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Prevention of desiccation induced postsurgical adhesion by thermosensitive micelles

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ABSTRACT

Tissue desiccation is a common cause of postsurgical peritoneal adhesion, which leads a variety of adverse consequences in clinic. Until now, many approaches have been advocated to prevent postsurgical adhesions, but the therapeutic effects were not very encouraging. In this work, biodegradable and thermosensitive poly(ε-caprolactone)-poly(ethylene glycol)-poly(ε-caprolactone) (PCL–PEG–PCL) micelles were prepared and assigned for anti-adhesion studies. The micelles were a free flowing sol at low temperature, but instantly inverted into a non-flowing gel at physiological temperature, which could serve as a potential candidate as anti-adhesion barrier. A novel cecum desiccation rat model was established and used for anti-adhesion studies. The micelles were sol state before use, which could cover the injured cecum unrestrictedly, and then formed gel in body temperature and adhered to the injured sites. All the rats in the control group developed adhesions with score 5 or 4, whereas none in the micelle-treated group developed adhesions with score 5 or 4 ($P < 0.001$, Mann–Whitney U test). Histopathological assessment also confirmed that the micelles exhibited excellent anti-adhesion effects on desiccation-induced peritoneal adhesion. Adhesiveness and degradation behavior of the micelles suggested that the micelles could adhere to the injured cecum and prevent adhesion in the critical time of healing process. The results indicated that the thermosensitive micelles could serve as an effective anti-adhesion barrier.
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1. Introduction

Tissue adhesion is a common complication after operation, which can be induced by many reasons, including infections, trauma, desiccation, ischemia, foreign bodies, etc [1]. Postsurgical adhesion causes many problems, such as female infertility, bowel obstruction, and difficulties in re-operations [2]. In open surgical procedures, organs are exposed for the convenience of operation, which leads the desiccation of the organs. In laparotomy, carbon dioxide pneumoperitoneum is often used to expand the surgical field, and the organs exposed to carbon dioxide also cause desiccation [3,4]. Desiccation induces injury of peritoneum which is composed by a layer of mesothelial cells. Under normal circumstances, mesothelial cells can secrete glycosaminoglycans and lubricants to protect tissues from abrasion and prevent adhesion formation. Injury of mesothelial cells reduces their function of secretion and in the same time cause an inflammatory response. Then, the inflammatory response increases vascular permeability, enhance release of plasma proteins-rich exudates, and eventually induce formation of adhesion [5]. Besides, tissue adhesion not only formed in the desiccation sites, but also in the tissues far from desiccation sites [6,7]. Therefore, it is necessary to prevent the desiccation induced tissue adhesion after surgery.

Because postsurgical adhesions occur in the early stage after surgery, numerous drugs or barriers were used during the surgery to prevent the formation of tissue adhesion [8,9]. Many drugs were used, including anti-inflammatory and antithrombotic drugs, but the anti-adhesion effects were not very encouraging, which was due to the fast absorption of drugs in abdominal cavity [10]. Many anti-adhesion barriers were also employed, including polymer solution, solid sheets, and *in situ* cross-linking hydrogels [11,12]. The *in vivo* residence time of polymer solutions was short, which dramatically reduced their anti-adhesion effects [13]. For the application of solid sheets, it was difficult to cover the whole injured peritoneal surfaces, and application of solid sheets in laparoscopy

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was inconvenience [14]. Recently, *in situ* cross-linking hydrogels were investigated as anti-adhesion barriers, however, the relatively long gelation time and risk of prolonged residence times *in vivo* (over 10 days) limited their application [15,16]. Thus, it is still necessary to develop a safe, effective and easy-to-use anti-adhesion barrier.

In our previous work, poly(ε-caprolactone)-poly (ethylene glycol)-poly(ε-caprolactone) (PCL–PEG–PCL) thermosensitive micelles have been prepared and characterized in detail [17,18]. Then, the micelles were assigned for adhesion prevention in sidewall defect-cecum abrasion rat model, in which the micelles showed excellent anti-adhesion activities [19]. Desiccation induced postsurgical adhesion is very common in both laparoscopic and open surgical procedures, which induces severe tissue adhesions. However, the anti-adhesion efficacy of the micelles on desiccation induced postsurgical adhesion is still unknown. In the present study, PCL–PEG–PCL thermosensitive micelles were assigned for anti-adhesion studies in a desiccation induced postsurgical adhesion rat model, which could simulate clinical circumstances.

2. Materials and methods

2.1. Materials, cells, and animals

Poly(ethylene glycol) (PEG, $Mn = 1000$), ε-caprolactone (ε-CL), stannous octoate ($Sn(Oct)_2$), and methyl thiazolyl tetrazolium (MTT) were purchased from Sigma-Aldrich company (St Louis, MO, USA). All reagents used in this article were of analytical reagent (AR) grade and used as received.

L929 cells (murine fibroblast cell line) were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA) and cultured in Roswell Park Memorial Institute 1640 medium (RPMI 1640, Gibco, Grand Island, NY, USA)) supplement with 10% fetal bovine serum (FBS, Gibco, Grand Island, NY, USA).

Female Sprague-Dawley (SD) rats, weighing 220 ± 20 g, were purchase from the Laboratory Animal Center of Sichuan University. Animals were housed under specific pathogen free (SPF) conditions, and provided with standard laboratory chow and tap water *ad libitum*. After quarantined for one week, all the rats were used for experiments. All animal care and experimental procedures were carried out according to Principles of Laboratory Animal Care.

2.2. Synthesis and characterization of PCL–PEG–PCL copolymer

Biodegradable PCL–PEG–PCL copolymer was synthesized according to our previous works [20]. In detail, PEG, ε-CL, and $Sn(Oct)_2$ were added into a dry glass ampoule under nitrogen atmosphere. The reaction system was kept at 130 °C for 6 h and then degassed under vacuum for another 1 h. The PCL–PEG–PCL copolymer was dissolved in dichloromethane and precipitated with cold petroleum, and then filtered and vacuum dried to constant weight at room temperature. The copolymer was characterized ^{1}H-NMR (Varian 400 spectrometer, Varian, USA) and FTIR (NICOLET 200SXV, Nicolet, USA).

2.3. Preparation and characterization of thermosensitive micelles

Thermosensitive PCL–PEG–PCL micelles were prepared according to our previous work [8]. Briefly, the obtained PCL–PEG–PCL copolymers were dissolved in deionized water (20 wt%) at 55 °C, and then cooled to 4 °C to form micelles. Particle size distribution of the micelles in aqueous solution was determined by a laser diffraction particle size analyzer (Nano-ZS 90, Malvern Instrument, UK) at 10 °C. Data were obtained from three individual samples, and all data were expressed as the mean ± standard deviation (S.D.).

Morphology of PEG–PCL–PEG micelles was investigated by transmission electron microscope (TEM, H-6009IV, Hitachi, Japan). Micelles were diluted with distilled water, negatively stained with phosphotungstic acid, placed on a copper grid covered with nitrocellulose, and dried at room temperature.

Thermosensitivity of PEG–PCL–PEG micelles was investigated by tube-inverting method [21,22]. Micelles were loaded into a 4 mL tightly screw-capped vial and then heated at the heating rate of 1 °C/min. The sol–gel–sol phase transition was visually observed by inverting the vials.

2.4. Cytotoxicity evaluation of the micelles

MTT assay was used to evaluate the cytotoxicity of PCL–PEG–PCL micelles in L929 cells. Briefly, L929 cells were plated into 96-well and incubated overnight. Then, the cells were exposed to a series of PCL–PEG–PCL micelles up to 1000 μg/mL. After incubated for 24 h or 48 h, MTT solution (5 mg/mL) was added. After 4 h, 200 μL of DMSO was added and the absorbance at 570 nm was measured by an ELISA reader (Multiskan Mk3, Thermo Scientific). Cell viability was calculated as the mean percentage of cell survival relative to that of untreated cells. Data were obtained from six individual experiments, and all data were expressed as the mean ± S.D.

2.5. Cecum desiccation adhesion model and treatment

Rat cecum desiccation model was introduced to investigate the anti-adhesion effects of PCL–PEG–PCL micelles. Rats were anesthetized by pentobarbital sodium (10 mg/mL) and an anterior midline incision was then made through the abdominal wall and peritoneum. The cecum was isolated and desiccated with sterile carbon dioxide (room temperature) at flow rate of 1 mL/s for 10 min to assure that the mesothelial cell layer was completely desiccation. Then, the desiccated cecum was returned to peritoneal cavity. Before complete closure, 1 mL of the micelles were extruded to coat the both the desiccated cecum. The incision was closed in two layers with 3/0 surgical silk suture. Eight rats received treatment of micelles and the other eight rats were treated with 1 mL NS which served as control. In the whole operation process, standard aseptic procedure was used. Fourteen days later, all rats were sacrificed with an overdose of intravenous sodium pentobarbital and examined for adhesion formation by two observers in a blinded manner. Animals were evaluated according to the following standard adhesion scoring system, which has been widely used in this field: score 0, no adhesion; score 1, one thin filmy adhesion; score 2, more than one thin adhesion; score 3, thick adhesion with focal point; score 4, thick adhesion with plantar attachment or more than one thick adhesion with focal point; score 5, very thick vascularized adhesion or more than one plantar adhesion.

2.6. Histopathological assessment and scanning electron microscopy (SEM) analysis

For histopathological examination, samples were obtained from the injured cecum and adhesion tissues containing cecum and other tissues. The samples were fixed by 4% paraformaldehyde, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E) and Masson trichrome staining under standardized conditions. The tissue slides were analyzed by a light microscopy (Olympus BX 45, Olympus, Hamburg Germany) by two pathologists in a blinded manner.

For SEM analysis, the tissue samples were fixed with 2.5% glutaraldehyde, dehydrated in a critical point apparatus, and examined by SEM (JSM-5900LV, JEOL, Japan) after a gold sputter coating.

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