions, not dissectible, tears organ when separated.

activity in PEGDC (2000) and (3350) appeared better and did not inhibit the cell viability. The cytotoxicity test showed that the hydrogel films of PEGDC (2000) and PEGDC (3350) do not show toxicity, whereas PEGDC 1000 showed some toxicity, albeit low. The cells were found to be viable and metabolically more active on the PEGDC/PEO hydrogel film with a higher mol-ecular weight of PEGDC, and the cell metabolic activity on all hydrogel films was better than the control-2D, as shown in Figure 1a,b.

Effect of the molecular weight of PEGDC on the gel fraction and swelling properties

Figure 2a shows the gel fraction of the PEGDC/PEO hydrogel films. The results showed that the gel fraction increased with decreasing molecular weight of PEGDC because of the increase in cross-linking density due to the higher double-

bond content in the lower molecular weight of PEGDC. Figure 2b shows the swelling ratio of PEGDC/PEO hydrogel film with various molecular weights of PEGDC as a function of swelling time. The swelling ratio decreased with increasing molecular weight of PEGDC from 651% in the PEGDC (3350)/ PEO hydrogel film to 509% in the PEGDC (1000)/ PEO film. The results indicate that the lower molecular weight of PEGDC decreases the water absorption capacity of the PEO/PEGDC hydrogel due to the increasing cross-linking of PEGDC together with radi-cals in the PEO backbones through radical coupling reaction.

Mechanical properties

Figure 3a shows the tensile strength of wet hydrogel film as a function of molecular weight of PEGDC; there was no significant difference in the tensile strength and elongation at break of each hydrogel (Figure 3b), parti-cularly at higher PEGDC contents. This indicates that all hydrogel films have good mechanical strength with 10% PEGDC for biomedical applications, such as an antiadhesion barrier.

Tissue adhesion

The adhesion strength of a hydrogel film is important to maintain the position of the film and cover up the wound site to ensure the wound healing process and minimize the adhesion with neighboring tissues. The tissue adhesiveness of a wet hydrogel film was

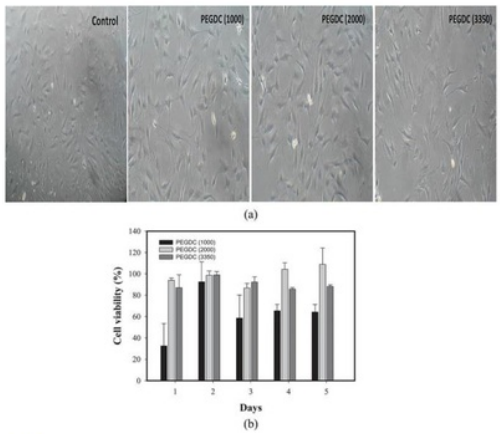

Figure 1. (a). NIH3T3 cells after the 24 h PEGDC/PEO hydrogel treatment. (b). Cell viability of the PEGDC/PEO hydrogels after the 24 h treatment.

determined by measuring the peak detachment force required to separate the film from the bovine intestine. As shown in Figure 4, the force recorded for the tissue adhesiveness reached the minimum value of 72.33 ± 4.93 cN at PEGDC (3350). The higher molecular weight of PEGDC (2000) increased the tissue adhesiveness to 86.67 ± 10.96 cN. The highest peak detachment force (116.33 ± 17.11 cN) was obtained at PEGDC (1000). This shows that the number of carboxyl groups enhanced the adherence of a cross-linked film.

Animal study

Three types of hydrogel films, PEGDC (1000)/PEO film, PEGDC (2000)/PEO film, and PEGDC (3350)/PEO film, and a control without a film were used in the animal study. No wound infection, sepsis, and dietary deficiency that would adversely affect the results were observed.

Table 2 list the grade of adhesion and the percentage of injured cecal surface area for all animals. The frequency of adhesion severity with the PEGDC group was less than those of the control, as shown in Figure 5. The grade of adhesion was assigned according to the former research.[25] The hydrogel with higher molecular weight of PEGDC showed better *in vivo* results.

Table 2. The grade of adhesion and the percentage of injured cecal surface area.

Rat		1	2	3	4	5	6	7	8	9	10
PEGDC-(1000) Guardix-SG[1]	Grade	2	2	2	2	2	2	2	0	0	1
	Area	3	4	3	3	3	3	3	0	0	2
PEGDC-(2000)	Grade	0	2	2	3	0	2	2	2	0	2
	area	0	3	3	4	0	3	3	3	0	3
PEGDC-(3350)	grade	0	1	2	1	2	2	0	0	0	1
	area	0	2	3	2	3	3	0	0	0	2
Control 10% PEGDC	grade	0	0	3	3	2	2	2	3	2	3
	area	0	0	4	3	4	3	2	4	4	4

molecular weight of PEGDC. The better adsorption of the cross-linked films induced less adhesion of the tissues with the cecum. On the other hand, the PEGDC (1000)/PEO showed worse results than that of the PEGDC(3330)/PEO hydrogel films. The increased post-operative adhesion could be related to the cytotoxicity of PEGDC (1000), which has a shorter poly(ethylene glycol) chain that should hide the inflammatory response against the carboxyl groups of PEGDC. The minimum tissue adhesion of PEGDC(3330)/PEO suggests that the cytotoxicity of the hydrogel films is more important than the adhesion of a gel film on the surface of a wound.

Conclusion

This study examined the influence of molecular weight of PEDC on the physical properties of hydrogel films and the antiadhesive effect of hydrogel film in rat model as a continuation of previous work. The decrease in molecular weight of PEGDC increased the gel fraction of the hydrogel film significantly due to the

increased number of double bonds in the 10% PEGDC/PEO hydrogels. The molecular weight had no significant effect on the tensile strength and elongation at break. The tissue adhesion increased considerably with decreasing molecular weight of PEGDC probably due to the decreased cell compatibility with the MW of PEGDC, while the swelling ratio decreased rapidly with decreasing molecular weight of PEGDC. The cytotoxi-city of the hydrogel films increased with decreasing MW of PEGDC. As a result, the antiadhesive effect of the hydrogel films with PEGDC (3350)/PEO was better than the other gel films, even though the gel film has a weaker interaction with the cecum surface.

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