Radiation-Induced Copolymerization of 2-Hydroxyethyl methacrylate and Polyethylene glycol methacrylate, and Its Protein Adsorption and Bacterial Attachment

Oh Hyun Kwon, Young Chang Nho∗†, and Young Moo Lee

Department of Industrial Chemistry, College of Engineering, Hanyang University, Seoul 133-791, South Korea
∗Radiation Application Division, Korea Atomic Energy Research Institute, Daejeon 305-600, South Korea

Received May 20, 2002; Accepted January 21, 2003

Abstract: In an attempt to produce biomaterial that shows the low levels of adsorption of proteins and adhesion of bacteria, 2-hydroxyethyl methacrylate(HEMA) and polyethylene glycol methacrylate(PEG-MA) co-monomers with ethylene dimethacrylate(EDMA) crosslinker were co-polymerized by using a γ-ray radiation technique. PEG-MA having a different molecular weight of polyethylene oxide was used for synthesizing copolymeric hydrogels for contact lenses. The degree of polymerization and water content of the hydrogels were examined. The protein adsorption on the hydrogel surfaces was investigated by electron spectroscopy for chemical analysis (ESCA). Bacterial attachments were evaluated by examining the adhesion on hydrogel surfaces by microscope and a viable cell counting method against Escherichia coli (E. coli) and Pseudomonas aeruginosa (P. aeruginosa). The amount of adsorption of protein and adhesion of bacteria on sample surface decreased significantly in the copolymer specimen of HEMA and PEG-MA, when comparing with the hydrogel of polyHEMA alone.

Keywords: radiation-induced copolymerization; 2-hydroxyethyl methacrylate; polyethylene glycol methacrylate; hydrogel; protein adsorption; bacterial attachment; biomaterial

Introduction

Hydrogels are used for biomaterials, such as soft contact lenses, artificial corneas and skin, and wound dressings, etc [1-5]. PolyHEMA-based hydrogels have been used in contact lenses for a long time because of the powerful optical properties, particularly transparency among the various plastics [6]. The contact lenses from hydrophilic polymer are softer and more easily accommodated for the eyes than hydrophobic polymer such as polymethylmethacrylate. Although soft contact lenses were accommodated much more comfortably by the wearer than the prior hard contact lenses, they have disadvantageous properties and have not been completely satisfactory. Hydrogel lenses are susceptible to pathological bacteria and fungi on their surfaces [7]. If not regularly cleaned and sterilized, or they are stored in contaminated solutions, pathogens can be easily absorbed by the lens materials due to their flexible, hydrophilic polymer structure. Because of their flexible and hydrophilic properties, proteins and other normal substances in the eye environment can be easily diffused through the lenses with use. Accumulation of such substances in a soft contact lens causes its discoloration and clouding with repeated cleaning and sterilization procedures practiced by the wearer. Therefore, it is important to provide soft contact lens which is resistant to penetration by pathological organism, and the adsorption of proteins and other migratory eye substances to prolong its life and optical effectiveness significantly.

Among the hydrophilic materials, a particularly effective polymer for protein-resistant surfaces appears to be PEO due to its unique solution and surface properties in water [8]. PEO surface has been introduced by physical adsorption of high molecular weight PEO or various PEO-containing block copolymers onto polymeric materials [9]. This process may be a simple and rapid means of
introducing PEO surfaces, if the PEO-containing copolymers can be adsorbed strongly onto the surfaces [10]. However, it is difficult to keep the immobilized polymers to permanently remain on the surface. Another process of introducing a permanent PEO surface is to use covalent binding of PEO or PEO derivatives onto polymeric substrates. However, this method is only possible if the surface has chemically active functional groups which can couple with PEO derivatives. This limits the application of this technique to certain biomaterials. One of the effective methods is the use of radiation to produce PEO groups on the polymer surface. In a previous paper [11], we synthesized PEO-grafted copolymer having low levels of protein adsorption by radiation-induced method. Therefore, we expected that copolymeric hydrogels of HEMA with PEO-MA would show low levels of protein adsorption and bacterial attachment.

Recently, soft contact lenses are widely produced on the base of polyHEMA. There are a great number of various hydrogel compositions, which contain compounds such as poly(vinyl pyrrolidone), poly(vinyl alcohol), poly(methacrylic acid), chitosan [12,13], and so on. Copolymerization is mainly used to improve mechanical properties of polyHEMA, the affinity for water, or oxygen permeability [14].

Polymerization using radiation is one of the useful hydrogel syntheses because of the uniform and rapid formation of the active radical sites in monomers without catalytic contamination. Although radiation-initiated copolymerization of HEMA with various compounds has been studied, the study on improving biocompatibilities such as protein adsorption and bacterial attachment of polyHEMA based-hydrogels for contact lenses is still not reported.

In this work, we synthesized hydrogels in different HEMA/PEG-MA ratios using radiation-induced method, with PEG-MA having a different molecular weight of PEO, and we examined the adsorption of protein and attachment of bacteria on the prepared hydrogels by ESCA and scanning electron microscopy(SEM), respectively.

Materials and Methods

Materials
HEMA was supplied from Junsei Chemical Co., Ltd. (Japan) and purified by passing it through a column packed with aluminium oxide(Aldrich Chemical Co., USA). PEG-MA(Nippon Oil & Fats Co. Ltd., Japan) with different PEO repeating unit was used without further treatment. EDMA(Polysciences, Inc., USA) was used as a crosslinking agent after purification by the same process as HEMA. Bovine serum albumin as model protein was supplied from Sigma Chemical Co.(USA). All other chemicals were reagent grade or with higher purity. The type of PEG-MA used in this experiment is shown in Table 1.

Irradiation
The γ-rays irradiation from Co-60 sources was carried out at an exposure rate of 2.02 kGy/h in the presence of air to a total dose of 2 - 20 kGy.

Polymerization Procedure
The polymerization experiments were conducted in two polypropylene(PP) plate moulds with a silicon sheet spacer having 30 × 45 mm inner dimensions and 2 mm thickness. The prepared PP moulds were degassed by bubbling nitrogen for 10 min. The pre-bubbled mixtures of HEMA and PEG-MA with a different molecular weight of PEO as well as HEMA only were injected to PP moulds with crosslinker by using a syringe. Then, the monomer solution in the PP mould was degassed for 10 min and sealed. The irradiation was carried out by placing the PP moulds including the monomer solutions with various composition at the front of γ-rays from Co-60 sources, at room temperature. After irradiation, the polymerized bulk polymers were quickly taken out of the PP moulds and soaked in a distilled water to remove the unreacted remaining monomers. After washing at 80°C for 24 h, purified polymeric hydrogels were obtained, and polished.

Measurement of Water content
The water content of the hydrogels which was synthesized from various HEMA/PEG-MA compositions was defined as the weight percent of water in the water-saturated hydrogels. The water content was determined as follows: Each hydrogel was soaked in water to water-saturation at room temperature and blotted between pieces of cellulose based-filter papers, and then weighed on a chemical balance. To obtain the exact data, the above experiment was repeated 3 times for each sample. The hydrogels then were dried to a constant

<table>
<thead>
<tr>
<th>Table 1. The Type of PEG-MA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbreviation</td>
</tr>
<tr>
<td>Ethylene Oxide No.</td>
</tr>
<tr>
<td>Molecular Weight</td>
</tr>
</tbody>
</table>
weight under vacuum at 60°C for 24 h above.

**Identification of Synthesized Polymers**

The synthesized polymers of HEMA/PEG-MA and HEMA were verified by ESCA. ESCA for examining the polymers was carried out with a V. G. SCIENTIFIC ESCALAB MK II spectrometer (UK) using monochromatized Al Kα X-ray radiation at 1486.6 eV operating at 10⁻⁹ mbar and 300 watt power at the anode, and photoelectron takeoff angle of 60°. High-resolution spectra for the carbon-1s and oxygen-1s were also obtained and computer curve-fitted employing a Gaussian model, using the same package software, to obtain the best binding energy values. To take into account some shift caused by charging of the sample surface, all spectra were adjusted by taking the carbon-1s peak at 284.6 eV as reference for the adventitious carbon contamination.

**Measurement of Tensile Strength**

The tensile mechanical strength of polyHEMA and poly(HEMA-co-PEG-MA) with the various HEMA/PEG-MA compositions was measured using a universal testing machine (INSTRON, Model 1130, USA), Tensilon CFT-200 equipped with pneumatic grips at room temperature. The dumb-bell-shaped specimens for testing strength were prepared by using a metal cutting die. All the specimens for tensile testing were measured after blotting between pieces of cellulose based-filter papers. The measurement was carried out at a crosshead speed of 100 mm/min and at a gauge length of 50 mm. For the exact data, the tensile testing of all the specimens was measured 5 times for each sample and averaged. From these experiments, tensile strength and elongation of all prepared samples were collected.

**Protein Adsorption**

Bovine serum albumin (Sigma Chemical Co., St. Louis, MO, USA) was diluted with a phosphate buffered solution (PBS, pH 7.4) to make a 1 w/v % solution. The polymers prepared under different conditions were hydrated in PBS 5 times at 37°C, and were placed in contact with the above protein solution in 24 polystyrene wall multi-vials (Multiwell™ Tissue Culture plate, Falcon 3047) at the same temperature for 1 h. The samples were washed with PBS, after incubation, and then washed with purified water to remove unabsorbed proteins. After vacuum drying for 24 h, the changes in protein adsorption of the polymer surfaces were investigated by ESCA. The changes in the nitrogen 1s peaks from the X-ray photoelectron spectroscopy survey scan spectra were examined as described above.

**Assessment of Bacterial Attachment**

Bacterial attachment was evaluated by microscopic examination on polymer surfaces and viable cell counting method against *E. coli* and *P. aeruginosa*. One loopful of the bacteria was inoculated in 150 mL of a nutrient broth (peptone 5.0 g/L, beef extract 3.0 g/L, pH 6.8) at 36°C for 24 h, and 1 mL from the former bacteria solution was cultured again in the nutrient broth at 36°C for 20 h in a test-tube shaker at 100 rpm. At this stage, the culture of *E. coli* and *P. aeruginosa* involves ca. 10⁷ and 10⁶ cells/mL, respectively. In this experiment, the used bacterial solution was ca. 10⁵ cells/mL. The polymers prepared under different conditions were cut into 1 × 1.5 × 0.2 cm pieces. The polymers hydrated in PBS 5 times at 37°C were immersed in 5 mL of solution for *E. coli* and *P. aeruginosa* to assess their bacterial attachment on the sample surfaces. After contacting at 36°C for 1 h in a test-tube shaker at 100 rpm, the polymer samples were quickly taken out of the bacterial solution. 1 mL of bacterial solution was added to 9 mL of distilled water, and several decimal dilution was repeated. From this diluted solution, the bacteria which did not attach themselves to the polymer samples, were counted by the spread plate method. After inoculation, the plates were kept at 36°C for 24 h, and the colonies were counted by the viable cell counting method as described before [15].

**Microscopic Examination of Bacteria**

The attached bacteria on the samples that remained adhered to the polyHEMA and poly(HEMA-co-PEG-MA) surfaces were fixed with a 2.5% glutaraldehyde in PBS for 10 min at room temperature. The bacteria fixed on the surfaces were dehydrated in an ethanol-grade series (50, 60, 70, 80, 90, and 100%) for 10 min after each was washed with PBS, and then allowed to dry on a clean hood at room temperature. The bacteria attached to the samples were examined by SEM (JSM-840A, JEOL Co., Japan) with a tilt angle of 45° at an accelerating voltage of 20 kV after gold deposition in a vacuum, as described before [16].

**Results and Discussion**

There have been various efforts to minimize or eliminate protein adsorption. Surfaces which show minimal protein adsorption may be important in many applications, not only for blood-contacting implant devices but also for other areas, such as biomembranes, biosensitive sensors, and contact lenses, etc [8].

Figure 1 shows the effect of the absorbed dose on the polymerization of HEMA in the presence of 0.8% (v/v) EDMCA crosslinker. As shown in Figure 1, the polymerization yield of polyHEMA in the presence of EDMCA increased rapidly as the absorbed dose increased up to 6 kGy, and then levelled off.
The effect of the EDMA crosslinker content on the HEMA polymerization at irradiation dose of 10 kGy was shown in Figure 2. The polymerization yield increased with an increased EDMA content, and then slightly decreased. The crosslinker appears to have a dual function, namely to enhance the copolymerization and also crosslink the PHEMA chains. During the polymerization HEMA in the presence of difunctional monomer, EDMA, one end of DEMA is bonded to the growing chain of HEMA while the other end is unsaturated and is free to initiate new chain growth. In principle this would increase the the yield of polymerization. However, it seems that when more than 10% crosslinker is mixed with HEMA, the shortage of compatibility between two components may lead to decrease the degree of polymerization.

The PEG-MA content and PEO chain length are one of the important factors controlling the biocompatibility on polymer surfaces. Therefore, we performed the copolymerization of HEMA and PEG-MA including various PEO chains. Figure 3 shows the effect of feed composition and the molecular weight of PEO on the degree of copolymerization of HEMA and PEG-MA with different PEO molecular weight in the presence of 1.0 %(v/v) EDMA crosslinker. As shown in Figure 3, the polymerization yield decreased as the PEG-MA content increased, and the high molecular weight of PEO in PEG-MA was favorable to the polymerization yield. It is interpreted in our cases that the high degree of polymerization in the long PEO chain is caused from molecular entanglement effect of the long PEO chain.

Figure 4 shows the water content of the samples prepared with HEMA and PEG-MA with the different PEO molecular weights when different compositions of HEMA and PEG-MA were copolymerized in the presence of 1.0% crosslinker. As shown in Figure 4, the water content increased with an increased PEG-MA content in comonomer as well as an increase in the molecular weight of PEO.

For the mechanical properties of the prepared polymer samples, the relation of the tensile strength with the
**Figure 4.** Effect of PEG-MA content in HEMA on the water absorption of polymer with different PEO molecular weights. EDMA crosslinker content 1.0%(v/v), absorbed dose 10 kGy.

**Figure 5.** The tensile strength and elongation percent at peak of poly (HEMA - co - PEO4/5-MA).

different HEMA/PEG-MA composition was examined, the result was shown in Figure 5. As shown in Figure 5, the tensile strength and elongation percent decreased with an increased PEG-MA content up to 75/25 v% of HEMA/PEG-MA composition and then levelled off, respectively.

The ESCA survey scan spectrum and carbon 1s core level spectra of polyHEMA and poly(HEMA-co-PEG-MA) samples are shown in Figure 6 and 7, respectively. As shown in Figure 6, the ESCA survey scan spectrum of poly(HEMA-co-PEG-MA) surface containing several PEO groups showed no significant differences from that of the polyHEMA sample containing unique PEO group. It may be because the PEO group of HEMA overlaps with that of the PEG-MA. Therefore, the carbon 1s core level spectra of samples prepared were investigated by using ESCA (Figure 7). The carbon 1s spectra were resolved into four characteristic peaks. The peaks at 288.6, 287.3, 286.3, and 284.6 eV on the surfaces of the samples indicate the functional groups of COO, C=O, C-O, and C-C, respectively. The peak at 286.3 eV of poly(HEMA-co-PEG-MA) increased with respect to polyHEMA.

The hydrophilicity and unique solubility properties of PEO produce surfaces that are in a liquid-like state with the polymer chains exhibiting considerable flexibility or mobility. PEO is the most flexible in water among common nonionic water-soluble polymers because it does not have bulky side groups in its structure and thus will not be hindered sterically in water. PEO surfaces in water with rapidly moving hydrated PEO chains and a large excluded volume tend to repel protein molecules which approach the surface. Bovine serum albumin was adsorbed onto polyHEMA and HEMA/PEG-MA copolymers with the various comonomer composition, and relative adsorbed amounts of proteins on the sample.
surfaces were evaluated by ESCA. The nitrogen peak from the peptide bonds was used as an indicator of surface protein adsorption. Figure 8 shows an ESCA survey scan spectra of samples prepared after protein adsorption. The nitrogen peak from the polyHEMA surface was much higher than poly(HEMA-co-PEG-MA). In the case of poly(HEMA-co-PEG-MA), the nitrogen peaks decreased with an increasing PEG-MA content.

Persistent infections associated with implant devices are a major clinical problem. These infections persist despite the use of antibiotics, thereby limiting the usefulness of many prosthetic devices. Bacterial infections are a significant problem because they frequently cannot be eradicated without surgical revision or implant removal, and infections can result in morbidity, amputation, or death [18]. The initial event in the development of such infections is the adhesion of bacteria to the implant surface, and therefore materials that are less adherent to bacteria would be preferred. Thus, we performed studies on bacterial attachment for the prepared samples.

Figure 9 shows the results of the bacterial adhesion to polyHEMA and poly(HEMA-co-PEG-MA) with the different PEG-MA content. The amount of *E. coli* and *P. aeruginosa* adhering to samples decreased significantly
in the presence of PEG-MA. The decrease in the bacterial adhesion on the prepared samples can be interpreted on the basis of non-fouling polymer surfaces of PEG-MA. Hoffman and coworkers [19] explained in their paper that the PEG-MA macromer surfaces has been shown to be resistant to fouling by attached cells. It may be explained that the poly(HEMA-co-PEG-MA) sample surface includes low interfacial free energy, hydrophilicity, high surface mobility and steric stabilization effects of PEG-MA. Also, the gram-negative bacteria, *E. coli* and *P. aeruginosa*, have very different motility. *E. coli* is motile by peritrichous flagella or nonmotile, where *P. aeruginosa* is motile by one or several polar flagella, that is, they are rarely nonmotile. Therefore, *E. coli* adhered much more than *P. aeruginosa* on sample surfaces. The adherene of *E. coli* and *P. aeruginosa* decreased 50% and 75%, respectively on the copolymer that was synthesized from 15/85 v% of HEMA/PEO4/5-MA, comparing with PHEMA.

Figure 10 shows the SEM photographs of samples prepared with different HEMA/PEG-MA compositions, on which *E. coli* was inoculated on and incubated at 37°C for 1 h. As shown in plates, the attached bacteria decreased significantly with an increasing PEG-MA macromer content.

**Conclusions**

In an attempt to produce biomaterial that shows low levels of adsorption of proteins and adhesion of bacteria, HEMA and PEG-MA were co-polymerized by using γ-ray radiation technique. The polymerization yield of polyHEMA in the presence of EDMA as a crosslinker increased rapidly as the absorbed dose increased up to 6 kGy, and then levelled off. The copolymerization yield decreased as the PEG-MA content in the mixture of HEMA and PEG-MA increased. The amount of *E. coli* and *P. aeruginosa* adhering to samples decreased significantly in the presence of PEG-MA. The adherene of *E. coli* and *P. aeruginosa* decreased 50% and 75%, respectively on the copolymer that was synthesized from 15/85 v% of HEMA/PEG-MA, comparing with PHEMA.

The appearance of the bacterial adhesion on the prepared samples can be interpreted on the basis of non-fouling polymer surfaces of PEG-MA. The amount of adsorption of protein on sample surfaces also decreased significantly in copolymer of HEMA and PEG-MA, comparing hydrogel of polyHEMA alone.

**Acknowledgement**

This project has been carried out under the Nuclear R&D Program by MOST.

**References**