Adhesion Barriers of Carboxymethylcellulose and Polyethylene Oxide Composite Gels

Lin-Shu Liu, Richard A. Berg
FzioMed, Inc., 170-A Granada Drive, San Luis Obispo, California 93401

Received 28 September 2001; revised 11 December 2001; accepted 17 December 2001
Published online 3 April 2002 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jbm.10211

Abstract: Composite gels and films of CMC and PEO have been used to separate healing tissues and have been demonstrated to reduce postsurgical adhesions in animal models of adhesion formation. Gels of CMC/PEO were studied here to elucidate the mechanism by which the combination of PEO with CMC is effective in reducing adhesions between tissues. CMC and PEO were demonstrated to undergo micro phase separation to form a two-phase system. Protein partitioning was measured in this system for albumin, fibrinogen, and gamma globulin. All of these proteins were found to partition preferentially into the CMC phase. When gels of CMC and PEO were examined for tissue adherence, the addition of PEO reduced the adherence of CMC to tissues. To further investigate the effects of PEO on tissue adherence of the gel, the extent of thrombus formation of citrated blood initiated by calcium chloride in CMC/PEO gels was measured in vitro. The extent of thrombus formation by CMC was reduced proportionally to the content of PEO in gels of CMC/PEO. A model was developed to explain how CMC and PEO contribute to the effectiveness of CMC/PEO gels that form a barrier between healing tissues to reduce postsurgical adhesions. In an open system PEO is released from the gel faster than CMC is dissolved, resulting in a shell structure with CMC coated by PEO. The PEO-rich outer layer functions to inhibit protein deposition and thrombus formation. The CMC-rich layer functions to anchor the gel to the tissue surface.

Keywords: carboxymethylcellulose; polyethylene oxide; protein partitioning; adhesions

INTRODUCTION

Postsurgical adhesions are a serious complication in many surgeries, as they result from a normal wound-healing process that is triggered by inflammation or tissue trauma. Peritoneal adhesions and adnexal adhesions develop in patients undergoing abdominal or gynecological surgeries. Peridural adhesions develop in patients undergoing spine surgery and perineural adhesions develop in patients undergoing peripheral nerve surgery. An important consequence of adhesion formation is the development of fibrosis that can cause a serious compromise of organ function in terms of bowel obstruction, infertility, or peridural fibrosis in the spine resulting in pain or neurological dysfunction.

CMC is a water-soluble anionic linear polymer that has been used in several medical applications and more recently as a component of an antiadhesion gel or membrane. PEO is a nonpolar, high-molecular-weight linear polymer of polyethylene oxide that has a large unfavorable free energy of interaction with proteins. It is a biocompatible polymer that has been used as a component of a tissue sealant and as a coating for medical devices and it has recently been used for conjugation to therapeutic proteins to extend their circulation time for drug delivery.

Solutions of CMC have been tested to inhibit adhesions in several animal models. To achieve sufficient coverage, the solutions were applied both intraoperatively and postoperatively. PEO has also been tested in animals as an antiadhesive solution. However, because of the difficulty of keeping PEO in contact with tissues, large volumes must be used.

More recently, a composite material composed of both CMC and PEO in the form of a film or a gel has been demonstrated to reduce adhesion formation in several animal studies of peritoneal or peridural adhesions. Composites of CMC and PEO in the form of both films and gels are tissue adherent and are effective in reducing adhesions. Because neither polymer was as effective when used alone as when used in the composite, the contribution of each component was evaluated here. It was shown that when CMC and PEO are formed into a composite gel, the unique prop-
TABLE I. Characteristics of PEO/CMC Gels

<table>
<thead>
<tr>
<th>Composition (%)</th>
<th>Viscosity $\times 10^6$ cps</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEO</td>
<td>CMC</td>
</tr>
<tr>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>97</td>
</tr>
<tr>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

The total concentration of polymers in each preparation was 3.3% (w/v).

Properties of each provide the basis for a mechanism to explain the composite's antiadhesive function.

MATERIALS AND METHODS

Materials

Sodium carboxymethylcellulose (CMC, mol.wt. $7 \times 10^5$ Daltons) was purchased from Hercules, Inc. (Wilmington, DE). Polyethylene oxide (PEO; mol.wt. $4.4 \times 10^6$ Daltons) was obtained from RITA, Inc. (Woodstock, IL). Fluorescein-PEG (mol.wt. $5 \times 10^3$ Daltons) was obtained from Shearwater (Huntsville, AL). PEO (mol.wt. 5000 Daltons), sodium citrate solution (4%, aseptically filled), as well as bovine fibrinogen and γ globulin were from Sigma Chemical Co. (St. Louis, MO). Bovine plasma albumin was from Calbiochem Inc. (La Jolla, CA). Methylene blue (0.05%, w/v, ACS grade), sulfuric acid (98%), phenol (double distilled) and other chemicals were from VWR (San Francisco, CA). Porcine intestine was from a local market. Citrated bovine blood was prepared by mixing one part of sodium citrate solution with nine parts of whole blood from a healthy adult bull (courtesy of Dr. William Plummer of the Animal Science Department, California Polytechnic State University, San Luis Obispo, CA).

Gel Preparation

Gels of various compositions (Table I) were prepared by blending CMC and PEO powders in deionized water containing 0.43% (w/v) NaCl and 0.47% (w/v) CaCl$_2 \cdot 2$H$_2$O at room temperature with the use of a heavy-duty blender (Arrow 2000, Arrow Engineering Co., Inc., Hillside, NJ). The polymers are blended to a smooth gel.

Gels thus formed were filtered through a stainless-steel membrane with the pore size of 53 μm (Millipore Corp., Bedford, MA), and loaded into 5-ml polypropylene syringes (Becton Dickinson & Co., Franklin Lakes, NJ). All gels were stored at 4 °C.

For a blood coagulation test, gels were cast on polystyrene petri dishes (100 × 15 mm, Fisher Scientific, Santa Clara, CA). Each dish contained 30 ml of the gel. For the protein-partitioning study, CMC and PEO (mol.wt. 8000) were dissolved in PBS separately. The polymers were mixed together as described in detail below. To study the kinetics of PEO released from the gel, gels were prepared with the use of fluorescein-PEG (mol.wt. 5000). For evaluation of the gel structure, gels were also formulated at a concentration of 6.7% w/v CMC in PBS containing methylene blue (50 ppm) and 0.764% w/v PEO in PBS and mixed as described above. The samples were centrifuged at 3500 rpm for 20 min. Photographs were taken after mixing and centrifugation.

Gel Viscosity

The viscosity of synthesized gels was measured at room temperature with the use of a digital viscometer (Brookfield model DV-II, Brookfield Engineering Laboratory, Inc., Stoughton, MA) at shear rate 0.5 rpm and spindle No. 29.

Gel Turbidity

The turbidity of gels was measured at 580 nm with the use of a UV spectrophotometer (Model 160U, Shimadzu, Japan) after any air bubbles entrapped in the gels were carefully removed by brief centrifugation.

Release of CMC and PEO from Gels

Studies on the release of CMC and PEO from gels were performed with PBS used as the release medium. Membranes of porcine intestine were mounted on the bottoms of petri dishes (diam = 50 mm) with double-sided adhesive tape. Gels were prepared with fluorescein-labeled PEO. An aliquot of 5.0 ml of each gel was evenly spread over the surface of the membrane. PBS (10 ml) was carefully loaded on the top of the gel layer, followed by incubation at room temperature under gentle shaking. At the time periods of 3 min, 10 min, 20 min, 1 h, and 2 h the dish was tipped to one side and 1.0 ml of PBS was pipetted from the solution above the gel and analyzed for the amount of PEO and CMC released.

The released PEO was quantified by measuring the absorbance of the fluorescein moiety attached at the PEG chain at 500 nm. The CMC released into the incubation medium was determined by measuring hexose content, by the method of phenol-sulfuric acid reaction, where the absorbance at 480 nm is measured after the CMC is incubated with phenol and sulfuric acid at 30 °C for 20 min.22

Protein Partitioning

Protein partitioning in an aqueous CMC/PEO system was performed as described by Johansson.23 Proteins tested in the current study were bovine plasma albumin, bovine fibrinogen, and bovine γ globulin. Solutions of 6.7% (w/v) CMC and 0.74% (w/v) PEO (mol.wt. 8000) were prepared separately as described in the paragraph on gel preparation, except that PBS was used as solvent instead of deionized H$_2$O. Proteins were added into the polymer solutions at the end of the blending step at a concentration of 0.01% (w/v). A volume of 45 ml of CMC/protein in PBS was placed in a 100-ml
was then placed on the gel surface, followed by the addition of 0.4 ml of 0.1 M CaCl₂ solution to initiate clotting. A large volume of deionized H₂O was added to the gel to stop the clot formation in 20 min. The thrombus was carefully removed from the gel with a clean, flat-end spatula, and fixed in PBS containing 2% glutaraldehyde for at least 4 h. The weights of clots were recorded after removing them from the fixing solution and removing the surface water by tapping with Kimwipes.

Empty polystyrene petri dishes were used as controls. Each sample was tested four times and the mean weights and standard deviations were calculated.

**Statistical Analysis**

All data are expressed as the mean ± standard deviation. Significance was determined with the use of a student's t test.

**RESULTS**

Gels were prepared by blending CMC with PEO at various ratios in the presence of CaCl₂ in an aqueous solution to form a composite gel. Gels were first characterized for viscosity by determining the resistance to shear force at 0.5 rpm and for turbidity by measuring the optical density at 580 nm. As shown in Table I, gel composed of CMC alone has the highest viscosity; gel prepared from PEO alone has a lower viscosity than that of CMC at the same concentration (% solids, w/v). After mixing CMC with PEO, the viscosities of composite gels varied as the function of PEO content. At the weight ratio of 50/50 of CMC and PEO, the composite gel showed the lowest viscosity.

The turbidity results of CMC/PEO gels as demonstrated by transparency are shown in Figure 1. At a concentration of 3.7% (w/v), gels prepared either from CMC alone or from PEO alone were transparent. However, for CMC/PEO com-
composite gels, the transparency of gels changed as the ratio of the two components changed. The gel composed of equal amounts of CMC and PEO had the highest turbidity. At higher concentrations of polymers, 7.5% (w/v), the transparency was easily seen as shown by photographs in Figure 2. Gels of CMC or PEO were transparent (Figure 2, Tubes 1 and 2); the mixture of CMC and PEO at high concentration was cloudy (Figure 2, Tube 3). Data from above viscosity and turbidity measurement demonstrated the existence of micro phase separation in the CMC/PEG gel system. This phase separation could be further demonstrated by centrifugation of the gel at 3500 rpm for 20 min to separate the system as a CMC phase in the bottom and a PEO phase in the top (Figure 2, Tube 4). In this study, a specific binder of CMC, methylene blue, was added into the system prior to mixing to visualize the CMC. As shown in the photo, there was no color that could be observed in the top phase, indicating that a complete phase separation occurred. Without centrifugation, the CMC/PEO composite gel retained the heterogeneous structure (Figure 2, Tube 3) at the temperature ranging from 4 to 25°C, demonstrating the physical stability of gel when stored in normal conditions.

Because the turbidity experiments indicated a phase separation of CMC and PEO, serum proteins were used to measure their partitioning between the phases with the use of a modified protein partitioning method. Because both phases of CMC and PEO contain more than 90% of buffer by weight, proteins can diffuse freely from one phase to another, driven by the affinity of proteins toward each polymer phase. As indicated by the results summarized in Table II, blood plasma proteins were preferentially partitioned into CMC-rich phase. There was an insignificant difference in the protein concentration observed in the CMC-rich phase among the three proteins, indicating that the CMC/PEG system is comparatively insensitive in the nature of proteins (Table II).

The stability of a composite gel in an open system, such as in contacting with body fluid or incubating with media, was investigated in vitro. An experiment was performed by coating the gel onto a piece of porcine intestine membrane, which was then incubated with the gel in PBS at room temperature. As shown in Figure 3, fluorescein-labeled PEO of molecular weight $5 \times 10^3$ was released from the gel at a much higher rate than that of CMC. The amount of PEO released was measurable after 3 min and increased over 60 min. Within 1 h, 25% of the fluorescein-labeled PEO was released from the gel coated onto the porcine intestine membrane into the PBS ($t_{1/2} = 108$ min). In contrast, there was no CMC in the release medium detected in the first 20 min period of incubation, and more than 97% of CMC was adhered to the tissue surface at the end of experiment (Table III, Figure 3). After 1 h of incubation, the gel was swollen enough to begin to dissolve and release CMC.

![Figure 3](image-url)  
**Figure 3.** Release of PEO and CMC from CMC/PEO gels. The gel was composed of 3.3% (w/v) CMC, mol.wt. $7 \times 10^5$ and 0.34% (w/v) fluorescein-PEO, mol.wt. $5 \times 10^3$. The gel was incubated with PBS in petri dishes covered with membranes of porcine intestine at ambient temperature. The amount of released CMC and PEO was detected by UV spectrometer as described in the Methods section. Data is expressed as mean ± SD (n=3).

![Figure 4](image-url)  
**Figure 4.** Tissue adherence of CMC/PEO gels as a function of the CMC content. Peak force for detachment was measured on a tape loop tester at ambient temperature. The gap between two tested tissues were adjusted to 2 ± 1 mm. Data expressed as mean ± SD (n=5).
that it is nonionic, yet highly soluble in water.\textsuperscript{29} This is due to its unique ethylene oxide monomeric unit, which displays a high degree of polymer solvent interaction in aqueous solutions. Because of the biocompatibility of PEO and its solubility in aqueous solution, polyethylene oxide has been used recently to coat a variety of materials to limit their interaction with proteins.\textsuperscript{30} One hypothesis to explain this property of PEO is that at room temperature, the dipole moment of ethylene oxide is maximal and PEO undergoes extensive hydration in water.\textsuperscript{31} This gives rise to a steric repulsion force between PEO and proteins that increases with the molecular weight of the polymer and the extent of surface coverage with PEO.\textsuperscript{30, 31} Adhesion formation requires the apposition of traumatized tissue with the suppression of fibrinolysis.\textsuperscript{2, 32} Typically, adhesion formation is initiated by the deposition of a fibrin network that bridges the apposed, traumatized tissues.\textsuperscript{2} A local temporary ischemia may contribute to adhesion formation by reducing the availability of fibrinolytic factors.\textsuperscript{32} Because tissue apposition is critical, adhesion prevention has been addressed primarily by developing materials that can be placed between healing tissues to provide a physical barrier to the transfer of proteins or cells between tissues. The properties of antiadhesive materials include their ability to provide a temporary barrier to reduce thrombogenicity and fibrin attachments between apposed tissues, which is recognized as a first step in adhesion formation.\textsuperscript{2} To be effective such barriers need to be present for a short time to prevent the apposed tissues from forming attachments that develop into adhesions; attract macrophages, fibroblasts; and blood vessels; and slowly become organized into scars.\textsuperscript{2} The presence of PEO with the CMC in the composites studied here would be hypothesized to reduce the partitioning of plasma proteins onto the coated tissue and reduce the extent of thrombus formation of whole blood, if present, thereby providing a temporary barrier to the development of adhesions.

PEO and CMC have each individually been used to inhibit adhesion formation. PEO itself has been tested in animals for adhesion prevention.\textsuperscript{18} One difficulty has been how to keep PEO in contact with tissues long enough for it to be effective. One novel approach has been to covalently cross-link the PEO to tissue in situ.\textsuperscript{33} In this case two different, reactive, PEO derivatives are directly administrated on the tissue site, where they react with one another to form a hydrogel that covers the tissue surface.\textsuperscript{15, 33} Polycarbohydrates have been tested as antiadhesion materials. Hyaluronic acid in the form of a gel has been tested\textsuperscript{34} and a polymer of glucose (icodextran) has been tested in animal studies.\textsuperscript{35} CMC has also been used to reduce postsurgical adhesions in several animal models\textsuperscript{11} and as a component in an anti-adhesive membrane consisting of HA and CMC.\textsuperscript{12}

In order to take advantage of the unique properties of both CMC and PEO, composites of these polymers have been developed to reduce postsurgical adhesions. The CMC/PEO composite materials described here have been shown to be effective antiadhesion barriers in several animal studies.\textsuperscript{20, 21}
Several properties of the individual polymers and properties of the composite may be responsible for the effectiveness of such composite gels. As demonstrated in this study, CMC is tissue adhesive and provides a barrier function. PEO inhibits the interaction of proteins with the CMC and functions to limit tissue interaction.

A scheme to explain these effects is presented in Figure 6. In an open system, PEO and the calcium-stabilized CMC form a network of interpenetrating polymers that undergo a microphase separation. In an open system, PEO is released from the gel at a rate faster than that of CMC, resulting in a PEa-rich layer. The PEa-rich outside layer plays a role in inhibition of protein deposition and thrombus formation on the surface of tissues. The CMC-rich layer anchors the gel to the tissue surface, functioning in tissue adhesion. Together CMC and PEO are an effective antiadhesive material because CMC coats and separates traumatized tissue, and PEO inhibits protein-protein interaction and is known to exclude proteins.

REFERENCES


