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In situ cross-linking carbodiimide-modified chitosan hydrogel for postoperative adhesion prevention in a rat model

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ABSTRACT

Background: Postoperative intra-abdominal adhesion often causes many complications. Chitosan fluid has been used in clinic to prevent intra-abdominal adhesion. However, fluid can be easily diluted and cannot stay on the wound site. As hydrogel is able to form stable physical barrier to separate injured tissues, we developed a chitosan hydrogel for better prevention of intra-abdominal adhesion in this study.
Methods: We synthesized a carbodiimide-derivatized chitosan gelatin (cd-CS-gelatin) hydrogel and investigated its rheological properties. A rat model was used to compare the anti-adhesion effect of chitosan hydrogel and fluid. The wounds were created with damage of the underlying muscle of the abdominal wall and the serosal layer of the cecum. They were coated with chitosan fluid or cd-CS-gelatin hydrogel. At day 14 after surgery, the animals were euthanized and intra-abdominal adhesion was assessed.
Results: The cd-CS-gelatin hydrogel solidified within 3 min after the mixing of the reagents. The cecum-abdomen adhesion occurred in all rats without anti-adhesion treatment. The application of cd-CS-gelatin significantly reduced the adhesion rate from 100% to 50%, compared the chitosan fluid only to 88%. The decrease of adhesion breaking strength also manifested that cd-CS-gelatin was more effective than chitosan fluid to reduce postsurgical intra-abdominal adhesion formation.
Conclusions: Chitosan hydrogel is more effective than chitosan fluid to prevent postoperative cecum-abdomen adhesion. It indicates that hydrogel could be a more promising state than liquid to prevent postoperative intra-abdominal adhesion.

1. Introduction

Intra-abdominal adhesion, which is induced by injuries during an abdominal operation, is a common postoperative complication >67% of patients with previous abdomen surgery suffered with intra-abdominal adhesion [1], which causes a number of complications such as chronic pelvic pain [2], intestinal obstructions [3], infertility [4], and so forth.

The methods to prevent intra-abdominal adhesion formation in clinic can mainly be attributed to one of two categories. One is improving surgical technique. For instance, the laparoscope is invented to minimize incision. However, this technique could not possibly be applied in many cases. The other category is to apply anti-adhesive biomaterials, which serve as physical barriers. The anti-adhesive biomaterials separate the injured tissue surface from surrounding tissues/organs. As a result, the intra-abdominal adhesions decrease accordingly. Gore-Tex® (W.L. Gore &Associates, Inc., Flagstaff, AZ), Interceed® (ETHICON Women's Health and Urology, Somerville, NJ) and Seprafilm® (Genzyme, Cambridge, MA) are the widely used biomaterials to prevent intra-abdominal adhesions in clinic [5]. Although the products can significantly reduce adhesion formation, their shortcomings, like undegradable and easily fragile once being wet, are not negligible [6]. It is necessary to develop the better anti-adhesive biomaterial products.

Chitosan is a deacetylated derivative of chitin, with a linear polysaccharide containing randomly distributed β-1,4-linked D-glucosamine and *N*-acetyl glucosamine residues [7]. It derived from crustaceans is nontoxic, renewable, biodegradable, as well as naturally abundant and easily functionalized. It has been taken into account for medical and pharmaceutical applications due to its interesting intrinsic properties [8], which allows it to be an ideal material to develop tissue engineering materials for the repair of skin, bone and cartilage [9,10]. In addition, chitosan is proven of hemostatic ability, antimicrobial action, and inhibiting fibroblast adhesion [11]. Therefore chitosan and its derivates could be the appropriate biomaterials for the prevention of intra-abdominal adhesion.

The commercial medical chitosan products approved by China Food and Drug Administration for preventing intra-abdominal adhesion

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formation are membranes and viscous fluids [12]. Modified chitosan medical anti-adhesion membranes BaiFeiMi® (Beijing Bailikang Biochemistries Co. Ltd., Beijing, China) and Nachitin® (Yantai Wanli Medical Equipment Co. Ltd., Shandong, China) were reported to be able to prevent post-surgical intra-abdominal complications, such as small bowel obstruction, and to improve the recovery of gastrointestinal function [13,14]. A medical chitosan fluid (Chitogel®, Shanghai Qisheng Biological Preparation Co. Ltd., Shanghai, China) was investigated in 125 patients, who underwent laparotomy in obstetrics and gynecology. It was found that chitosan fluid could effectively prevent postoperative adhesions [15]. In another clinical investigation, the application of a medical chitosan fluid (Shu Yi Ning®, Shijiazhuang Yishengtang Medical Products Co. Ltd., Hebei, China) significantly decreased the occurrence of intestinal adhesion and pelvic pain in 180 cases of vaginal hysterectomy [16].

Membranes and fluids of medical chitosan products are convenient in clinical applications. However, membranes are hardly used in laparoscopic surgery, and the fluids can be easily diluted by the exudates from the wounded tissue and subsequently prone to flowing to other locations in the abdominal cavity instead of residing on the injured area. Recently, we reported that a stable hydrogel formed from a chemically activated sodium hyaluronate solution (cd-HA-gelatin), resulting in a significant reduction in the incidence, score, and breaking strength of intra-abdominal adhesion in a rat model [17]. In this study, we developed a chitosan-derived hydrogel to prevent intra-abdominal adhesion in a SD rat model. The effect of the chitosan hydrogel and the chitosan fluid on intra-abdominal adhesion prevention was investigated by the incidence, the severity, the area, the breaking strength, and the work of separation of the cecum-abdomen adhesion.

2. Materials and methods

2.1. Materials and animals

Medical chitosan fluid (Chitogel®) was purchased from Shanghai Qisheng Biological Preparation Co. Ltd. (Shanghai, China). Gelatin from porcine skin was provided by Rousselot Gelatin Co. Ltd. (Guangdong, China). 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and chitosan (degree of deacetylation ≥ 75%) were purchased from Sigma-Aldrich (USA).

The animal experiment in this study was designed according to the Guidelines for Animal Experiments of Shenzhen Institutes of Advanced Technology (SIAT). Thirty-two specific-pathogen-free (SPF) Sprague-Dawley rats (8- to 10-week-old, weighing 230–260 g) were used in this experiment at SIAT and the Shenzhen PKU-HKUST Medical Center Experimental Animal Center. The animals were housed in SPF condition, and kept ad libitum access to food and tap water.

2.2. Preparation of cd-CS-gelation hydrogel

Chitosan and gelatin were first dissolved in phosphate buffered saline solution (5.0–5.5). The EDC was dissolved in the same buffer solution. The cd-CS-gelatin hydrogel was prepared by mixing chitosan-gelatin and EDC solutions (volume ratio = 1:1). The final concentrations of EDC, chitosan, and gelatin in the hydrogel were 0.25%, 0.5%, 5%, respectively.

2.3. Rheological analysis

Rheological properties of cd-CS-gelatin hydrogel and medical chitosan fluid were characterized with a Physica MCR302 rheometer (Anton Paar, Austria) with a parallel plate system. In oscillatory mode, 0.3 ml chitosan and gelatin solution was mixed with the equal volume of EDC solution using a double syringe with a mixing chamber at 37 °C. Following the mixed solutions applied to the bottom plate, the top plate was immediately lowered and kept a gap of 0.7 mm to the bottom plate. The storage modulus and loss modulus were measured under a condition of 10% strain and 1-Hz-frequency oscillation. The gelation time was defined as the time point when the value of storage modulus exceeded loss modulus.

2.4. Surgical procedures

Thirty-two rats were used in this study. The animals were anesthetized with sodium pentobarbital (40 mg/kg) by intraperitoneal injection and the abdomens were shaved and disinfected with iodophor. A 5-cm incision in skin was made along the midline of rat abdomen. The cecum was taken out from the abdominal cavity and placed on a piece of gauze. All rats were randomly assigned to one of 4 groups ($n = 8$): 1) sham, 2) adhesion, 3) chitosan fluid, and 4) cd-CS-gelatin hydrogel.

In the sham group, the cecum was put back into the cavity and the abdomen was closed with a 4/0 Vicryl running suture, and the skin incision was closed with 4–0 silk interrupted suture.

In the adhesion group, the ceca of rats were abraded with a toothbrush for 100 strokes, and homogeneous petechial hemorrhage over a 1×2 cm area on the serosal layer was created. Then a 1×2 cm segment of parietal peritoneum, 1 cm lateral to the midline incision, was resected from the superficial layer of underlying muscle of each rat. The injured cecum was returned to its original location. The abraded serosal layer was in contact with the injured abdominal wall.

In the chitosan fluid group, after injuries were created on cecal surface and abdominal wall as described above, 0.6 ml medical chitosan (Chitogel®, Shanghai Qisheng Biological Preparation Co. Ltd., Shanghai, China) was applied onto the injured area of the cecum and the abdominal wall.

In the chitosan hydrogel group, the abraded cecal surface and the injured abdominal wall were both covered with 0.6 ml mixture of carbodiimide-modified chitosan solution and gelatin solution (cd-CS-gelatin). The mixed cd-CS-gelatin solution formed a stable hydrogel in 3 min. After treatment, the abdominal wall and skin were closed as described above.

A single person (FY) performed all procedures concerning cecum abrasion and parietal peritoneum dissection. All rats were sacrificed to assess adhesion formation at day 14 after the surgery.

2.5. Adhesion score

An over-dose of sodium pentobarbital (120 mg/kg) was intraperitoneally injected to euthanize the rats on the 14th day after the surgery. The abdomen was re-opened by a U-shaped laparotomy. The cecum-abdomen adhesion was assessed with the incidence, the severity and the area according to the previously established scoring system [18,19] with minor modifications (Table 1). Four independent investigators who were blind to the treatments examined the cecum-abdomen adhesion of each rat.

2.6. Mechanical evaluation of intra-abdominal adhesion

A customized mechanical testing system was employed to assess the cecum-abdomen adhesion [20], based on the protocol in our published work [21]. In brief, the excised cecum-abdomen complexes were mounted on the device horizontally. The cecum was held tightly with

Table 1
Adhesion scoring criterion.

Grade	Adhesion severity	Adhesion area
0	No adhesions	No adhesions
1	Thin filmy adhesion	0–25% of initial injured area
2	More than one thin adhesion	25–50% of initial injured area
3	Thick adhesion with focal point	50–75% of initial injured area
4	Thick adhesion with planar attachment	75–100% of initial injured area