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Photocrosslinkable gellan gum film as an anti-adhesion barrier

Ming-Wei Lee[a,b,*], Hui-Fang Tsai[a,b], Shu-Min Wen[b], Chia-Hui Huang[b]

[a] Department of Clinical Laboratory, Chung Shan Medical University Hospital, Taichung, Taiwan
[b] School of Medical Laboratory and Biotechnology, Chung Shan Medical University, Taichung, Taiwan

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ABSTRACT

The purpose of this study was to develop a gellan gum-based film which could be photocrosslinked for medical applications. Gellan gum was grafted with cinnamate to yield the photo crosslinkable polymer (gellan gum-cin). This material had 14.7% of its D-galacturonic residues reacted with cinnamate groups and displayed maximum absorption at 254 nm. Investigation of the photochemical properties showed that the crosslinking efficiency was 82% after 16 min of UV irradiation. The anti-adhesion films prepared from gellan gum-cin polymers exhibited high gel contents (88 ± 2%) and suitable mechanical properties. When implanted into rats, the gellan gum-cin film exhibited the most promising anti-adhesion potential in 2 out of 10 rats without forming any tissue adhesion. Furthermore, the gellan gum-cin film could effectively inhibit inflammation in rats based on the results of fluid leukocyte analyses. The gellan gum-cin film thus has potential in clinical applications.

1. Introduction

Gellan gum is a linear, anionic extracellular polysaccharide from *Pseudomonas elodea* with repeating tetrasaccharide units of D-glucose, D-glucuronic acid, D-glucose, and L-rhamnose (Jansson, Lindberg, & Sandford, 1983; O'Neil, Selvendran, & Morris, 1983). Gellan gum is a food additive that functions as a stabilizer, thickening agent, and structuring and versatile gelling agent in a wide variety of foods. Recently, gellan gum has been investigated as a candidate material for biomedical engineering because of its biocompatibility and low cytotoxicity (Oliveira et al., 2010; Silva-Correia et al., 2011). Gellan gum has also been tested as a drug-delivery carrier, cell carrier, guided bone-regeneration material, and wound dressing (Chang et al., 2010; Lee, Chen, & Tsao, 2010). The stable cross-linked structure of gellan gum can be obtained in the presence of metallic cations or by forming bonds between gellan gum molecular chains and chemical cross-linkers, such as 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC). Although the polysaccharide can be cross-linked with Ca^{2+} ions, the mechanical properties of polysaccharide are fragile and less malleable (Ichibouji, Miyazaki, Ishida, Sugino, & Ohtsuki, 2009). When implanted, tissue calcification occurs, which limits the biomedical application of Ca^{2+}-cross-linked gellan gum. In addition, chemical cross-linkers can be cytotoxic due to dosage responses and cross-linker residues (Powell & Boyce, 2006).

To develop a non-toxic method of cross-linking gellan gum that can be applied in biomedicine is the main purpose of this research work. Crosslinking via the photodimerization of polymeric systems has been utilized in various applications. In this study, we designed a new photocrosslinkable gellan gum molecule that contains a cinnamate moiety and may be used for medicinal purposes. The crosslink mechanism is based on the π-electron density of the photoactive chromophore, with dimerization of the cinnamate groups occurring presumably as a result of [2 + 2]π-electron cycloaddition (Dong et al., 2005). The reaction does not require the addition of a light-sensitive initiator. Cinnamate is a natural tropane alkaloid found within the Erythroxylum coca plant that possesses anti-inflammatory and non-toxic properties (Ballabeni et al., 2010). In this study, cinnamate functions not only as a cross-linking agent but also as an anti-inflammatory drug.

Various types of films made of polysaccharides have been reported to reduce adhesion formation, including Dextran-70, Interceed, and Seprafilm™ (Robertson et al., 2010), but have not fully satisfied the requirements for clinical implementation. An ideal adhesion-prevention product should be resolvable, non-reactive, easy to apply, and capable of being fixed. In a previous study, we demonstrated that gellan gum could prevent fibroblast adhesion and migration. In this report, we describe the evaluation of the efficacy of a photosensitive gellan gum film (denoted as GG-Cin film) in reducing postoperative adhesion formation in a rat model.

* Corresponding author at: Department of Clinical Laboratory, Chung Shan Medical University Hospital, Taichung, Taiwan. Tel.: +886 4 24730022x12412; fax: +886 4 23248171.
E-mail address: d880430@csmu.edu.tw (M.-W. Lee).

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2. Methods

2.1. Dissolution of gellan gum in dimethyl sulfoxide (DMSO)

To render gellan gum soluble in DMSO, the sodium ions of gellan gum were exchanged with the lipophilic tetrabutylammonium (TBA) ion (Oudshoorn, Rissmann, Bouwstra, & Hennink, 2007). Ion exchange was performed using Dowex® 50W-X8 cation-exchange resin (1.8 mmol/g exchange capacity; Fluka 44519). The Dowex® resin was incubated with a large excess of TBA (1:2.77 molar ratio of the exchange capacity of Dowex® to TBA) dissolved in 50 ml deionized water for 1 h and washed extensively with water. Next, the resin was transferred into 1% (w/w) gellan gum solution in water (1:10 molar ratio of the carboxyl groups of gellan gum to Dowex®-TBA) and mixed for 2 h at room temperature. The mixture was then centrifuged for 10 min at 3000 rpm to remove the resin. The obtained gellan gum-TBA solution was lyophilized and used for chemical modification with a photosensitive group.

2.2. Synthesis of gellan gum-cinnamate in DMSO

Gellan gum-TBA was dissolved in DMSO (1%, w/w). Cinnamyl bromide was dissolved in DMSO to a concentration of 4% (w/w). A mixture of gellan gum-TBA solution and cinnamyl bromide solution was stirred at 50 °C for 48 h. The mole ratio of cinnamate to gellan gum carboxyl residues was 5:1. The gellan gum-cinnamate (GG-Cin) product was purified by ethanol precipitation. The purified gellan gum-cin was analyzed by ^{1}H NMR (500 MHz, Bruker Advance DRX500).

2.3. Photochemical properties of gellan gum-cin

The photoreactivity of gellan gum-cin was studied by dissolving it in DMSO to a concentration of 0.1% (w/v) and exposing to UV light at 254 nm using a mercury lamp (Cole-Parmer 9815-series lamps 100 W) for different intervals of time. After each irradiation period (2 min), UV spectra were recorded on a scanning spectrophotometer (Milton Roy Spectronic 3000 array). The crosslinking efficiency was determined by calculating the percent conversion of photoactive chromophores using the following equation (Dong et al., 2005):

$$\%\ \text{crosslinking} = \frac{A_t - A_0}{A_\alpha - A_0} \times 100$$

where A_0, A_t, and A_α are respectively the absorbance values at time 0, time t, and time α after which no further changes were observed in the absorbance.

2.4. Preparation of gellan gum-cin film

Gellan gum-cin (0.2 g) was dissolved in 1.5 ml DMSO and then mixed with 13.5 ml deionized water. The solution was poured onto a glass dish (diameter 5 cm) and evaporated at 50 °C until the weight of the film was constant. To prevent dissolution of the non-cross-linked gellan gum-cin film in the aqueous solution, the film was immersed in ethanol and irradiated with UV light (Cole-Parmer 9815-series Lamps 100 W) for 30 min. The cross-linked gellan gum-cin film was washed with 95% ethanol three times and then dried at room temperature.

2.5. Characterizations of the cross-linked gellan gum-cin film

An electrical thickness tester (mitutoyo, MDC-25 SB) was used to measure the thickness of the gellan gum-cin film. We used the FTIR-L396A (Perkin-Elmer) to analyze the properties of the chemical functional groups of the cross-linked gellan gum-cin film. The analysis of the gel content of the cross-linked gellan gum-cin film was performed as follows. After drying, we weighed the cross-linked film (W_1) and then swelled it in DDW at 37 °C for 24 h. After removing the wet film from the solution, the film was dried in a vacuum oven for 12 h at 60 °C and then weighed again (W_2). The gel content (%) was 100 (W_2/W_1).

The gellan gum-cin film was cut into 1 cm × 5 cm pieces (Mathew & Abraham, 2008). We then used the H1-KS testing machine (Tinius Olsen) with a crosshead speed of 5 mm/min to measure the mechanical properties of the gellan gum-cin films and to automatically record the mechanical parameters.

2.6. Animal implant study

Twenty Sprague-Dawley rats (200–250 g) were tested 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 until the serosal surface was disrupted and hemorrhaged but not perforated. The denuded peritoneal wall was then covered with a gellan gum-cin film (diameter: 1.0 cm). The rats in the control group were not covered with any anti-adhesion film. Contact between the cecum and opposing peritoneal wall was maintained in all animal groups with two nonoccluding loops of 4/0 polypropylene sutures placed 2 cm apart. After completion of the procedure, the abdomen was closed in a double layer using 4/0 polypropylene in a continuous fashion. The experimental rats were sacrificed on day 3 or 7 after surgery to examine the process of adhesion formation at the injured site (Peng et al., 2011). Adhesions were scored in a blinded manner according to the method of Zuhlke HV et al. (Table 1), where grade 0 indicates no adhesions and grade 4 indicates firm extensive adhesions that are dissectable only with sharp instruments and almost unavoidable organ damage. 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 their thin sections were examined after staining with hematoxylin–eosin (H–E).

2.7. Peritoneal fluid analysis

Peritoneal fluids were collected before the operated animals were sacrificed on day 3 or 7 after surgery. The peritoneal fluid was aspirated through a pipette with a bulb tip after 3 ml of the DMEM containing heparin was injected into the peritoneal cavity. Turk's solution (0.01% Giemsa stain and 3% acetic acid) was used to stain white blood cells, and the number of neutrophils in the collected fluid was determined by cell counting using a hemocytometer.

2.8. Statistical analysis

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

Table 1
Grading of adhesion according to Zuhlke (grade description).

0	No adhesions
1	Filmy, fibrin adhesions, easily removed by blunt dissection (mild)
2	Fibrous adhesions, easily dissected (moderate)
3	Thick fibrous adhesions, dissectable (severe)
4	Thick fibrous adhesions, not dissectable without damage to the adherent tissue (very severe)