# Prevention of Surgical Adhesions with Barriers of Carboxymethylcellulose and Poly(ethylene glycol) Hydrogels Synthesized by Irradiation

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**ABSTRACT:** Biocompatible and biodegradable hydrogels based on carboxymethylcellulose (CMC) and poly(ethylene glycol) (PEG) were prepared as physical barriers for preventing surgical adhesions. These interpolymeric hydrogels were synthesized by a γ-irradiation crosslinking technique. Sections (1.5 cm × 1.5 cm) of the cecal serosa and an adjacent abdominal wall were abraded with a bone burr until the serosal surface was disrupted and hemorrhagic but not perforated, and the serosa of the cecum was sutured to the abdominal wall 5 mm away from the injured site. The denuded cecum was covered with either CMC/PEG hydrogels or a solution from a CMC/PEG hydrogel. A control rat serosa was not covered. Two weeks later, the rats were killed, and the adhesions were scored on a 0–5 scale. No treatment showed a significantly higher incidence of adhesions than the CMC/PEG hydrogels or solutions from the CMC/PEG hydrogels. This study demonstrated that CMC/PEG hydrogels could prevent intra-abdominal adhesion in a rat model. © 2005 Wiley Periodicals, Inc. J Appl Polym Sci 96: 1138–1145, 2005

**Key words:** adhesion; crosslinking; hydrogels; irradiation; radiation; swelling

## INTRODUCTION

Postsurgical adhesions are abnormal tissue attachments that result from cuts or abrasions to tissues during surgery. These adhesions develop as part of the normal wound-healing response of the tissues to the trauma and occur in over two-thirds of all abdominal surgical patients. The consequences of these adhesions are varied and depend on the surgical site involved. Problems may include pain, infertility, obstruction of the intestines, and even an increased risk of death after cardiac surgery. The process of adhesion formation initially involves the establishment of a fibrin framework and normal tissue repair. The normal repair process allows for fibrinolysis alongside a mesothelial repair. However, in surgical adhesion formation, the fibrin matrix matures as the fibroblasts proliferate into the network, and angiogenesis occurs, resulting in the establishment of an organized adhesion within 3–5 days. After major abdominal surgery, 60–95% of patients develop adhesions.[1] Adhesions are responsible for about 60% of bowel obstructions and 20% of all infertility as well as substantial costs associated with adhesiolysis and hospitalization.[2–4]

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Interventional attempts to prevent the formation of postsurgical adhesions have included the use of hydroflotation techniques and barrier devices. Hydroflotation involves the instillation of large volumes of polymer solutions such as dextran or carboxymethylcellulose (CMC)[5,6] into the surgical space in an attempt to keep the organs apart. However, this technique has produced marginally beneficial effects in animals and humans. Synthetic barrier membranes made from oxidized regenerated cellulose (Interceed) or polytetrafluoroethylene (Goretex surgical membrane) have demonstrated some limited inhibition of adhesion formation in humans.

Resorbable barrier materials that have received research attention include Dextran-70, hyaluronic acid,[7] Poloxamer 407,[8] Interceed (Johnson & Johnson Medical, Inc., Arlington, TX), CMC, fibrin glue, sodium hyaluronate/CMC, and amnion. Only two products have been approved for clinical use: Interceed[9,10] and Seprafilm (Genzyme Corp., Cambridge, MA).

Hydrogels are most often defined as two-component systems in which one of the components is a hydrophilic polymer, insoluble in water because of three-dimensional networks connecting as chains, and the second one is water. These systems may swell in water up to an equilibrium state and retain their original shape. The factors responsible for water sorption by hydrogels include hydration, which is connected to the presence of such chemical groups as —OH,

—COOH, —$CONH_2$, —CONH—, and —$SO_3H$, the existence of capillary areas, and the differences in the osmotic pressure. The forces that make hydrogel dissolution impossible are connected to the existence of covalent bonds between the individual polymer chains, although they may also have the characteristics of electrostatic or hydrophobic interactions. Polymer gels have a very low modulus of elasticity and, therefore, cause minimal mechanical irritation. They usually show good biocompatibility with blood, bodily fluids, and tissues. In recent years, much attention has been focused on the research and development of polymer hydrogels for biomaterials, such as contact lenses, wound dressings, enzyme immunoassays, catheters, and drug-delivery systems.

Irradiation has been recognized as a very suitable tool for the formation of hydrogels. The radiation process has various advantages, such as easy process control, the possibility of joining the hydrogel formation and sterilization in one technological step, and no necessity of adding any initiators and crosslinkers possibly harmful and difficult to remove. They make irradiation the method of choice for the synthesis of hydrogels.

Water-soluble polysaccharides such as CMC, carboxymethyl starch, carboxymethyl chitin, and carboxymethyl chitosan are crosslinked by radiation in more than 10% aqueous solutions.[11]

The goal of this study was to evaluate the efficacy of CMC/poly(ethylene glycol) (PEG) hydrogels as barriers for reducing postsurgical adhesions in a rat cecal abrasion model.

## EXPERIMENTAL

### Materials

CMC [weight-average molecular weight ($M_w$) = $7.0 \times 10^5$, degree of substitution (DS) = 0.9] and PEG ($M_w = 4 \times 10^3$) were supplied by Aldrich Chemical Co. (Milwaukee, WI) Distilled water was used as the solvent in all the experiments.

### Preparation of the hydrogels

CMC/PEG (various weight compositions; see Table I) was dissolved in distilled water at 25°C and then mixed with a physical blender at room temperature to produce CMC/PEG solutions. The dried content of CMC/PEG was 11.2–12.3 wt %, and the CMC/PEG weight composition was 88/12, 85/15, 82/18, or 79/21. The homogeneous paste was then put into a cavity between plastic plates with a 2-mm spacer. The paste was exposed to 25 kGy of $\gamma$ rays to make the hydrogels.

**TABLE I**
**Formulations for the Hydrogels**

| Hydrogel composition | | | |
|---|---|---|---|
| | | CMC/PEG Composition | |
| Abbreviation | CMC/PEG concentration (%) | CMC (%) | PEG (%) |
| Cp88/12 | 11.2 | 88 | 12 |
| Cp85/15 | 11.6 | 85 | 15 |
| Cp82/18 | 11.9 | 82 | 18 |
| Cp79/21 | 12.3 | 79 | 21 |

CMC $M_w = 7.0 \times 10^5$; DS = 0.9; total dose = 2.5 Mrad; dose rate = 5 kGy/h; PEG $M_w = 4.0 \times 10^3$.

### Preparation of the gel solutions from the hydrogels

A sample of CP88/12 (Table I) was diluted with distilled water and agitated mildly to make a 2 wt % concentration of CMC/PEG. This gel solution, which consisted of small segregated particles of the hydrogels in water, was used as a coating to prevent intraabdominal adhesion in a rat model.

### Degree of swelling

The degree of swelling could be described as the water absorptivity of the hydrogels. The gel samples were immersed in distilled water at room temperature until the gel collapsed. After the water on the surface of the swollen gels was removed with cellulose paper, the mass was determined. The degree of swelling was defined as follows:

$$\text{Water absorptivity} = \frac{W_s - W_d}{W_d} \quad (1)$$

where $W_s$ is the weight of the swollen gels and $W_d$ is the initial CMC/PEG weight.

### Gel strength

A cylindrical hydrogel specimen, 4.8 mm high and 12 mm in diameter, was used for the compressive strength tests. The compressive strength tests were conducted with an Instron model 4400 universal testing machine (Canton, MA) at room temperature. A cylindrical hydrogel specimen was placed on the base, and the probe was lowered until contact was made. The probe was then lowered at 10 mm/min until a 70% relative deformation and then raised. The compressive strength used in this experiment was the value measured at 70% relative deformation. The mechanical properties of the hydrogels were obtained by the determination of the compressive strength.

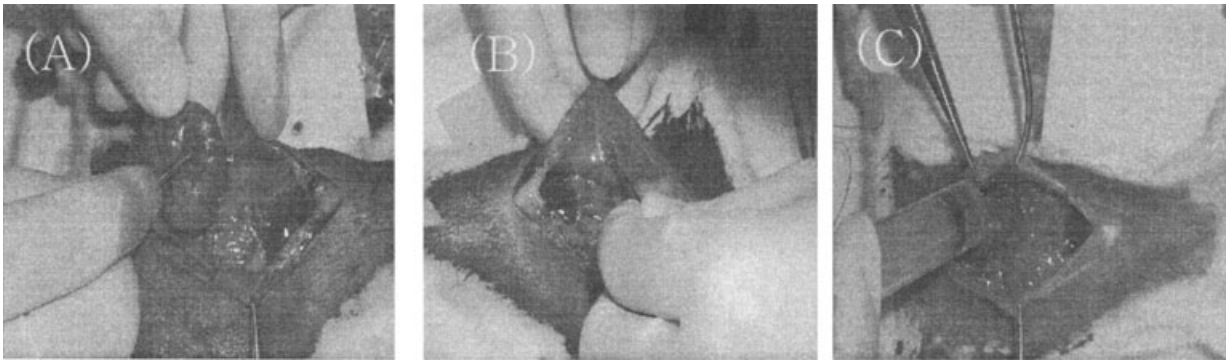


**Figure 1** Treatment procedure for the cecal serosa and adjacent abdominal wall for an adhesion evaluation: (A) an abrasion injury of the cecal serosa, (B) an abrasion injury of the adjacent abdominal wall, and (C) an application of a solution prepared from a hydrogel (Cp88/12) to the injury.

### Adhesive force

The adhesive force was obtained by the measurement of the force required to break the contact between the CMC/PEG hydrogel and the mucosa layer of the porcine intestine with an Instron model 4400 tensile tester. CMC/PEG hydrogels (Cp88/12; thickness = 2 mm) and porcine intestine were cut (1 cm × 1 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). The rats were anesthetized with an intramuscular injection of ketamine (200 mg/kg), and their ventral hair was removed with electric clippers. With an aseptic technique, a 7-cm incision was made on the midline of 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 hemorrhagic but not perforated. The serosa of the cecum was sutured to the abdominal wall 5 mm away from the injured site (Fig. 1).

To evaluate the effects of a CMC/PEG hydrogel (Cp88/12) or a 2% gel solution from a CMC/PEG hydrogel (Cp88/12) as a physical barrier for the prevention of an intra-abdominal adhesion in a rat model, we divided 30 female rats into three equal groups. Group I was the control; in group II, a sheet (2 mm) of the CMC/PEG hydrogel was laid over the viscera; and in group III, a gel solution from the CMC/PEG hydrogel was coated over the viscera. Ten of the animals from each group were killed on postoperative day 14, and the adhesion severity and strength were scored according to Vlahos et al.'s experiment.[12] The adhesion severity was classified as follows (Table II): (0) no adhesion, (1) one thin filmy adhesion, (2) two or more thin filmy adhesions, (3) a thick adhesion with a focal point, (4) a thick adhesion with a planer attachment; , and (5) a very thick vascularized adhesion. The adhesion strength was classified as follows (Table II): (1) the adhesion was filmy and easily torn with very light pressure, (2) the adhesion was substantial and needed moderate pressure to tear, (3) the adhesion was heavy and required significant pressure to rupture, and (4) the adhesion was very heavy and difficult to rupture.

Some of the model rats were killed 3, 7, and 14 days after surgery to observe the absorption of the CMC/PEG hydrogel (Cp88/12) at the injured site.

At death, the abdominal wall of the injured site and the opposed cecum were removed and fixed in a 10 wt % formalin solution. The specimens were dehydrated in a graded series of ethanol and in toluene and then

**TABLE II**
**Classification of the Adhesion Severity and Strength**

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

[a] According to Vlahos et al.[12]

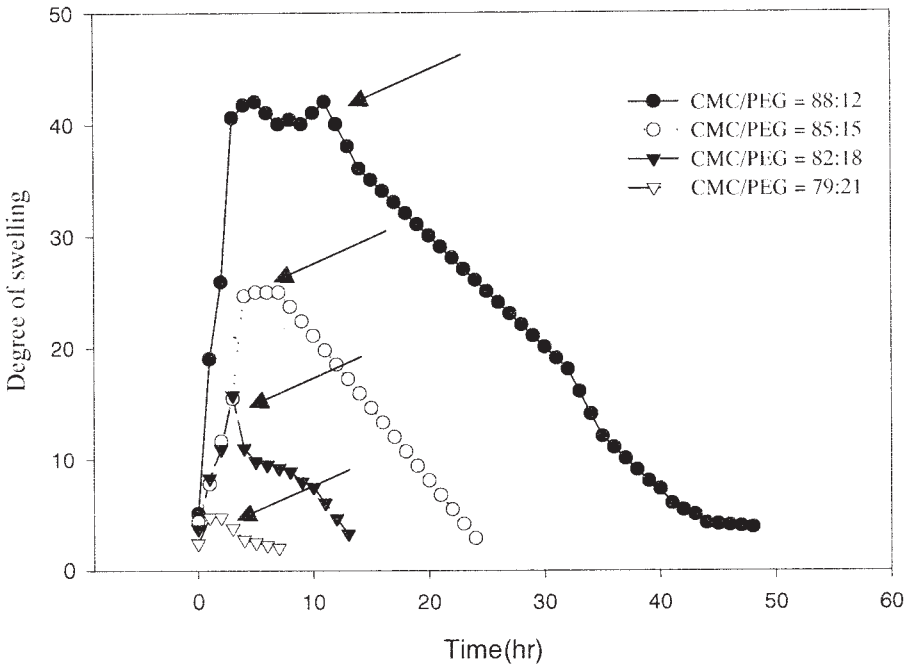


**Figure 2** Swelling behavior of the hydrogels prepared with the formulations listed in Table I. The arrows show the starting time for disintegration.

embedded in paraffin. Sections were cut at 4–5 $\mu$m. The tissues were processed by the standard procedure for histological examinations, and their thin sections were examined after they were stained with hematoxylin and eosin.

## RESULTS AND DISCUSSION

### Physical properties

There are several methods for preparing crosslinked hydrogels, such as radiation and chemical processes. Radiation reactions use electron beams, $\gamma$ rays, X-rays, or ultraviolet light to excite a polymer and produce a crosslinked structure. Chemical crosslinking requires at least one difunctional, low-molecular-weight crosslinking agent. This agent usually links two longer molecular weight chains through its difunctional or multifunctional groups. Radiation crosslinking can be easily adjusted through the control of the radiation dose, and it is reproducible. All the samples prepared with the formulation in Table I had very soft and flexible properties. When the prepared hydrogels

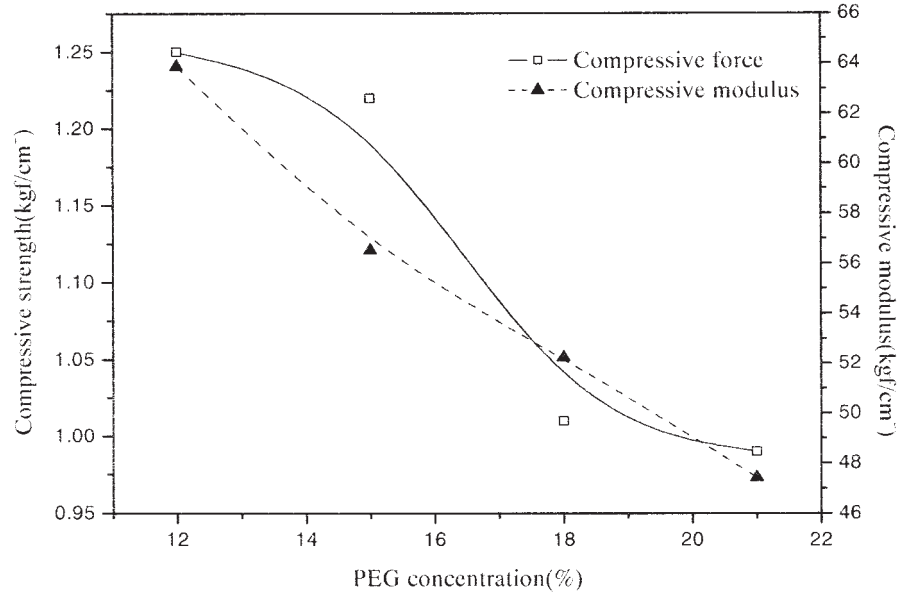


**Figure 3** Compressive strength and modulus of the hydrogels prepared with the formulations listed in Table I.

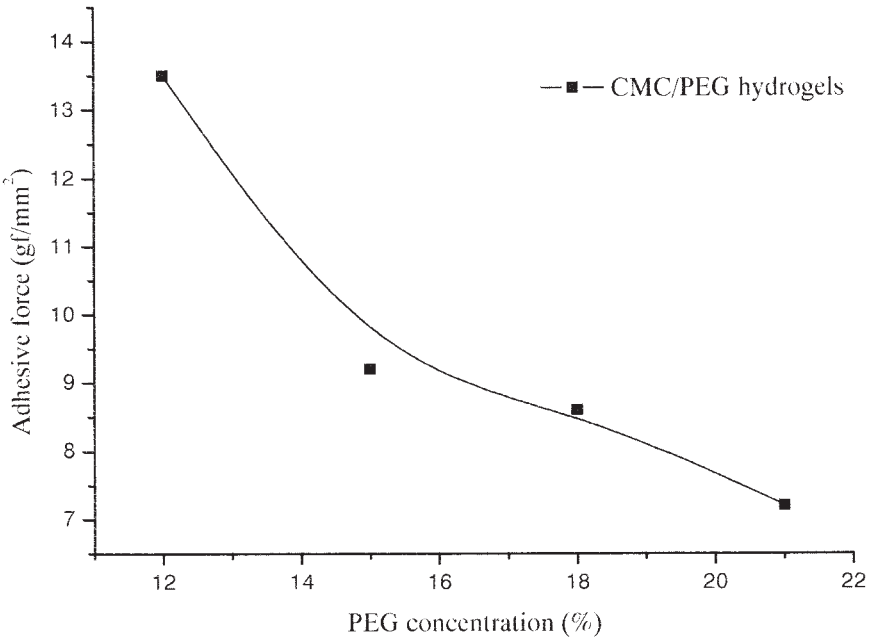


**Figure 4** Adhesive force of the CMC/PEG hydrogels and the mucosa layer.

were kept in water for more than 48 h, we were unable to evaluate the gel percentage because the forms of the hydrogels disintegrated. Figure 2 shows the degree of the swelling behavior of the hydrogels that were synthesized by $\gamma$ irradiation. The swelling percentage decreased as the PEG concentration in CMC/PEG increased. As the concentration of PEG increased, the hydrogels dipped in water were very weak and vulnerable. Therefore, the swelling percentage decreased with an increasing PEG concentration, and this led to the high disintegration rate. The hydrogels were disintegrated 48 (Cp88/12), 24 (Cp85/15), 13 (Cp82/12), and 7 h (Cp79/21) after they were dipped in water.

Crosslinking transforms a linear polymer into a three-dimensional molecule, and this results in a significant increase in the molecular mass, lower solubility in organic solvents, and improved mechanical properties. Degradation results in a decrease in the molecular mass and has the opposite effect on the physical properties of the polymer. Crosslinking and

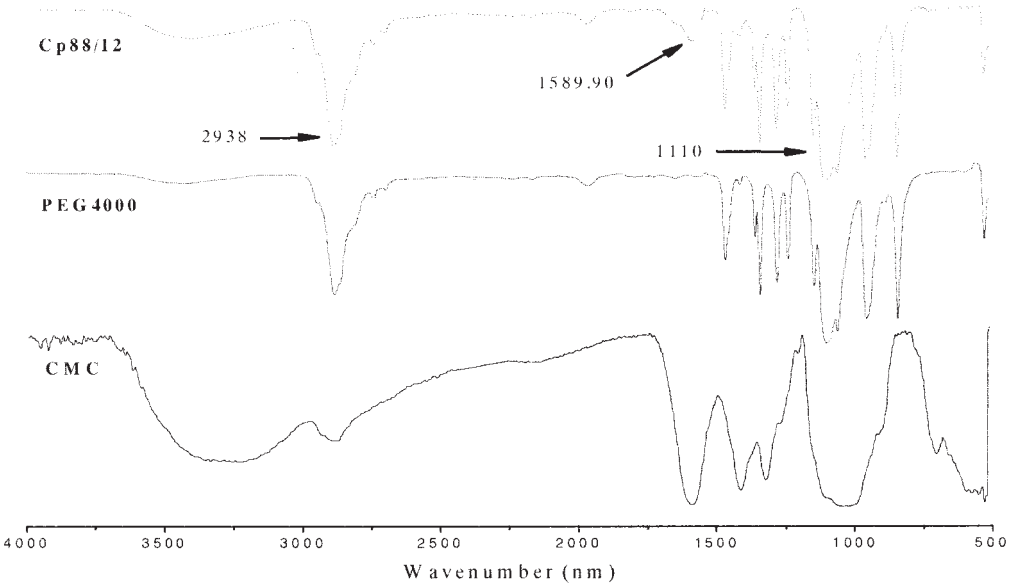


**Figure 5** FTIR spectra of CMC, PEG, and CMC/PEG [the CMC/PEG film was prepared from a hydrogel with CMC/PEG (88/12 w/w)].

**TABLE III**
**Evaluation of the Adhesion for Rats Treated with the CMC/PEG Hydrogel (Cp8812) or a Solution from the CMC/PEG Hydrogel (Cp8812)**

| Aggregation | Adhesion degree | Adhesion area ($cm^2$) | Adhesion strength |
|---|---|---|---|
| Controls | 4.6 ± 0.5 | 3.61 ± 0.24 | 3.6 ± 0.51 |
| 2% solution (Cp88/12) | 0.2 ± 0.42[a] | 0.0125 ± 0.04[a] | 0.3 ± 0.94[a] |
| Hydrogel (Cp88/12) | 0.1 ± 0.3[a] | 0.0104 ± 0.03[a] | 0.2 ± 0.63[a] |

[a] $p < 0.05$ versus controls.

degradation occur simultaneously. However, the ratio of their rates depends on the chemical structure of the polymer, its physical state, and its irradiation state. Polymers generally can be divided into those that predominantly crosslink and those that predominantly degrade. CMC and PEG were crosslinked in a homogeneous mixture with water. As the molecular weight of PEG in these experiments was low, an increase in the PEG concentration in the CMC/PEG solution resulted in a decrease in the gelation of the hydrogels.

The compressive strength used in this experiment was the value measured at 70% relative deformation. The gel strength of the hydrogels was obtained by a determination of their compressive strength (Fig. 3). The compressive strength and modulus decreased as the concentration of PEG in CMC/PEG increased. Because low-molecular-weight PEG did not contribute to the crosslinking, an increase in the PEG concentration in CMC/PEG resulted in a decrease in the compressive strength and modulus of the hydrogels.

Figure 4 shows the adhesive force between the CMC/PEG hydrogels and the mucosa layer. A mucoadhesion that adheres to the mucosa layer may be useful for fixing hydrogels onto the viscera. The higher the PEG concentration was in the CMC/PEG hydrogels, the lower the adhesive force was. It is thought that the carboxylic groups in CMC are important for a mucoadhesion.

Figure 5 shows FTIR spectra of CMC, PEG, and a CMC/PEG film prepared from a CMC/PEG hydrogel. PEG showed absorption features at 2938–2976 $cm^{-1}$ (C—H) and around 1110 $cm^{-1}$ (C—O—C). The intensities of the carboxyl band at 1589 $cm^{-1}$, which was not observed for pure PEG, are shown for the CMC/PEG film prepared from a CMC/PEG hydrogel.

## Animal studies

Adhesions are unwanted tissue growths occurring between the layers of adjacent bodily tissues and internal organs. Adhesions commonly form during the 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 of the adhesion scoring are summarized in Table III. The control animals formed dense adhesions between the cecal and the abdominal wall. Animals treated with the CMC/PEG hydrogel (Cp88/12) or a 2 wt % solution from the CMC/PEG hydrogel (Cp88/12) had a significantly lower average adhesion score than the controls. There was not much difference in the adhesion scores between groups II and III. At day 14, no residual HA/CMC hydrogels were visible in the treated animals. The mechanism of action by which CMC reduces adhesion formation is not clear. CMC, when implanted intraperitoneally, attracts fluid in its surroundings and thereby prevents the serosa from peritoneal contact; this is called the hydroflotation effect. In addition, there is evidence to suggest that CMC/PEG coats intraperitoneal surfaces and re-

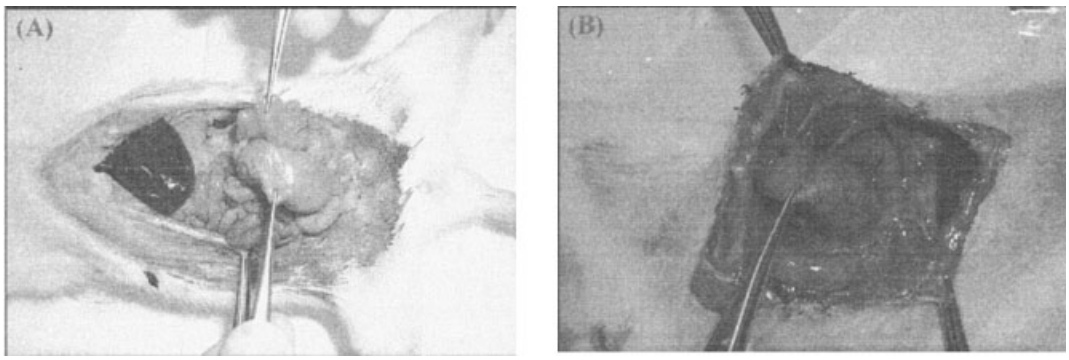


**Figure 6** Rat necropsy performed 14 days after an operation to determine the difference in the adhesion between (A) the control and (B) a solution prepared from a hydrogel (Cp88/12).

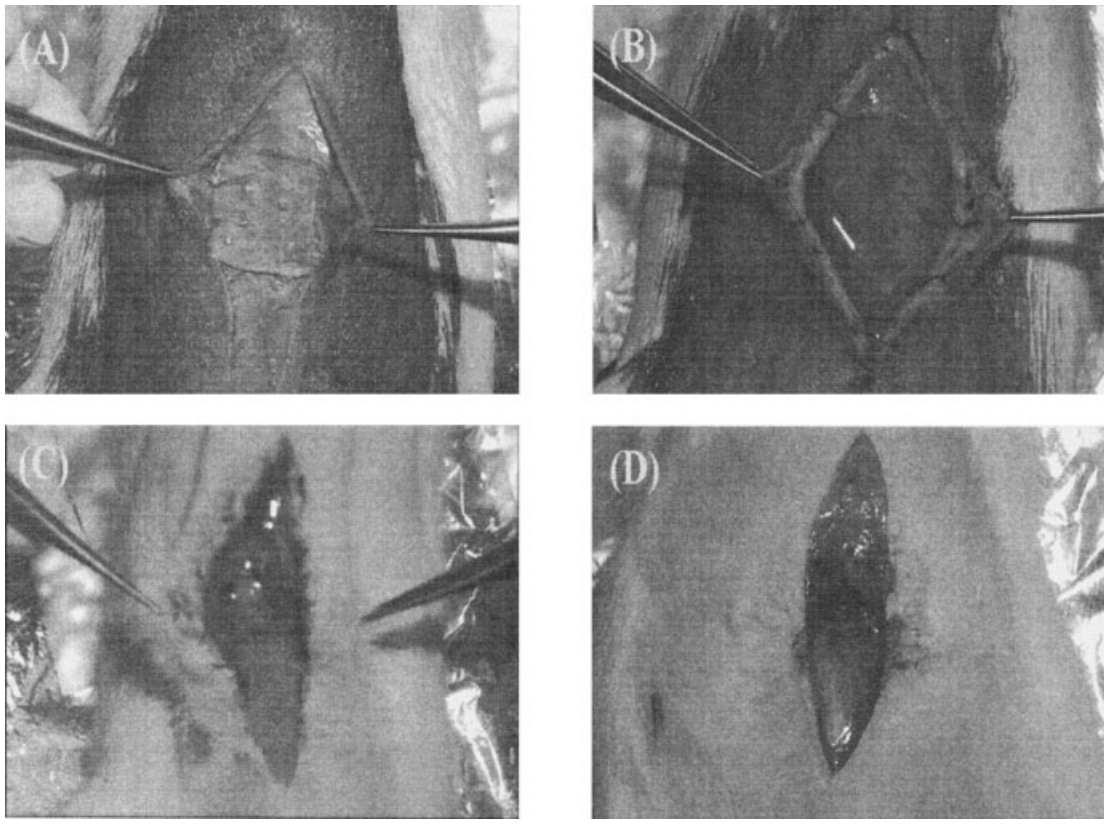


**Figure 7** Procedure for the bioresorbability of a CMC/PEG hydrogel: (A) immediately after an application of the hydrogel, (B) 3 days after an application of the hydrogel, (C) 7 days after an application of the hydrogel, and (D) 14 days after an application of the hydrogel.

duces the direct apposition of traumatized structures; this is called the siliconizing effect.[13] The other proposed mechanism for the action of CMC involves its effect on fibroblastic and cellular activities.[14]

At the time of application, the hydrogels adhered readily to the serosal. In both the control and experimental groups, the celiotomy incisions healed normally. The sutures holding the bowel to the abdominal wall were still in place (Fig. 6).

The bioresorbability of the CMC/PEG hydrogel (Cp88/12) between the skin and the abdominal layer of the rat was evaluated by the observation of the

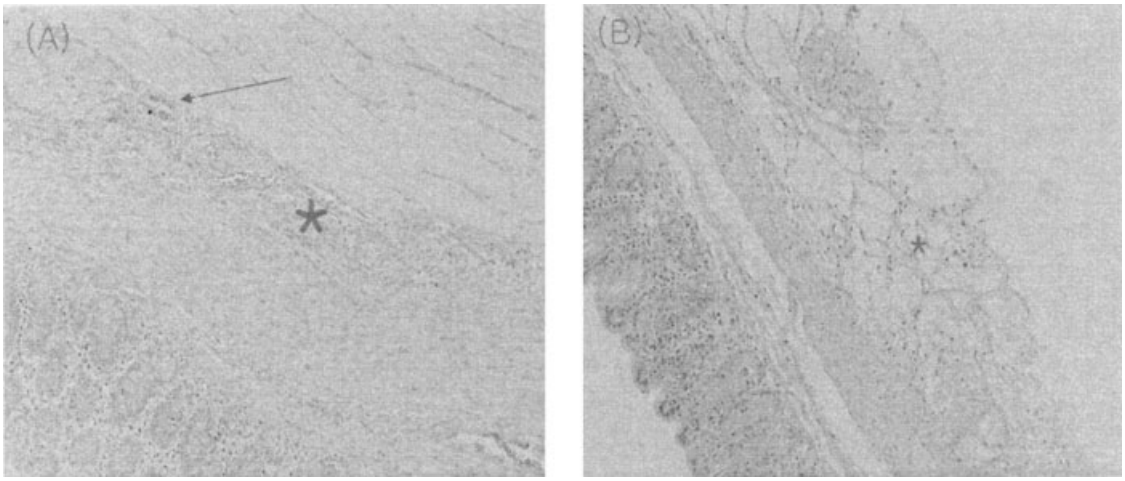


**Figure 8** Histological appearance of tissue in part of the cecal serosa and abdominal wall: (A) the control and (B) an application of a hydrogel .

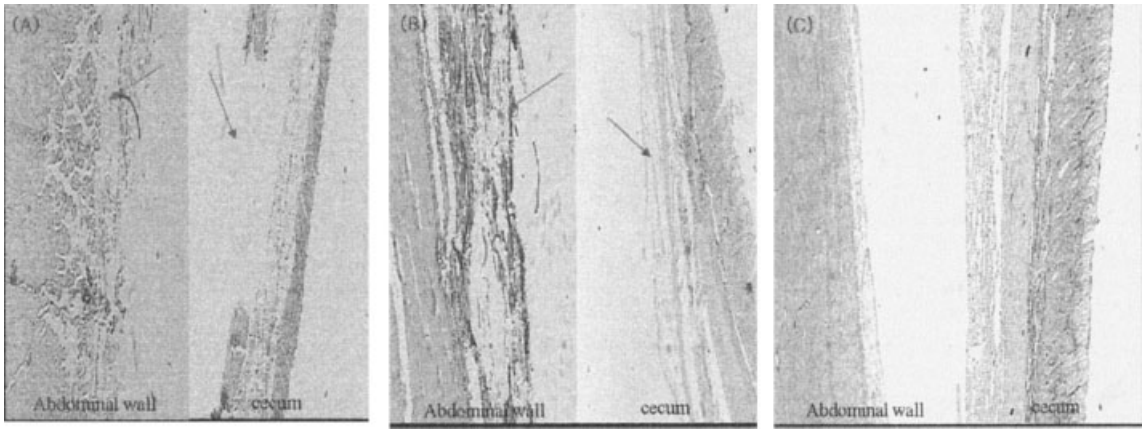


**Figure 9** Histological appearance of the tissue in part of the cecal serosa the abdominal wall: (A) 12 h after an application of a 2 wt % solution from a hydrogel, (B) 3 days after an application of a 2 wt % solution from a hydrogel, and (C) 7 days after an application of a 2 wt % solution from a hydrogel.

forms of the hydrogels on the cecal serosa on the 3rd, 7th, and 14th days after surgery. The hydrogel was found to be a loose gel on the 3rd postoperative day and was slightly detectable at the 7th day, but it was almost not detectable on the 14th postoperative day (Fig. 7). The mechanism of the bioresorbability of CMC or PEG is not well known.

Figure 8 shows the histological appearance of the tissue in a part of the cecal serosa and the abdominal wall. In the control, a dense fibrous adhesion between the intestine (cecal serosa) and the abdominal wall was found, and variable inflammatory cells and a neovascular structure were observed in the granulation tissue. The serosal area and the surrounding fat tissue showed mild inflammatory cell infiltrations. On the other hand, the rat tissue treated with the hydrogel showed no granulation tissue formation.

Figure 9 shows the histological appearance of the tissue in a part of the cecal serosa and the abdominal wall 12 h, 3 days, and 7 days after the application of the hydrogels to the injuries. We can detect a focal abdominal wall and mucosal erosion and the completely formed muscular tissue and a mesothelial area by wound healing.

## CONCLUSIONS

Hydrogels based on CMC/PEG were prepared as physical barriers for preventing surgical adhesions with radiation. These interpolymeric hydrogels were synthesized by a $\gamma$-irradiation crosslinking technique. Animals treated with a CMC/PEG hydrogel (Cp88/12) or a gel solution from a CMC/PEG hydrogel (Cp88/12) had a significantly lower average adhesion score than the controls. At day 14, almost no residual HA/CMC hydrogels were visible in the treated animals. Hydrogels prepared by radiation significantly reduced the postsurgical adhesions in a rat cecal abrasion model.

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