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Thermosensitive methyl cellulose-based injectable hydrogels for post-operation anti-adhesion

Yongli Zhang[a], Chunjuan Gao[b], Xiulan Li[c], Chen Xu[d], Yang Zhang[c], Zhiming Sun[a,*], Yu Liu[b], Jianping Gao[b,*]

[a] Huanhu Hospital, Tianjin 300060, PR China
[b] School of Science, Tianjin University, Tianjin 300072, PR China
[c] Tianjin Orthopedic Hospital, Tianjin 300211, PR China
[d] Beijing 302 Hospital, 300060, PR China

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ABSTRACT

Thermosensitive methyl cellulose (MC)-based injectable hydrogels for post-operation anti-adhesion were prepared by integrating polyethylene glycol (PEG), carboxymethyl cellulose (CMC) and chitosan sulfate (CS-SO_3) with MC sols. The viscosity of the MC-based sols depended on the sol composition, especially the amount of CMC. The gelation temperature of the sols was tuned by adjusting the concentrations of K^+ and other components to obtain an MC-based sol that transformed to a gel at body temperature. The composition of the sol also affected the gel strength. Adding PEG decreased the repulsions between the CMC and CS-SO_3 macromolecules and thus increased the gel strength. The efficacy of the MC-based injectable hydrogels as barriers for reducing postsurgical adhesions was evaluated using a rat cecal abrasion model. The PEG and CS-SO_3 loaded MC-based injectable hydrogels were effective in reducing adhesion formation and reduced adhesiolysis difficulties.

1. Introduction

Adhesions are basically abnormal attachments between tissues and organs following surgery, trauma, infection, or other harmful events and may be congenital or acquired (Ellis, 1982; Trew, 2006; Wiseman, 1994). They can result in intestinal obstruction, female infertility, ureteral obstruction and chronic pelvic pain (Hershlag & Cherney, 1991; Tito, 1996), all of which are major health problems which can cause death and result in high financial costs (Coleman & Moran, 2000). In addition, adhesions can create significant difficulties for surgeons engaging in reoperative surgical procedures because they increase the time to reenter the abdomen, limit exposure of portions of the abdominal cavity, and increase the risk for inadvertent enterotomies (Coleman & Moran, 2000; Tito, 1996). The health care cost for adhesiolysis and the treatment of adhesion-related conditions in the United States was estimated to be more than 1.3 billion dollars in 1994 (Ray, Thamer, & Perry, 1994). Consequently, finding effective methods to prevent adhesions is an important task. Even though much effort has made in the prevention of post-surgical adhesions, the formation of peritoneal adhesions continues to be a significant and common side effect of intra-abdominal surgery.

Currently there are no definitive strategies to prevent the formation of peritoneal adhesions (Genevieve, Boland, & Weigel, 2006). One of the most widely used anti-adhesion strategies is the application of physical barriers (Al-Musawi, 2001; Bennett, Torchiana, Wiseman, & Sawhney, 2003; Pressato et al., 2002), which can separate and isolate the wounded tissue after surgery. This mechanically limits tissue apposition during the critical period of mesothelial repair and healing, and effectively prevents adhesion formation (diZerega, 1994).

Polyethylene glycol (PEG), a water soluble polymer, has been tested in animals as an anti-adhesive barrier (Sullivan et al., 1991). However, large volumes of the material must be used because of the difficulty in keeping PEG in contact with the tissues (Nagelschmidt, 1997).

Cellulose and its derivates have been widely used in biomedical applications due to their excellent biocompatibility (Huang et al., 2012; Miao et al., 2011). Oxidized regenerated cellulose (Interceed®) has good clinical efficacy in preventing adhesion formation when it is used properly in the absence of blood or peritoneal fluids (Kathleen et al., 2003). Sodium hyaluronate/carboxymethyl cellulose (HA/CMC) (Seprafilm), a bioreabsorbable membrane, has reduced the formation of adhesions

* Corresponding authors. Fax: +86 22 274 034 75.
E-mail addresses: zmsun@163.com (Z. Sun), jianpingg@eyou.com (J. Gao).

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in animal models and in human studies and has been approved for clinical use. Methyl cellulose (MC) has inverse thermal gelling properties. At low temperature, MC can dissolve in water to form a solution. As the temperature increases, hydrogen bonds between the polymer and surrounding solvent break, and hydrophobic junctions form to produce a gel (Li et al., 2001). The gelation temperature depends on the salt in the MC solution. The gelation temperature decreases as the salt concentration increases because water molecules tend to surround the salts (Xu, Wang, Tam, & Li, 2004), thus reducing the solubility of the MC in the water (Liang, Hong, Chung, Lin, & Chen, 2004). MC has previously shown good biocompatibility when used as scaffolds (Tate, Shear, Hoffman, Stein, & LaPlaca, 2001; Wells et al., 1997).

Natural sulfated polysaccharides and their synthetic analogs such as dextran sulfate, heparin, mannan sulfate, chitosan sulfate (CS-SO_3) and chondroitin sulfate display unique biologic activities and are widely used in biology and medicine (Bannikova, Sukhanova, Vikhoreva, Varlamov, & Galbraikh, 2002; Nishimura, Kai, & Shinada, 1998; Yoshida et al., 1990). Adhesions are in part due to phosphatidylserine-positive erythrocyte-thrombospondin interactions. The anti-adhesive effects of heparin have been previously investigated and it was reported that the inhibition of red blood cell-endothelial adhesion was mediated via P-selectin and soluble thrombospondin (Fu, Ji, Yuan, & Shen, 2005; Miyata et al., 1988; Setty et al., 2008; Sun et al., 2003). CS-SO_3 is structurally similar to the heparin (Hirano et al., 1985; Jayakumar et al., 2007) and would be expected to possess similar properties.

At present, several anti-adhesion barriers have been approved by the Food and Drug Administration of the United States of America (FDA, USA) and they include Interceed® (Johnson and Johnson Patient Care, Inc., New Brunswick, NJ); Seprafilm® (Genzyme Corporation, Cambridge, MA); expanded polytetrafiuorethylene (Preclude®, W.L. Gore&Associates, Inc., Flagstaff, AZ); biodegradable polylactide (Surgiwrap™, MAST Biosurgery, Inc., San Diego, CA); and 4% icodextrin solution (Adept®, Innovataplc and Baxter Healthcare Corporation, Deerfield, IL). The last one is easy to result in leakage of fluid from the surgical site owing to its liquid state. The other four anti-adhesion barriers are all solid films and have shown significantly reducing postoperative adhesions. But they still have some shortage, for example, Preclude® is not bioresorbable and must be removed via a second operation except it is to be left in place, Surgiwrap™ need to be fixated to reduce the potential for migration. In addition, it is impossible for these films to be used as anti-adhesion barriers in minimal invasive surgeries.

In view of the unique properties of MC, PEG and CS-SO_3, a combination of these components may create an effective anti-adhesion material. When PEG and/or CS-SO_3 are incorporated into MC gels, the PEG or CS-SO_3 macromolecules should diffuse to the surface and inhibit adhesion (Fu et al., 2005; Zhang et al., 2011). CMC is one of the components of the anti-adhesion membrane, Seprafilm. It is usually used as a thickener and can effectively increase solution viscosity. Here, CMC was mainly used to increase the viscosity of the MC-based sols to a suitable value so that the sols did not overflow before they changed to gels. However, the viscosity cannot be too high, otherwise, it is difficult for the sol to be injected through a needle and spread on the desired area. So herein, MC-based injectable hydrogels for post-operation anti-adhesion were fabricated from MC, PEG, CMC and CS-SO_3. They have the advantages of both liquid and film anti-adhesion barriers and can be applied in minimal invasive surgeries. The relationships between the sol composition, the sol viscosity and the gelation temperature were investigated. A rat cecal abrasion model was used to evaluate their anti-adhesion efficiency.

2. Experimental

2.1. Materials

CMC (degree of substitution: 2.1), MC (degree of substitution: 1.8) and PEG (10,000) were from Huzhou Zhanwang Medical Co. Chitosan (85% deacetylated, average molecular weight of 10×10^4) was purchased from Zhejiang Yuhuan Marine Biochemical Co., Ltd. Chlorosulfonic acid, formamide and the other chemicals were from Tianjin Chemical Co. All the chemicals were used as received. All concentrations in this article are weight concentration, except the specially stated ones. The numbers of replicates for the characterization experiments (except those of animal studies) are set at five.

2.2. Preparation of chitosan sulfate

Formamide (10 mL) was added to a three-neck flask in an ice bath, and then chlorosulfonic acid was added dropwise in order to maintain the temperature below 5 °C. About 1.0 g of chitosan powder was added to the stirred mixture and the mixture was then heated to 60 °C and allowed to react for about four hours. A small amount of water was added to dissolve the product, and the un-dissolved materials were removed by filtration. Then 20 mL of ethanol was poured into the stirred solution to precipitate the CS-SO_3. The raw CS-SO_3 was rinsed three times with ethanol, centrifuged and dried in vacuum to obtain CS-SO_3 powder. The S content of the CS-SO_3 was measured by an inductively coupled plasma (ICP) optical emission spectrophotometer (Vista-MPX, Varian) at a high frequency power emission of 1.5 kW and a plasma air flow of 15.0 L min^{-1}).

2.3. Preparation of MC-based sols

The desired amounts of MC, CMC, PEG and CS-SO_3 were weighed (their weight ratios are listed in Table 1) put into a flask, and mixed. The flask was then put in a water bath at 85 °C after the desired amount of water was added. When the powder was well dispersed with vigorous stirring, certain amount of water was added to make the total weight of the sol be 100 g and then the temperature was cooled to 0 °C. The mixture was stirred at 0 °C until a transparent sol was obtained and then stored in a refrigerator.

2.4. Viscosity measurement of the MC-based sols

A falling-ball viscometer (Gilmont Instruments, IL, USA), mounted in a constant-temperature chamber was employed to determine the viscosity of the MC-based sols. The viscometer was first filled with a MC-based sol, degassed by vacuum, allowed to equilibrate for 10 min at 20 °C, and then the time of ball descent was measured and the viscosity was calculated by the equation (Sampedro, Muñoz-Clares, & Uribe, 2002):

$$\text{Viscosity} = K \cdot (d_b - d_l) \cdot t$$

where K is the viscometer constant, t is the time of ball descent, and d_b and d_l are the density of the ball (2.53) and the sol (g/mL) respectively. The density of the sol was measured by weighing 1 mL of the given sol at 20 °C. The experimental data were reproducible and the standard deviations were smaller than 3%.

2.5. Determination of gelation temperature

The reversible sol–gel transition temperatures of the MC-based sols were measured by a test tube tilting method (Bain et al., 2012). To measure the gelation temperature by this process, the sol was