Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.JournalofSurgicalResearch.com

Effect of "phase change" complex on postoperative adhesion prevention

Xiao-Dong Li, BS,[a,b,1] *Dong-Lin Xia, MS,*[a,c,1] *Ling-Ling Shen, BM,*[d] *Hong He, MN,*[b,e] *Chao Chen, MS,*[a,c] *Yu-Fei Wang, MS,*[a,c] *Yan-Pei Chen, MS,*[a] *Ling-Yan Guo, BN,*[d] *and Hai-Ying Gu, PHD*[a,b,c,*]

[a] *School of Public Health, Nantong University, Nantong, China*
[b] *Nantong Tongda Chemicals Safety Evaluation Center Co Ltd, Nantong, China*
[c] *Institute of Analytical Chemistry for Life Science, Nantong University, Nantong, China*
[d] *Institute of Nautical Medicine, Nantong University, Nantong, China*
[e] *Affiliated Hospital of Nantong University, Nantong, China*

ARTICLE INFO

Article history:
Received 13 August 2015
Received in revised form
26 November 2015
Accepted 22 December 2015
Available online 30 December 2015

Keywords:
Postoperative adhesion
Phase change
Chitosan
CsA
Adjustable
Degradable

ABSTRACT

Background: Postsurgical peritoneal adhesion is a major clinical problem. Numerous anti-adhesion products have been studied, but none could be easily used to provide a physical barrier. In this study, we developed a "phase change" anti-adhesion barrier for reducing peritoneal adhesion by cross-linked copolymerization of O-carboxymethyl chitosan (CMC) and $CaCl_2$ and addition of cyclosporin A (CsA).

Materials and methods: The CMC–$CaCl_2$–CsA compound was characterized by equilibrium swelling rate, weight loss, releasing effect, and coagulation test, and its biosafety was characterized by acute oral toxicity, hemolysis, and cytotoxicity. Intestinal adhesion model was applied on 64 Sprague–Dawley rats, which received CMC, CMC–$CaCl_2$, or CMC–$CaCl_2$–CsA treatment. At postoperative days 7 and 14, the rats were euthanized, and adhesions were graded by an investigator blinded to the treatment groups, using a predetermined adhesion scoring system. The cecum and adhesion tissue were stained with hematoxylin and eosin and antibodies for matrix metalloproteinase-9 and TIMP-1 for further histopathologic examination.

Results: The phase change anti-adhesive material exhibited effective blood clotting and were nontoxic in clotting experiments and acute toxicity test. The degradation rate could be adjusted using phosphate-buffered solution with varying pH. Adhesions were significantly reduced in the CMC–$CaCl_2$–CsA treatment group compared with the control group ($P < 0.001$). Expression of matrix metalloproteinase-9 was stronger in CMC–$CaCl_2$–CsA treatment group at 7 days after surgery.

Conclusions: "Phase-change" adhesive can undergo changes after application, and it inhibits the formation of abdominal adhesions after surgery. The material is convenient for using by surgeons and provides an effective tool for intestinal adhesion prevention.

* *Corresponding author*. School of Public Health, Institute of Analytical Chemistry for Life Science, Nantong University, Nantong Tongda Chemicals Safety Evaluation Center Co Ltd, No. 9, Seyuan Road, Nantong, Jiangsu Province, China. Tel./fax: +86 513 81188058.
E-mail address: hygu@ntu.edu.cn (H.-Y. Gu).

[1] These authors equally contributed to this work.

0022-4804/$ – see front matter © 2016 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jss.2015.12.033

1. Introduction

In clinical studies of patients who had surgeries, the incidence of intra-abdominal adhesions was shown to be about 90% [1]. Postsurgical peritoneal adhesions may cause inflammation, chronic pain, bowel obstruction and even female infertility, and other complications [2]. Therefore, many tools, such as nonabsorbable spacers, absorbable spacers, viscous solutions, and other, have been studied to prevent postsurgical adhesions [3–5]. Significant amount of research has been conducted on mechanical barriers [6], such as hyaluronic acid/carboxymethyl cellulose [7], oxidized regenerated cellulose [8], expanded polytetrafluoroethylene [9], bioabsorbable gels [10], and fluid agents.

In theory, inert materials that prevent contact between the damaged serosal surfaces during the first few critical days after surgery allow separate healing of the injured surfaces and may help to prevent adhesion formation. However, such materials are inconvenient to use, and their stiffness and hydrophobicity limit their application. Solutions are more convenient to use, but their efficacy of preventing adhesion is poor. Thus, the current approaches cannot prevent formation of postsurgical adhesions completely. It is worthwhile therefore to develop an easy-to-use postoperative adhesion prevention solution, which would change to a solid acting as a mechanical barrier, and decompose after adhesion recovery.

Chitosan is used to prevent postsurgical adhesion formation due to its numerous qualities, such as being biocompatible, biodegradable, hemostatic, nontoxic, and capable to reduce fibroblast adhesion [11,12]. O-carboxymethyl chitosan (CMC) is a deacetylated derivative of chitin with multiple important biological characteristics, such as antimicrobial activity, regulation of immune function, promotion of wound healing, and inhibition of scar formation. *In vivo* studies have demonstrated that CMC can effectively inhibit the proliferation of fibroblasts although it does not have an effect on the growth of epidermal cells [13]. As CMC is water-soluble, it could not accumulate in injured area after abdominal surgery, which affected the efficacy of its anti-adhesion action.

Cyclosporin A (CsA) is a potent immunosuppressive drug, and previous studies have found that it also has effects on the cell proliferation and collagen synthesis of human fibroblasts [14]. At appropriate concentrations, CsA can effectively suppress proliferation of a variety of cells, including conjunctival fibroblasts. As it is important to control fibroblast proliferation and collagen synthesis as soon as possible after abdominal surgery to prevent adhesions, CsA represents a major anti-adhesion material.

In this study, a type of "phase change" material for use in postoperative adhesion prevention was prepared by cross-linked copolymerization of O-CMC and $CaCl_2$ and addition of CsA. This material is liquid when applied, then cross-links to form a colloidal compound providing a physical barrier in intra-abdominal space, and decomposes on healing. We assess the effect of "phase change" adhesive on adhesion formation in a widely accepted rat intestinal injury model. Our results demonstrate that the "phase change" adhesive can undergo a phase change after application, and it inhibits the formation of abdominal adhesions.

2. Materials and methods

2.1. Materials

O-CMC (deacetylation degree $\geq$ 90%) was purchased from BoMei Chemicals (China). Cyclosporin A (CsA) was provided by YuanYe Chemicals (China). $CaCl_2$ was purchased from Tianjin GuangFu Fine Chemical Research Institute. Methylthiazolyldiphenyl-tetrazolium bromide (MTT) was provided by the Beyotime Institute of Biotechnology, China. All other chemicals used were of analytical grade. Mouse fibroblast cell line L_{929} was purchased from Shanghai Boye Institute of Biotechnology, China.

2.2. Preparation of CMC–$CaCl_2$–CsA solution

At room temperature, 20 mL of 2% physiological saline solution of CMC was added into a three-neck flask, which was placed on a magnetic stirrer for 30 min. Then, 36 mg of CsA was added under constant stirring for 5–10 min. The resulting solution is referred to as solution A. The solution B was prepared by dissolving 3.31 g of $CaCl_2 \cdot 2H_2O$ in 100-mL physiological saline at a concentration of 2.5%. The solutions A and B were disinfected using CO_{60} radiation. To contrast the adhesion prevention effect, CMC–$CaCl_2$ was prepared as mentioned previously without adding CsA.

2.3. Characterization of CMC–$CaCl_2$–CsA solution

The CMC–$CaCl_2$–CsA compound was made by adding 16 mL of solution B to the 20 mL of solution A, mixing by shaking for 5 min and letting stand for 15 min, at which point the white suspension formed a compound.

Equilibrium swelling ratio (ESR) of the compound was assessed according to the protocol described by Li *et al.* [15]. Briefly, 2.0 g of dry compounds (W_{dry}) was immersed in sealed tubes containing PBS with the pH of either 5.0, 7.4, or 8.0 and then placed in a thermostatic shaker maintaining 37°C and weighed (W_{wet}). ESR was calculated using the following equation: $ESR = (W_{wet} - W_{dry})/W_{dry}$.

CMC–$CaCl_2$–CsA compound was dried and weighed (W_0). Then, it was immersed in 10 mL of PBS with the pH of either 5.0, 7.4, or 8.0. At different time points, the samples were dried and weighed (Wt). The weight loss percentage (ΔW%) was determined using the following equation: $\Delta W(\%) = (W_0 - W_t)/W_0 \times 100\%$.

In vitro release was tested as follows: triplicate 1.0 g samples of compound were weighed into vials, and 100 mL of saline solution was added as the release buffer. The vials were then placed into an electric-heated thermostatic water bath and kept at 37°C. Aliquots were taken and replaced with fresh saline solution. The content of CsA at each time point was determined using high performance liquid chromatography.

Coagulation *in vivo* was tested as follows: 1 mL of test sample and 0.5 mL of saline were added into every tube. Obtained from the carotid artery of rabbits, 2 mL of rabbit blood was sequentially added to each tube, and each tube was placed in a 37°C water bath. We stopped tube incubation once the blood clotted and recorded the clotting time of each sample.