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

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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

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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

Table 1
Animal experimental design and difficulty of adhesiolysis and adhesion sites with the MC-based injectable gels.

	Group 1	Group 2	Group 3	Group 4	Group 5
Number of animals (*n*)	8	8	8	8	8
Treatment[a]	Control	MC6/CMC2	MC6/CMC2/PEG1	MC6/CMC2/CS-$SO_3$1	MC6/CMC2/PEG1/CS-$SO_3$1
Difficulty of adhesiolysis (mean value)	3.75	2.50	1.75	1.62	1.38
0 (*n*)		1	1	2	2
1 (*n*)		1	2	1	2
2 (*n*)	1	2	3	3	3
3 (*n*)	2	1	2	2	1
4 (*n*)	3	3			
5 (*n*)	2				
Adhesion sites (*n*)					
Cecum–cecum	3	2	4	3	4
Cecum–intraperitoneal fat	2	3	2	3	2
Cecum–small bowel	2	2	1		
Cecum-abdominal wall	1				

[a] The number behind each component represents the % concentration of that component.

sealed in a 20 mL glass tube and placed in a super thermostatic water bath. The temperature of the bath was then increased at a rate of 2 °C per minute below 30 °C. When the water temperature reached 30 °C, the rate was adjusted to 0.2 °C/min to observe the gelation process of the samples. At a certain temperature the sol was completely converted into a gel. The gel did not flow with the tilting of the test tube. This characteristic temperature is called the gelation temperature.

2.6. Measurement of gel strength of the MC-based gels

The gel strength was measured using a texture analyzer (TA-XT2, Stable Micro Systems). The measurements were performed on the gels prepared from the MC-based sols after the gel had stood for 12 h at 36 °C. A cylindrical plunger 10 mm in diameter operating at cross-head speed of 1 mm/s was used for the tests (Hurtado-Ponce & Umezaki, 1988).

2.7. Swelling ability of the MC-based xerogels

A weighed amount of MC-based xerogel (obtained from freeze-drying method) was soaked in distilled water and weighed at regular intervals until its weight became constant. Tests were performed in triplicate, and the average swelling ability (*SA*) was calculated as:

$$SA\left(\frac{g}{g}\right) = \frac{W_e - W_0}{W_0}$$

where W_0 is the average initial weight (g) of the dry sample and W_e is the weight of the sample in the swelled state.

2.7.1. In vitro cell culture and cell morphology

Fibroblast cells were cultured according to a previously described method (Wu et al., 2010). The films were irradiated with ^{60}Co γ-ray for 24 h and then placed in the wells of a culture plate (24-well multiplate, Iwaki, Japan). A suspension of fibroblasts (3×10^4 cells/mL) in Eagle's minimum essential medium containing 10% fetal calf serum was added to the wells. The cells were then cultured at 37 °C in 5% CO_2–95% air for the desired period of time. The morphologies of the cells attached to the composite films were observed with a phase-contrast microscope (IMT-2, Olympus, Tokyo, Japan) at 5 h, 1 day, 3 day, 5 day and 7 day. For cell staining, the cells growing on the films were fixed in 10% neutral buffered formalin solution, dehydrated through an ethanol gradient, and then stained with Wright–Giemsa solution (Scytek) at room temperature for 10 min.

2.8. Evaluation of anti-adhesion properties using an animal model

Forty Wistar rats (half male and half female) with body weights of 200–250 g were used for the anti-adhesion experiments (Yeo et al., 2006). The animals were randomly divided into five experimental groups of eight according to Table 1. Rats were anesthetized by intramuscular injection of 3% pentobarbital (30.00 mg/kg), and then their ventral hair was shaved and their abdomens were cleansed with alcohol. Using an aseptic technique, a 2–3 cm incision was made on the midline of the abdominal wall, and a 0.5 × 0.5 cm surface of the cecum was scraped with a scalpel blade until it was damaged and hemorrhaging, but not perforated. The damaged cecum surface was then covered with MC-based injectable gels (1.0 × 1.0 cm). Rats in the control group were only washed with saline solution and were not covered with any anti-adhesion barriers. After the above procedures, the cecum and the small bowel were returned to their original locations. The abdominal incision was closed with 4–0 chromic catgut and 3–0 silk with double layers.

The animals were then given free access to food and water. Fourteen days after the surgery, the rats were sacrificed and the incisions were reopened and the injured sites were examined for adhesions by the naked eye. The difficulty of adhesiolysis was evaluated grossly by a surgeon (blinded trial) using the six point scoring system shown in Table 2 (Lodge et al., 2008). The same surgeon used a yes/no questionnaire to determine whether adhesions had formed over the abraded cecum, between the cecum and at three other locations: the small bowel, the abdominal wall, and in the intraperitoneal fat.

3. Results and discussion

The MC-based injectable gels were fabricated from MC, CMC, PEG and CS-SO_3. The CS-SO_3 was prepared under various conditions and then characterized by ICP (Fig. 1) and IR (Fig. S1) (Holme & Pedin, 1997). The CS-SO_3 has a stronger S=O stretching vibration peak at 1402 cm^{-1} than CS. The ICP results indicate that the S content of the CS-SO_3 was influenced by the reaction conditions.

Table 2
Adhesion severity scoring system.

Level	Definition
0	No adhesions
1	Loose filmy adhesions that can be separated by blunt dissection
2	Less than 50% of adhesions requiring sharp dissection for separation
3	More than 50% of adhesions requiring sharp dissection for separation
4	Serious jury
5	Full-thickness injury

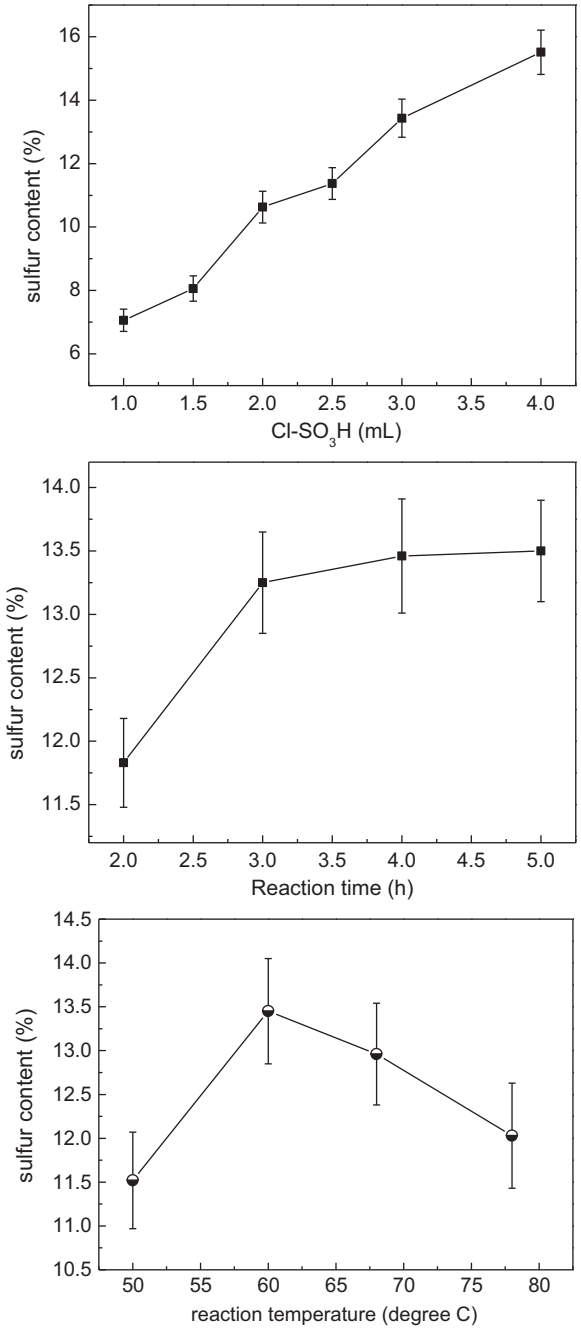

Fig. 1. S content of CS-SO_3 prepared at different conditions.

The S content increased as the concentration of chlorosulfonic acid increased. It took about 5 h for the reaction to be complete and the best reaction temperature was 60 °C. At higher temperatures, more side reactions occurred which resulted in a lower S content in the CS-SO_3. As long as the S content is higher than 7.5%, the CS-SO_3 is water soluble.

The effect of the MC sol composition on the viscosity of the sol was tested. The results show that CMC greatly increase the viscosity to the desired value of 43.2 Pa S (Fig. 2).

3.1. Gelation temperature

Thermal sensitive polymers have potential applications in biomedical engineering. Poly(*N*-isopropylacrylamide) (PNIPAM) is a well-known example and it has a low critical solution

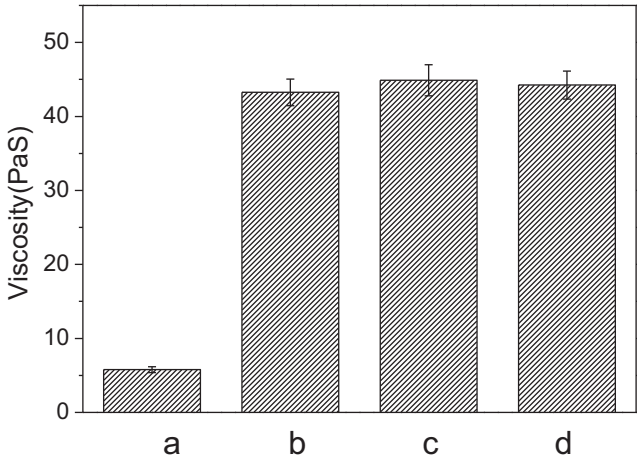

Fig. 2. Viscosity of MC-based sols for MC6 (a), MC6/CMC2 (b), MC6/CMC2/PEG1 (c), and MC6/CMC2/PEG1/CS-$SO_3$1 (d).

temperature (LCST) at around 33 °C. Below the LCST, PNIPAM is soluble in water and forms an aqueous solution. When the solution is heated and reaches the LCST, PNIPAM becomes insoluble (Ha, Lee, Chong, & Lee, 2006). This characteristic has resulted in the extensive study of PNIPAM for controlled drug release systems and injectable gels (Ha et al., 2006; Kurisawa, Yokoyama, & Okano, 2000). MC also has inverse thermal gelling properties which are similar to those of PNIPAM. As the temperature increases, the hydrogen bonds between the polymer and the surrounding solvent break, and hydrophobic junctions form to produce a gel (Li et al., 2001). However, its gelation temperature (GT) is usually much higher than body temperature, but it can be adjusted by adding additives (including salt) or by changing the concentration of MC.

Table 3 shows the effect of the sol composition on the GT of MC-based sols (√ represents a GT of 36 °C; × represents GT above 36 °C). The GT decreased as the MC concentration increased, and the pure MC sol turned to a gel at 36 °C when the MC concentration was higher than 6%. For the samples only made of MC, the concentration of MC affected the GT. When the MC is 8%, they can change to gel even the concentration of KCl is zero. When other components are integrated, if MC is more than 5%, the sample can also gel but the concentration of KCl is at least 0.0410%. These MC-based sols with GTs of 36 °C should be good candidates for injectable thermosensitive MC-based hydrogels for post-operation anti-adhesion.

Table 3
MC-based sols gelled at human body temperature (36 °C).

Entry	Composition of MC-based sols[a]	36 °C	K^+ (%)
1	MC5	×	0.0560
2	MC6	×	0.0560
3	MC7	√	0.0387
4	MC8	√	0
5	MC3/CMC1	×	0.0560
6	MC4/CMC1	×	0.0560
7	MC5/CMC2	√	0.0560
8	MC6/CMC2	√	0.0526
9	MC5/CMC1/PEG3	√	0.0480
10	MC5/CMC2/PEG2	√	0.0480
11	MC6/CMC1/PEG3	√	0.0480
12	MC6/CMC2/PEG1	√	0.0410
13	MC6/CMC2/PEG2	√	0.0410
14	MC6/CMC2/CS-$SO_3$1	√	0.0410
15	MC6/CMC2/PEG1/CS-$SO_3$0.5	√	0.0410
16	MC6/CMC2/PEG1/CS-$SO_3$1[a]	√	0.0410

[a] The number behind each component represents the % concentration of that component.

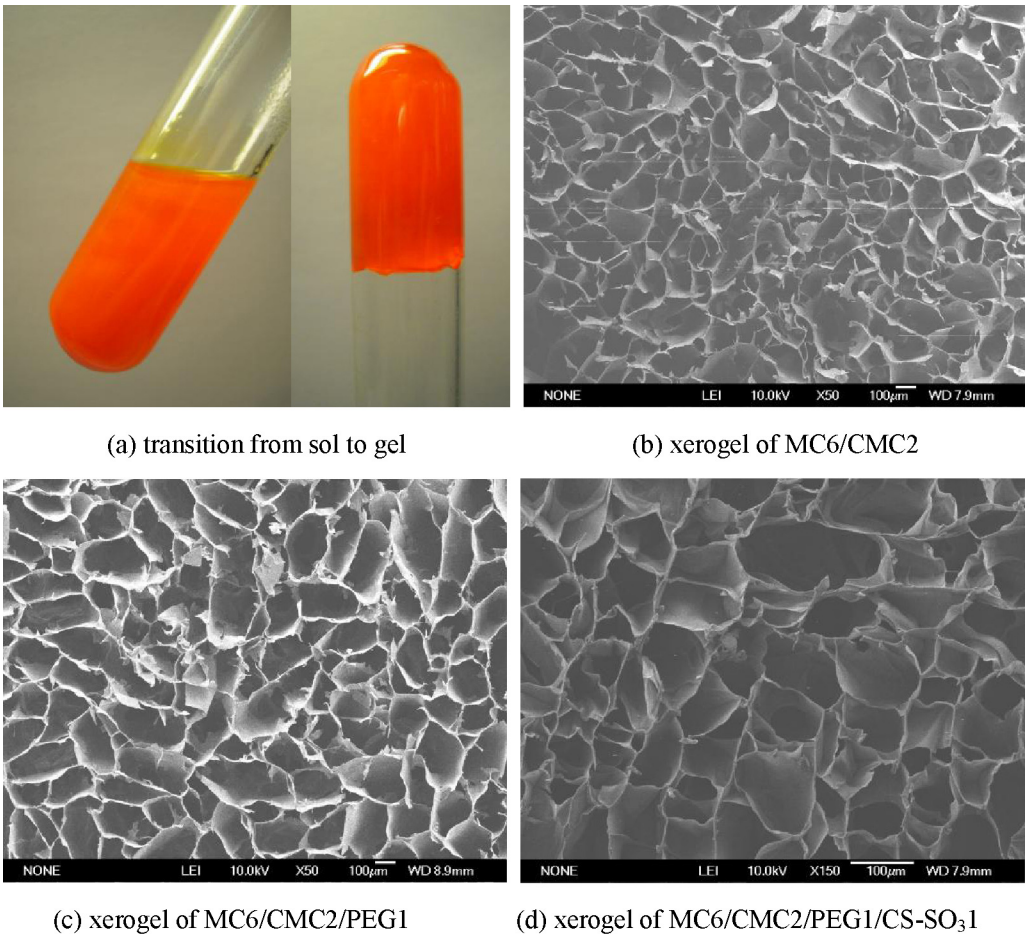

Fig. 3. Photograph of the transition from MC-based sol to gel at 36 °C (a), and SEM photos of the xerogels obtained from gels by a freeze-drying method (b–d).

The above MC based sols undergo sol–gel transition as shown in Fig. 3a. In order to clearly observe the transition, the sol was dyed with Sudan red (however methylene blue and methyl blue are better when biocompatability is a concern since they are often used as dyes in biotechnology). The SEM photos of the xerogels prepared by a freeze-drying method are shown in Fig. 3b–d and the results show a porous structure. The pores are uniform and interconnected with sizes of about 150 μm.

3.2. Gel strength of the MC-based gels

As shown above, the MC sols turn to gels under suitable conditions. Gel strength is one of the important properties of a gel. The strength of gels with different compositions was tested and the results are shown in Fig. 4. The results show that the strength depends on both the MC concentration (MC7 and MC8) and the composition of the sol. There are strong repulsions between the CMC macromolecules which may reduce the strength of the gels. When PEG is present it reduces the repulsions between the CMC macromolecules and so the gel strength increases (comparing MC6/CMC2 and MC6/CMC2/PEG1, MC6/CMC2/CS-$SO_3$1 and MC6/CMC2/PEG1/CS-$SO_3$1). Since PEG has a low molecular weight,

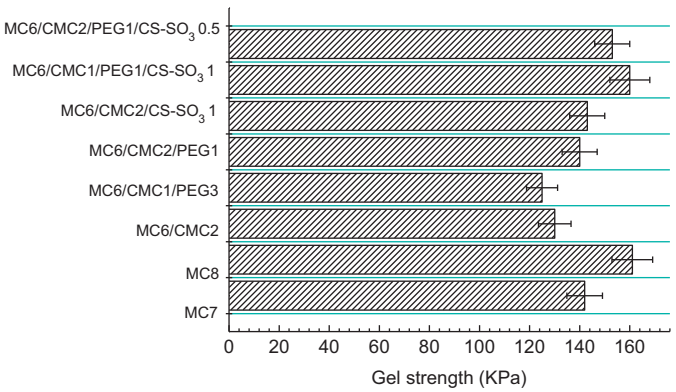

Fig. 4. Gel strength of MC-based gels.

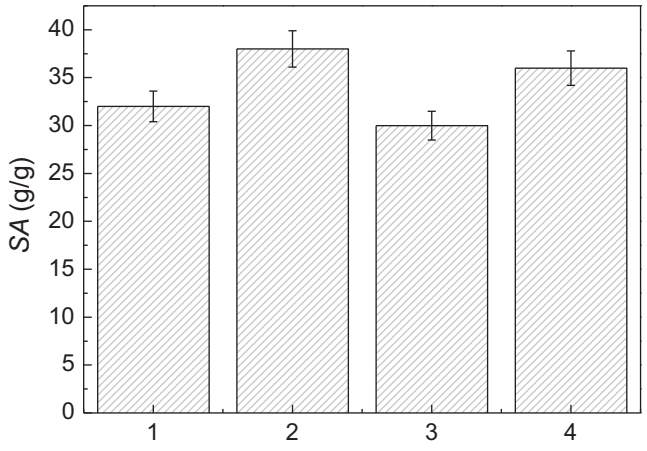

Fig. 5. Swelling ability of MC6/CMC2 (1), MC6/CMC2/PEG1 (2), MC6/CMC2/CS-$SO_3$1 (3), and MC6/CMC1/PEG1/CS-$SO_3$1 (4) xerogels.

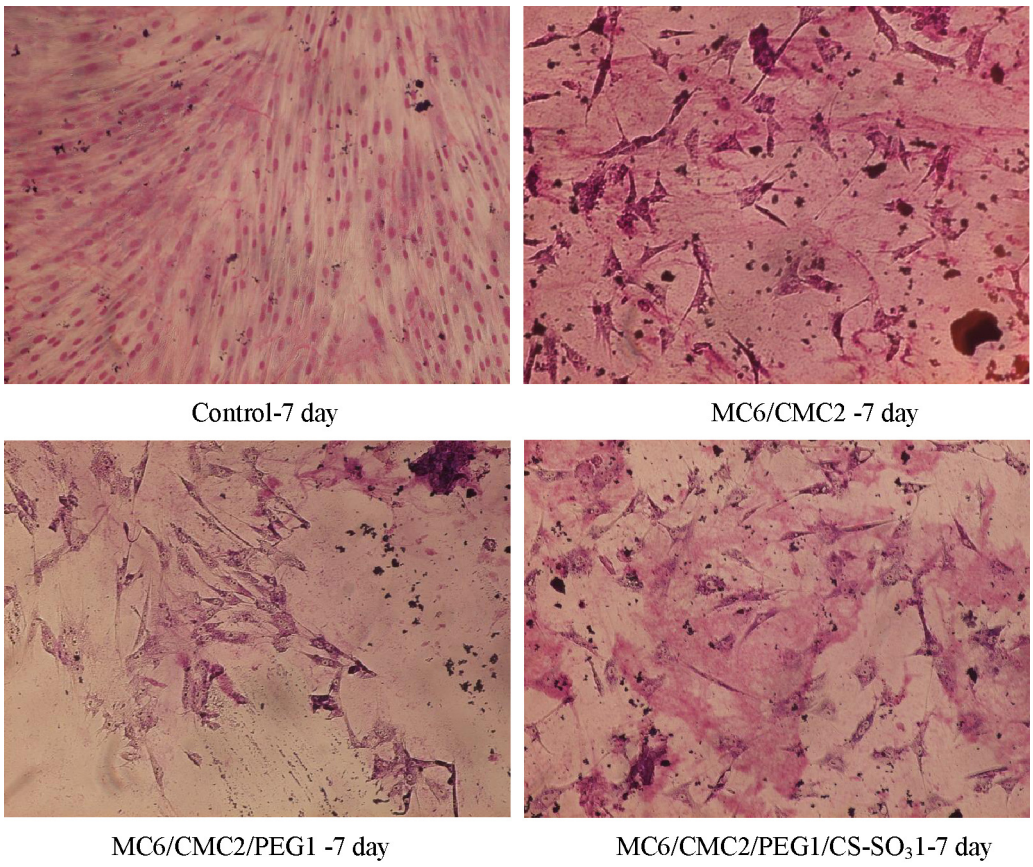

Fig. 6. Microscope images of the fibroblasts cultured on the MC-based gels.

it reduces the gel strength if it is present in excess (comparing MC6/CMC2/PEG1 and MC6/CMC2/PEG3). CS-SO_3 contains negative SO_3^- groups which repel each other with the —COO^- groups on CMC. These repulsions reduce the gel strength. However CS-SO_3 also has un-reacted —NH_3 groups which reinforce the interactions between the macromolecules via hydrogen bonds with the —OH and —COO^--groups in the MC and CMC macromolecules (Dhar, Akhlaghi, & Tam, 2012). These hydrogen bonds increase the gel strength. The opposing actions of SO_3^- and —NH_3 seem to cancel each other so the gel strength is not affected by CS-SO_3 (comparing MC6/CMC2, MC6/CMC2/PEG1 and MC6/CMC2/CS-$SO_3$1). So the strength depends on the composition of the sols, but the difference is not big (within 25%).

3.3. Swelling ability of the MC-based xerogels

Hydrogels usually contain a large amount of water. They swell when they are soaked in water and contract when they are dried. Fig. 5 shows the swelling ability of some of the MC-based xerogels. Obviously, PEG increases the swelling ability of the MC-based xerogels while CS-SO_3 almost has no effect on the swelling ability. PEG is a hydrophilic polymer, and adding PEG is beneficial for water absorption. So the swelling ability of MC6/CMC2/PEG1 is higher than that of MC6/CMC2. When CS-SO_3 was added, the —SO_3^- groups on the CS-SO_3 macromolecules and the —COO^- groups on the CMC macromolecules repelled each other, which weakened the interactions between the macromolecules. At the same time, the —NH_3 groups on the CS-SO_3 macromolecules formed hydrogen bonds with the —OH and —COO^- groups on the MC and CMC macromolecules which reinforced the interactions between these macromolecules. The net result of the two effects was that no statistical difference was observed in the swelling ability when CS-SO_3 was added.

3.4. Cell cultured on the MC-based gels

Fibroblasts were cultured on the different MC-based gels and the results are shown in Figs. 6 and S2. The morphologies of the fibroblasts cultured on the different MC-based gels were different. After 5 h of culture, most of the fibroblasts on the culture plate (control) and the MC/CMC gels were flatten and adhered onto the surfaces. However no fibroblasts adhered to the MC/CMC/PEG and MC/CMC/PEG/CS-SO_3 gels. After 3 day of culture, the fibroblasts on the control were completely flattened and spread out but only a few fibroblasts on the surface of the MC/CMC gel were flattened and spread out. In contrast, at 3 day, there were no fibroblasts on the surface of the MC/CMC/PEG and MC/CMC/PEG/CS-SO_3 gels.

The fibroblasts on the culture plates (CP) near the materials were also observed after culturing for 5 and 7 day. Significant differences in the cell morphologies and numbers were also found among these samples. In the control group, the adhesion and spread of the cells were extensive and the fibroblasts were all oriented in the same direction. Some of the cells on the CP near the MC/CMC gel were attached and spread out, but for the MC/CMC/PEG and MC/CMC/PEG/CS-SO_3 gels, few cells were observed. In addition, the number of living cells near the MC-based gels was much fewer than that on the CP. These results clearly indicate that the MC/CMC/PEG and MC/CMC/PEG/CS-SO_3 gels exhibit strong repulsions to the fibroblasts which is due to the presence of the PEG and CS-SO_3 macromolecules (Zhang et al., 2011). CMC is a water soluble polymer so it can diffuse into the culture solution from the gel. It then leaves pores on the gel surface which makes the surface uneven. A rough surface prevents the attachment and spreading of cells, so

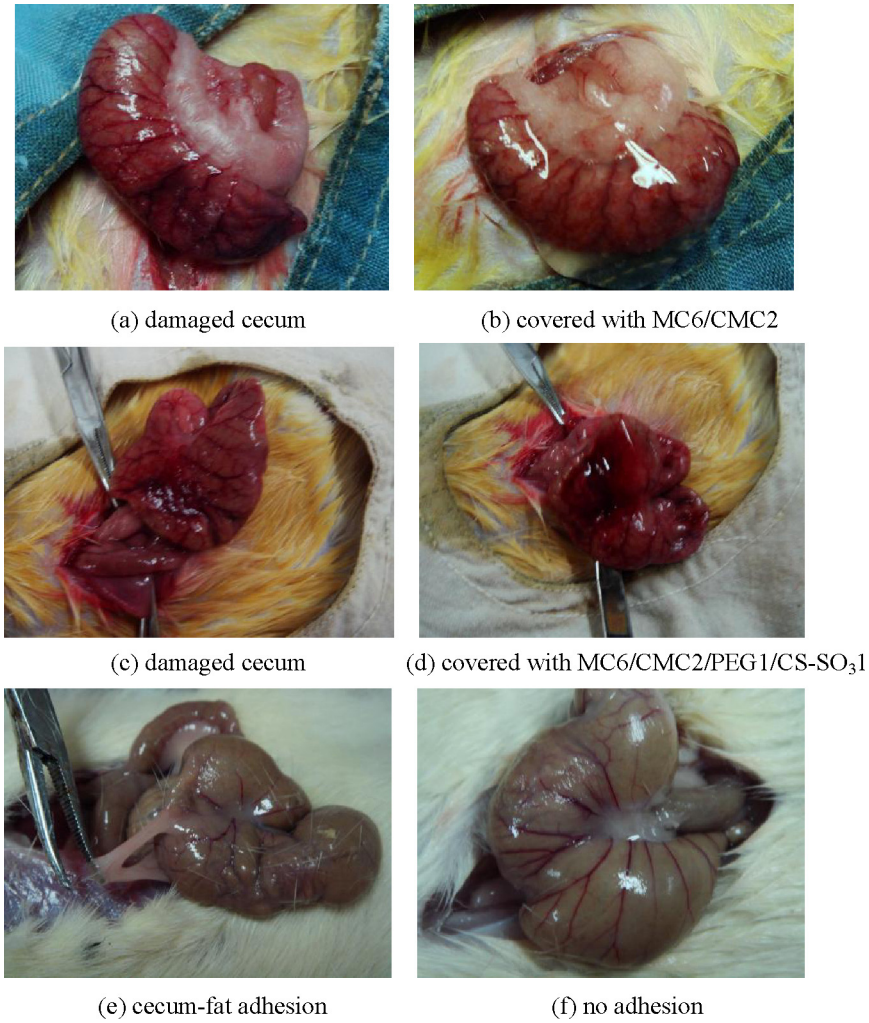

Fig. 7. Damaged rat cecum (a and c), treated by covering with MC6/CMC2 gel (b) and MC6/CMC2/PEG1/CS-$SO_3$1 gel (d), an example of post-surgery adhesion patterns (e) and no adhesion (f).

the gels provide a steric barrier which prevents cell adhesion onto the gel surface (Jayakumar et al., 2007; Nishimura et al., 1998).

3.5. Evaluation of the anti-adhesion efficacy of the MC-based injectable gels in an animal model

A damaged rat cecum surface was used as a model to evaluate the anti-adhesion efficacy of the MC-based injectable gels. After the damaged cecum surfaces were created (Fig. 7 a and c), they were covered with MC-based injectable gels (Fig. 7b and d), and then the abdominal incision was closed. Fourteen days later, the rats were sacrificed, the incision was reopened and the injured site was examined for adhesions by the naked eye. There are five possible outcomes: no adhesion (Fig. 7f), cecum–cecum adhesion, cecum–abdomen adhesion, cecum–small bowel adhesion, and cecum–intraperitoneal fat adhesion (Fig. S3). The adhesion severity scoring system in Table 2 was used to evaluate the samples (Lodge et al., 2008). The difficulty of adhesiolysis and the number of adhesion sites that occurred after using the MC-based injectable gels as anti-adhesion barriers are listed in Table 4. The rats treated with the MC-based injectable gels had lower difficulty of adhesiolysis scores than those in the control group. The case of adhesion obviously decreased. Though some adhesion cases happened, they were not serious adhesion and no cecum–abdomen adhesion occurred in the rats treated with the MC-based injectable gels. There were no statistically significant differences between the MC6/CMC2/CS-$SO_3$1 and MC6/CMC2/PEG1 groups (1.62 versus 1.75), but the adhesiolysis was less difficult for those treated with the MC6/CMC2 gels (2.5). This demonstrated that PEG or CS-SO_3 can improve anti-adhesion ability of MC/CMC gels. Of all the MC-based injectable gels, MC6/CMC2/PEG1/CS-$SO_3$1 had the lowest difficulty of adhesiolysis score. This may be attributed to the synergic effect of PEG and CS-$SO_3$1 which resulted in fewer adhesions forming. The above results indicate that MC/CMC gels as barriers can decrease adhesion and this anti-adhesion effect can be strengthened by integrated PEG or/and CS-SO_3. The current used anti-adhesion barriers are either solid films or liquid solution. They all have their shortages. Adept® is used in gynecological laparoscopic procedures and its usage requires no change to routine surgical practice, or any special training, but it is easy to result in leakage of fluid from the surgical site and brought abdominal distension and discomfort (Menzies et al., 2006). Interceed® showed limited effectiveness in the presence of blood or peritoneal fluids (Rodgers et al., 2003), Surgiwrap™ and Preclude® need to be fixated to reduce the potential for migration (Lamoutte & Chatterji, 2006; Trew, 2006), and Seprafilm® required careful handling at open operation and it is difficult to use laparoscopically (Beck et al., 2003; Rodgers et al., 1998). The MC-based injectable hydrogels are in liquid state, so they can be used in laparoscopically and their usage required no change to routine surgical practice, or any special training. They transform to hydrogels at body temperature, which prevents leakage of fluid and migration of film. So MC-based injectable hydrogels have the

advantages of both liquid and solid anti-adhesion barriers and will have potential for future application in clinical applications.

4. Conclusions

MC-based injectable gels were fabricated by blending MC with CMC, PEG and/or CS-SO_3 and they were then used to prevent post-surgical adhesions which can cause severe clinical complications. The gelation temperature was tuned by adjusting the concentration of K^+ and other components so that the MC-based sols transformed to gels at body temperature. The addition of CMC significantly affected the MC sol viscosity. The concentration of CMC in the MC-based sols was adjusted to give a suitable viscosity so that the sol did not overflow before changing into a gel and so that the sol was easily injected and spread on the desired area. The addition of PEG decreased the repulsions between the CMC and CS-SO_3 macromolecules, which increased the strength of the MC-based gels. The growth and morphology of fibroblasts cells on the surface of the MC based gels showed that gels with PEG exhibited minimal cell adhesions on their surfaces. The animal model experiments showed that the MC6/CMC2/PEG1, MC6/CMC2/CS-$SO_3$1, and MC6/CMC2/PEG1/CS-$SO_3$1 injectable gels were more effective than the control and MC6/CMC2 in preventing adhesion formation. These results show that MC-based injectable hydrogels have potential for future use in clinical applications.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2013.09.001.

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