Reduction of Postsurgical Adhesion Formation with CM-Chitosan Hydrogel Barriers Prepared by Using γ-Irradiation

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ABSTRACT: To evaluate the efficiency of carboxymethylchitosan (CM-chitosan)-based hydrogels as barriers for reducing postsurgical adhesions, CM-chitosan was synthesized to simplify the hydrogel-making process, and the CM-chitosan solutions were cross-linked by using γ-ray irradiation to create the desired hydrogels instead of using chemical cross-linking reagents. The prepared CM-chitosan hydrogels were characterized by a FTIR spectroscopy, swelling behavior, gel-fraction content,and mechanical property such as gel strength of a hydrogel and the results showed a good swelling behavior and mechanical properties. Also, the radiation-induced CM-chitosan hydrogels significantly reduced and inhibited the postsurgical adhesions in the rat models. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 116: 3682–3687, 2010

Key words: adhesion; cross-linking; gels; irradiation; swelling

INTRODUCTION

The formation of postsurgical adhesions after an abdominal operation is common and can result in a significant morbidity for a patient. For example, adhesions are a major cause of intestinal obstructions and can lead to fistula formation, postsurgical reproductive organ complications, a ureteral obstruction, and chronic abdominal–pelvic pain. Furthermore, pericardial adhesions after a cardiac operation can result in complications during preoperative cardiac procedures.[1] To prevent the formation of postsurgical adhesions hydroflotation techniques and barrier devices have developed as interventional attempts.[2] However, this technique has produced marginally beneficial effects in animals and humans.[3] Also, synthetic barrier membranes have demonstrated some limited inhibition of adhesion formation in humans.[2] Hydrogels have received increasing attention in biomedical and biochemical applications, because of their permeability, biocompatibility, and biodegradability.[4–6] Therefore, resorbable hydrogel barriers such as a hyaluronic acid (HA) hydrogel have been known to play a role in normal wound healing and in inhibiting pericardial and peritoneal adhesion formation. However, the HA hydrogel has also proved unattractive in a clinical setting because of its high cost and limited efficacy.

In recent years, interest has been focused on chitosan hydrogels owing to their excellent water-absorption ability and water retention ability for a wound dressing.[7] However, their applications are restricted because of its poor solubility and its lack of an amphiphatic nature.[8] Therefore, chemically modified chitosan derivatives, particularly, carboxymethylchitosan (CM-chitosan), which has with structural similarities to HA, have received attention in research as a novel resorbable hydrogel barrier. This is because CM-chitosan significantly reduces the incidence and severity of postoperative adhesion formation (Fig. 1).[9–12] Structurally, the CM-chitosan has carboxymethyl substituents on some or both the amino and primary hydroxyl sites of the glucosamine units of the chitosan structure.[13] Thus, in comparison to chitosan, the CM-chitosan not only dissolves in acidic as well as neutral and basic solutions but it also has unique chemical, physical, and biological properties, such as high viscosity, large hydrodynamic volume, low toxicity, biocompatibility, and easy hydrogel-making process.[14]

To prepare these CM-chitosan hydrogels, chemical cross-linking has been reported as one of the cross-linking methods.[15] However, in this method, cross-

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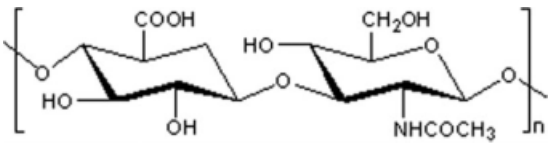

Figure 1 Chemical structure of hyaluronic acid (HA).

linking agents, such as glutaraldehyde, diisocynates, carbodiimides, and acyl azide are necessary to initiate the process, which reduces their biocompatability, due to their inherent cytotoxicity.[16] γ-Ray irradiation is a very suitable method for the formation of hydrogels. Its main advantages, compared to the other methods, are no necessity to add any initiators and crosslinkers to start the initiation process, hence the final product only contains polymer in its structure and the final product does not require further purification.[8] Moreover, γ-ray irradiation usually allows for the combination of a synthesis and sterilization of polymeric materials in one technological step, thus reducing the costs and production time.[17]

In this study, we used carboxymethylchitosan (CM-chitosan) to simplify the hydrogel-forming process, and the CM-chitosan solutions were crosslinked by using γ-ray irradiation to create the desired hydrogels. The radiation-induced CM-chitosan hydrogels were evaluated for the efficacy as barriers for reducing postsurgical adhesions in a rat cecal abrasion model.

EXPERIMENTAL

Materials

Chitosan ($M_W = 3.0 \times 10^5$) with a degree of deacetylation of $\sim$ 90.8% was obtained from Chito123, Korea. Glycerol, isopropyl alcohol, sodium hydroxide, and chloroacetic acid were purchased from Showa Denko, Japan. Unless otherwise stated, all the other used chemicals and reagents were of an analytical grade and were used without any further purification.

Synthesis of carboxymethylchitosan (CM-chitosan)

CM-chitosan was synthesized by a previously reported method with slight modifications (Fig. 2).[13,18,19] Chitosan powder (20 g) was suspended in 200 mL of isopropyl alcohol and stirred at room temperature. 8.4 mL of 10 *N* aqueous NaOH solutions, divided into six equal portions, was then added to the stirred slurry for 1 hr. Subsequently, chloroacetic acid (24 g) was added, in five equal portions (4.8 g), at 5 min intervals and then the reaction mixture was stirred at 60°C for 3 h. Then, the reaction mixture was adjusted to pH 7.0 by an addition of cold distilled water and acetic acid. The resulting mixture was filtered and the solid product (CM-chitosan) was thoroughly washed with methanol. The resultant CM-chitosan was vacuum-dried in an oven at 60°C and we obtained the desired product (28.7 g) as a yellowish solid.

Preparation of CM-chitosan hydrogels

10 wt% of glycerol was added to various weight compositions (5–15 wt%) of CM-chitosan to provide smoothness for the resultant. The CM-chitosan solutions were prepared by being dissolved in distilled water at room temperature for 30 min, with stirring, to become homogeneous mixtures. Then the homogenized solutions were poured into a Petri dish to a thickness of 2 mm and irradiated at a dose of 10 to 200 kGy (10 kGy/h dose rate) by using a ^{60}Co γ-ray source (Fig. 2).

Fourier transformed infrared spectroscopy

FTIR was used to confirm the chemical structure of the CM-chitosan. All spectra were recorded by attenuated total reflection-Fourier transform IR spectroscopy (ATR-FTIR, Bruker TENSOR 37, Germany). The IR spectra of chitosan and CM-chitosan were recorded in the range of 500 to 4000 cm^{-1} using KBr pellets.

Mechanical properties

The mechanical properties of the CM-chitosan hydrogel were obtained by determination of the gel strength. The tests were conducted by using an Instron UTM (model 4467, Canton, MA) analyzer set at a grip length of 15 mm and a crosshead speed of 50 mm/min at room temperature. At least five measurements for each sample were recorded and a mean value was calculated. The gel strength (G_s)

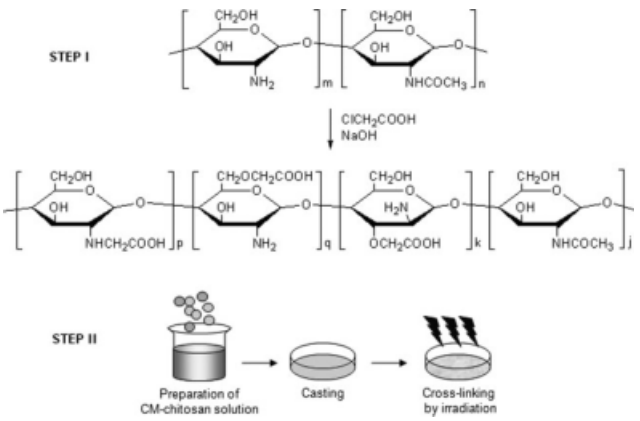

Figure 2 Schematic representations of the carboxymethylation of chitosan (step I) and radiation-cross-linking procedure (step II). The CM-chitosan was synthesized by previously reported method with slight modifications.

was given by $G_s = F_B \times \Delta D$, where F_B is the applied force at break and ΔD is the elongated distance of the gel.

Gel-fraction content

The gel-fraction content was estimated by measuring its insoluble part after immersing it in distilled water at room temperature for 48 h. The remaining hydrogel also was kept in a 50°C oven for 2 days to completely dry the hydrogels. The gel-fraction content (G_c) was defined gravimetrically by G_c (%) = (W_d/W_i) × 100. Where W_d is the oven-dried gel weight after the swelling for 48 h, and W_i is the initial weight of the dried hydrogels.

Swelling of hydrogel

The swelling of the CM-chitosan hydrogel was measured by a previously reported method.[20] The dried polymeric samples were immersed in distilled water at room temperature. The hydrogel was weighed after the equilibrium state of the swelling ratio (i.e. maximum swelling ratio) had been reached. The swelling ratio was calculated as follows (in grams of absorbed solvent per gram of dried gel): Swelling ratio (%) = $(W_s - W_d)/W_d \times 100$, where W_s is the weight of the hydrogel in the swollen state and W_d is the weight of the insoluble part of hydrogel after extraction with water. Also, the effects of acid and base on the swelling behavior of the CM-chitosan hydrogels were performed by determining the swelling ratio.

Adhesive force

The adhesive force was obtained by the measurement of the force required to break the contact between the CM-chitosan hydrogel and the mucosa layer of the porcine intestine with an Instron UTM (model 4467, Canton) analyzer. CM-chitosan hydrogels (thickness = 2 mm) and porcine intestine were cut (1.5 cm × 1.5 cm). The hydrogels were attached to the native porcine intestine under a force of 50 gf/cm^2 for 2 min. The peak force required to detach the hydrogels from the intestine was measured.

Animal testing

Female Wistar rats (250–300 g) were purchased from Kyeryong Science Co. (Daejon, Korea). Food and water for all animals were provided *ad libitum* during the acclimation period. To maintain the animals under a specific pathogen free environment, temperature control was set at 23 ± 1°C and humidity control was set at 55 ± 10%. Ventilation was performed 15 to 20 times/h, noise level was kept below 45 and light was at 150 to 600 Lx between 09 : 00 a.m. and 09 : 00 pm. The rats were anesthetized with ketamine (200 mg/kg). A midline incision was made in the abdominal wall, and a section (1.5 cm × 1.5 cm) of the cecal serosa and the adjacent abdominal wall were abraded with a bone burr until the serosal surface was disrupted and made hemorrhagic but not perforated. The serosa of the cecum was sutured to the abdominal wall 5 mm away from the injured site. To evaluate the effects of a CM-chitosan hydrogel as a physical barrier for the prevention of an intra abdominal adhesion in a rat model, the rats were randomly separated into two groups of 30 female rats. Group I was the control. In Group II, the CM-chitosan hydrogel sheet was laid over the viscera. Animals were permitted to feed after operation for 2 weeks. Animals were killed on postoperative day 14, and the adhesion severity and strength were examined according to Vlahos et al.'s[21] method. The adhesion severity and strength were shown in Table I.

RESULTS AND DISCUSSION

Synthesis of carboxymethylchitosan (CM-chitosan)

The CM-chitosan was successfully synthesized and the FTIR spectra of chitosan and the CM-chitosan are compared in Figure 3 without more detailed chemical points, i.e. possibilities of by-reactions under these synthetic conditions employed. As shown in the spectrum, an absorption band that appears at near 1650–1550 cm^{-1} was assigned to the carboxylate ion. The characteristic peak (1601 cm^{-1}) in the spectrum of the CM-chitosan was assigned to carboxylic acid salt (—COO$^-$ stretch), suggesting that there were carboxymethyl groups in the CM-chitosan. Furthermore, in both spectra, the peaks (1070

TABLE I
Classification of the Adhesion Severity and Strength

Adhesion severity	
0	No adhesion
1	One thin filmy adhesion
2	Two or more thin filmy adhesion
3	Thick adhesion with focal point
4	Thick adhesion with planar attachment
5	Very thick vascularized adhesion
Adhesion strength	
1	Adhesion was filmy and easily torn with very light pressure
2	Adhesion was substantial and needed moderate pressure to tear
3	Adhesion was heavy and required significant pressure to rupture
4	Adhesion was very heavy and difficult to rupture

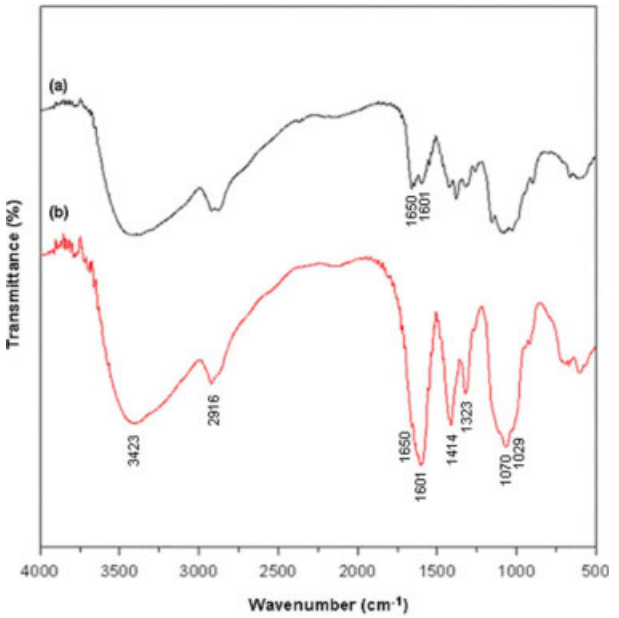

Figure 3 FTIR spectra of chitosan (a) and CM-chitosan (b). FTIR was used to confirm the chemical structure of the CM-chitosan. The samples were scanned from 500 to 4000 cm^{-1}. Both spectra indicate that a carboxymethyl group introduced into —CH_2—OH at the C6 position of the CM-chitosan. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

and 1029 $cm^{-1)}$ were assigned to the secondary hydroxyl group (—CH—OH in cyclic alcohols, C—O stretch) and the primary hydroxyl group (—CH_2—OH in primary alcohols, C—O stretch), respectively, and the peak intensity ratios (1029 cm^{-1}/1070 cm^{-1}) decreased. This result indicates that a carboxymethyl group introduced into —CH_2—OH at the CM-chitosan. Notable functional groups were also detected at amide band (—CONH—) around 1650 cm^{-1} and —NH_2 deformation at 1601 $cm^{-1.}$ The new absorption peak in the cross-linked CM-chitosan appears at 1663 cm^{-1} (—CONH—) after γ-irradiation. The irradiation activated the carboxylic groups, and formed the amide bonds in the amino groups of CM-chitosan (—NH_2).

Characteization of CM-chitosan hydrogel

The radiation-cross-linked CM-chitosan hydrogels were successfully prepared and these hydrogels showed excellent or equivalent swelling behavior and mechanical properties. In Figure 4, the CM-chitosan was cross-linked in a high concentrated aqueous solution (more than 10 wt %) but at a lower concentration these materials (at 5 wt %) resulted in degradation by irradiation. Figure 4 presents the gel-fraction content of the CM-chitosan hydrogel as a function of CM-chitosan contents and the irradiation dose. The gel-fraction content usually increased till 45–65%. But at a high dose, the gel-fraction content

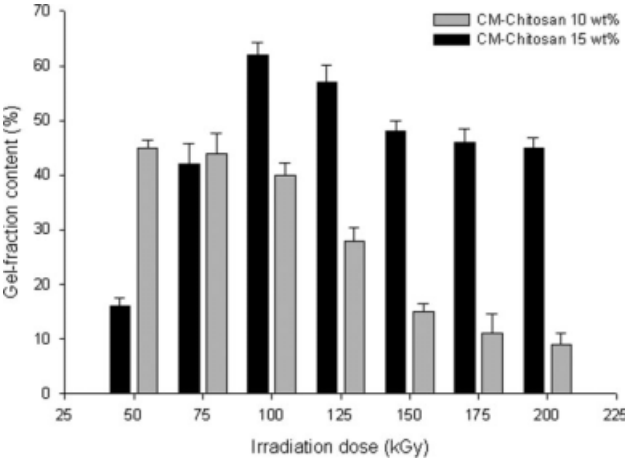

Figure 4 Gel-fraction contents of CM-chitosan hydrogels as a function of irradiation dose.

decreased slightly due to a degradation of the hydrogel. A 15 wt % of CM-chitosan solution is preferable to form gels, but when the irradiation dose exceeds 100 kGy, the gel-fraction content decreases due to the resultant degradation.

The degree of swelling of the gels with different CM-chitosan contents as a function of irradiation dose was investigated (Fig. 5). The swelling of both hydrogels was increased with the absorbed dose at an early stage, after reaching a maximum and then the 15 wt % gels rapidly decreased with the dose in a comparison with the 10 wt % gels. This is because of the cross-linking density in the network. In practice, the cross-linking density in hydrogel increases rapidly with increasing irradiation doses, resulting in a rise of network elasticity contributions. These crosslinks restrict the extensibility of the polymer chains induced by swelling and thus counter any tendency for dissolution. Thus the swelling ratio (%) reduces with the increase of the network. In

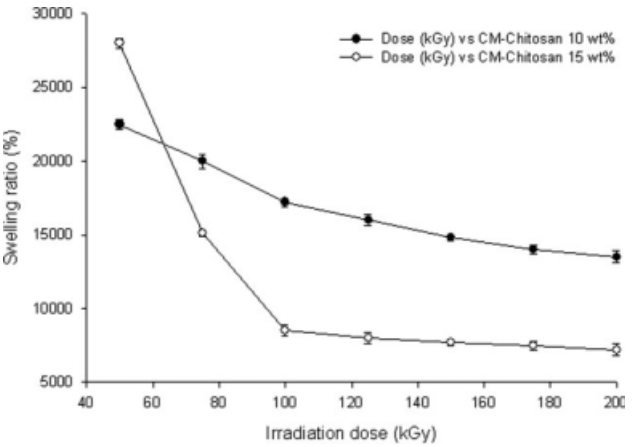

Figure 5 Swelling ratio of 10 wt % and 15 wt % of CM-chitosan hydrogels obtained by irradiation at different irradiation doses.

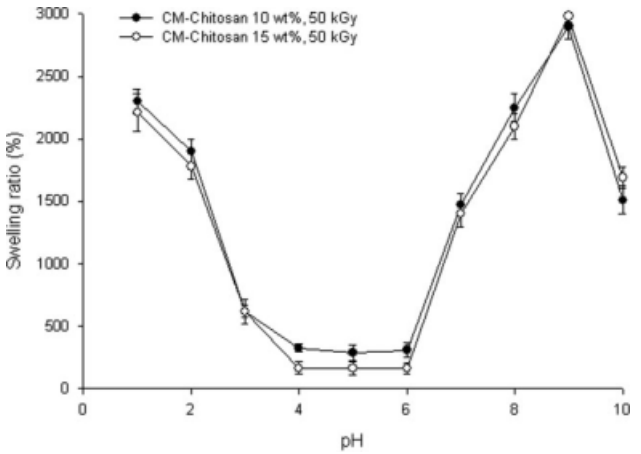

Figure 6 Swelling ratio of 10 wt % and 15 wt % of CM-chitosan hydrogels at different pH values.

addition, increasing concentration also produces more cross-linking, which reduced the swelling ratio. Thus the swelling corresponds well to the cross-linking behavior. The hydrogel made from the 15 wt % concentration showed a maximum swelling ratio of 28,000, and the gel from the concentration of 10 wt % showed a maximum swelling ratio of 23,000. In addition, the effects of pH value on the swelling of CM-chitosan hydrogels (10 and 15 wt %, 50 kGy) are shown in Figure 6. The CM-chitosan hydrogels showed pH-sensitive swelling behavior. The hydrogels swelled at low pH (<2) and 6 < pH < 9 but deswelled in the range of pH 4–6. This is because the carboxyl and amino groups in the CM-chitosan gel form oppositely charged networks which could change the charge state of the ionic groups varying with pH. The protonated amino group is the dominant charge in acidic solution (<4) and the unprotonated carboxyl group is the dominant charge in the basis (>6) and the gel thus swelled. However, at pH 4–6, most of the ionic groups are absent due to protonation of the carboxyl group and deprotonation of the amino group. On this account, the gel deswelled in this region.

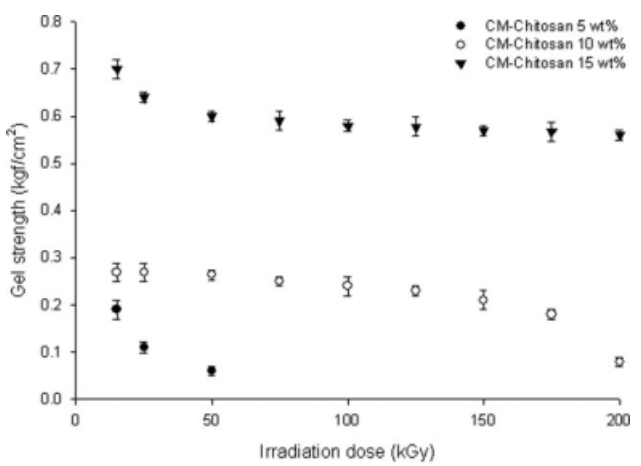

Figure 7 Gel strength of CM-chitosan hydrogels as a function of irradiation dose.

The effects on the mechanical properties of the concentration of CM-chitosan in the hydrogels and the irradiation dose were investigated (Fig. 7). The tensile strength of the hydrogels increased with increasing the CM-chitosan content and decreasing irradiation dose. These results were due to the increase of the cross-linking density, and the higher absorbed doses which led to a degradation and destruction of the network structure. The gel strength of 0.7 kgf/cm^2 was obtained in the 15 wt % CM-chitosan hydrogels at 15 kGy dose, and 0.25 kgf/cm^2 was obtained in the 10 wt % CM-chitosan hydrogels at 15 kGy dose.

Animal studies

Adhesions are unwanted tissue growths occurring between the layers of adjacent bodily tissues and internal organs. These adhesions commonly form during a healing that follows surgical procedures, and when present, adhesions can prevent the normal motions of those tissues and organs with respect to their neighboring structures. The results concerning the adhesion degree are summarized in Table II. The control animals formed dense adhesions between the cecal and the abdominal wall. In Group II, the CM-chitosan hydrogel treated animals had a significantly lower average adhesion degree than the controls. At day 14, no residual CM-chitosan hydrogels were observed in the treated animals (Fig. 8). The mechanism by which CM-chitosan reduces the adhesion formation is not clear. CM-chitosan, when implanted intraperitoneally, attracts fluid in its surroundings and thereby prevents the serosa from peritoneal contact; this is called a hydroflotation effect. In addition, there is evidence to suggest that CM-chitosan coats the intraperitoneal surfaces and reduces a direct apposition of traumatized structures; this is called a siliconizing effect. The adhesion formation is essentially an inflammatory reaction leading to fibrinogen influx and fibrin deposition. So, the other proposed mechanism for CM-chitosan involves its effect on the fibroblastic and cellular activities. The CM-chitosan may reduce adhesions by forming barriers to the

TABLE II
Evaluation of the Adhesion for Rats Treated with the CM-chitosan Hydrogel

Sample	Adhesion degree	Adhesion area (cm^2)	Adhesion strength
Control	4.6 ± 0.54	3.55 ± 0.51	3.6 ± 0.54
Hydrogel	0.2 ± 0.44[a]	0.016 ± 0.03[a]	0.2 ± 0.44[a]

[a] P value < 0.05 versus controls.

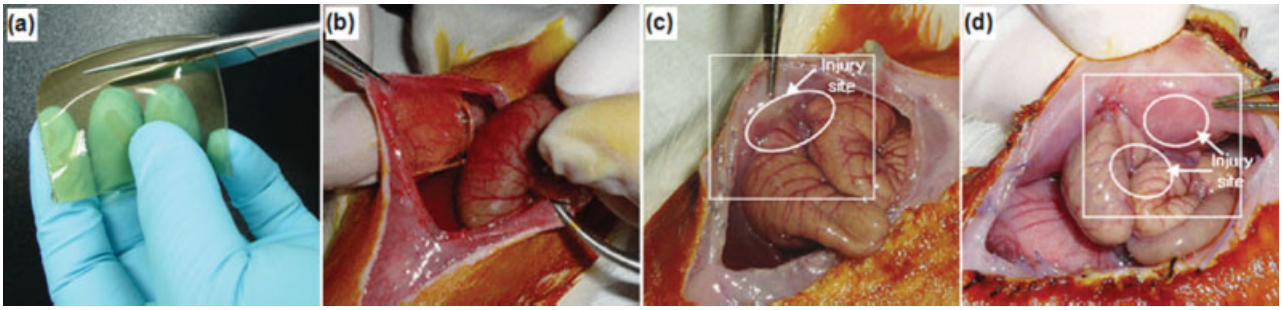

Figure 8 Animal tests for prevention of the intra abdominal adhesion by CM-chitosan hydrogel. (a) Surface photo of CM-chitosan hydrogel. Rat necropsy performed (b) immediately after an application of the CM-chitosan hydrogel, 14 days after an operation to determine the difference in the adhesion between the non-treated and (c) the treated CM-chitosan hydrogel (d). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

activities of inflammatory cells or factors. Alternatively, the fibrin deposition may be retarded with the addition of CM-chitosan, because coagulation and fibrin matrix development have been shown to coincide and the HA has been shown to inhibit platelet aggregation.[22,23] Therefore, it is reasonable to speculate that this is a potential idea for CM-chitosan's anti-adhesion activity. Recently, other anti-adhesion agents have been found to have limitations,[24–27] however, we demonstrated here that hydrogels prepared by irradiation method significantly reduced postsurgical adhesions without decreasing the mechanical strength when compared with the chemical cross-linking method.[28–30]

CONCLUSIONS

A water-soluble chitosan (CM-chitosan) was successfully synthesized and biocompatible CM-chitosan hydrogels were prepared, by a γ-irradiation cross-linking technique, as physical barriers for preventing surgical adhesions. The most efficient cross-linking process was obtained for the CM-chitosan concentration of 15 wt % at 15 kGy and these CM-chitosan hydrogels can provide satisfactory properties, such as excellent gel-fraction content, a high swelling behavior, and good mechanical properties for an application. Animals treated with a CM-chitosan hydrogel had a significantly lower average adhesion degree than the controls. These results imply that the CM-chitosan hydrogel prepared by an irradiation has the potential for many future applications, especially as a barrier for the prevention of surgical adhesions.

References

1. Ellis, H. Surg Gynecol Obstet 1971, 133, 497.
2. Alponat, A.; Lakshminarasappa, S. R.; Yavuz, N.; Goh, P. M. Am Surg 1997, 63, 818.
3. Wuster, S. H.; Bonet, V.; Mayberry, A.; Hadolinott, M.; Williams, T.; Chassdry, I. H. J Surg Res 1995, 59, 97.
4. Matsuyama, H.; Teramoto, M.; Urano, H. J Membr Sci 1997, 126, 151.
5. Muhlebach, A.; Muller, B.; Pharisa, C.; Hofmann, M.; Seiferling, B.; Guerry, D. J Polym Sci Part A: Pol Chem 1997, 35, 3603.
6. Sudhamania, S. R.; Prasada, M. S.; Sankarb, K. U. Food Hydrocolloids 2003, 17, 245.
7. Ishihara, M.; Obara, K.; Ishizuka, T.; Fujita, M.; Sato, M.; Masuoka, K.; Saito, Y.; Yura, H.; Matsui, T.; Hattori, H.; Kikuchi, M.; Kurita, A. J Biomed Mater Res 2003, 64, 551.
8. Zhao, L.; Mitomo, H.; Yoshii, F.; Kume, T. J Appl Polym Sci 2004, 91, 556.
9. Kennedy, R.; Costain, D. J.; Mcalister, V.; Lee, T. D. G. Surgery 1996, 120, 866.
10. Zhao, L.; Mitomo, H.; Nagasawa, N.; Yoshii, F.; Kume, T. Carbohydr Polym 2003, 51, 169.
11. Rosiak, J. M.; Fumio, Y. Nucl Instr Meth B 1999, 151, 56.
12. Yoshii, F.; Zhao, L.; Wach, R. A.; Nagasawa, N.; Mitomo, H.; Kume, T. Nucl Instr Meth B 2003, 208, 320.
13. Hayes, E. R. U.S. Pat. 4,619,995 (1986).
14. Muzzarelli, R. A. A.; Tanfani, F.; Emanuelli, M.; Mariotti, S. Carbohydr Res 1982, 107, 199.
15. Muzzarelli, R. A. A. Chitin; Pergamon Press: Oxford, UK, 1977.
16. Roberts, G. A. F.; Taylor, K. E. Macromol Chem Phys 1989, 190, 951.
17. Rosiak, J. M.; Ulanski, P. Radiat Phys Chem 1999, 55, 139.
18. Lingyun, C.; Yumin, D.; Xiaoqing, Z. Carbohydr Res 2003, 338, 333.
19. Muzzarelli, R. Eur. Pat. 212,688 (1986).
20. Zhao, L.; Xu, L.; Mitomo, H.; Yoshii, F. Carbohydr Polym 2006, 64, 473.
21. Vlahos, A.; Yu, P.; Lucas, C. E.; Ledgerwood, A. M. The Am Surgeon 2001, 67, 15.
22. Shushan, A.; Mor-Yosef, S.; Avgar, A.; Laufer, N. J Reprod Med 1994, 39, 398.
23. Buckman, R. F.; Bukman, P. D.; Hufnagel, H. V.; Gervin, A. S. J Surg Res 1976, 21, 67.
24. Holmdahl, L. E.; A1-Jabreen, M.; Risberg, B. Wound Rep Reg 1994, 2, 171.
25. Guyton, D. P.; Guy, J.; Sloan, K.; Evans, D. M. Surg Forum 1989, 40, 34.
26. Connolly, J. E.; Smith, J. W. Surg Gynecol Obstet 1960, 110, 417.
27. Ellis, H. Surg Gynecol Obstet 1961, 113, 547.
28. Darren, J. C.; Renee, K.; Curtis, C.; Vivian, C. M.; Timothy, D. G. L. Surgery 1997, 121, 314.
29. Belluco, C.; Meggiolaro, F.; Pressato, D.; Pavesio, A.; Bigon, E.; Donà, M.; Forlin, M.; Nitti, D.; Lise, M. J Surg Res 2001, 100, 217.
30. Juan, Z.; Robert, S. L.; Clive, E.; Timothy, D. G. L. J Thorac Cardiovasc Surg 2008, 135, 777.