Materials Science and Engineering C 33 (2013) 3704–3710

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Effect of chemical composition on corneal cellular response to photopolymerized materials comprising 2-hydroxyethyl methacrylate and acrylic acid

Jui-Yang Lai *

Institute of Biochemical and Biomedical Engineering, Chang Gung University, Taoyuan 33302, Taiwan
Biomedical Engineering Research Center, Chang Gung University, Taoyuan 33302, Taiwan
Molecular Medicine Research Center, Chang Gung University, Taoyuan 33302, Taiwan

ARTICLE INFO

Article history:
Received 7 March 2013
Received in revised form 23 April 2013
Accepted 29 April 2013
Available online 7 May 2013

Keywords:
Photopolymerized materials
2-hydroxyethyl methacrylate
Acrylic acid
Chemical composition
Corneal cellular response

ABSTRACT

Characterization of corneal cellular response to hydrogel materials is an important issue in ophthalmic applications. In this study, we aimed to investigate the relationship between the feed composition of 2-hydroxyethyl methacrylate (HEMA)/acrylic acid (AAc) and material compatibility towards corneal stromal and endothelial cells. The monomer solutions of HEMA and AAc were mixed at varying volume ratios of 92:0, 87:5, 82:10, 77:15, and 72:20, and were subjected to UV irradiation. Results of electrokinetic measurements showed that an increase in absolute zeta potential of photopolymerized membranes is observed with increasing the volume ratios of AAc/HEMA. Following 4 days of incubation with various hydrogels, the primary rabbit corneal stromal and endothelial cell cultures were examined for viability, proliferation, and pro-inflammatory gene expression. The samples prepared from the solution mixture containing 0–10 vol.% AAc displayed good cytocompatibility. However, with increasing volume ratio of AAc and HEMA from 15:77 to 20:72, the decreased viability, inhibited proliferation, and stimulated inflammation were noted in both cell types, probably due to the stronger charge–charge interactions. On the other hand, the ionic pump function of corneal endothelial cells exposed to photopolymerized membranes was examined by analyzing the Na^+,K^+-ATPase alpha 1 subunit (ATP1A1) expression level. The presence of material samples having higher anionic charge density (i.e., zeta potential of −38 to −56 mV) may lead to abnormal transmembrane transport. It is concluded that the chemical composition of HEMA/AAc has an important influence on the corneal stromal and endothelial cell responses to polymeric biomaterials.

1. Introduction

Hydrogels have been known to be composed of hydrophilic polymer networks, which are formed via physical or chemical interactions [1]. While the networks of physical gels are held together by molecular entanglements and secondary forces, the networks observed in chemical gels are cross-linked by covalent bonds [2]. Hydrogel biomaterials have gained increasing interest due to their great potential in various ophthalmic applications, such as drug delivery [3], regenerative medicine [4], wound repair [5], and tissue replacement [6]. In our laboratory, the gelatin was modified with poly(*N*-isopropylacrylamide) to develop in situ forming hydrogels for intracameral delivery of antiglaucoma medications [7]. These materials were also used to fabricate thermo-responsive and biodegradable hydrogel carriers for corneal endothelial cell sheet engineering [8,9]. More recently, functional copolymer hydrogels based on 2-hydroxyethyl methacrylate (HEMA) and acrylic acid (AAc) were designed for applications in anterior lamellar keratoplasty to eliminate the risk of corneal endothelial rejection associated with penetrating keratoplasty [10]. Other keratoprosthetic biomaterials such as poly(methyl methacrylate) (PMMA) [11] and polydimethylsiloxane (PDMS) [12] have also received much attention due to their excellent optical properties. However, as compared to the rigid PMMA or hydrophobic PDMS, PHEMA is more advantageous because it contains more hydroxyl groups to interact with water that facilitates mass transport through the keratoprosthetic hydrogels. Copolymerization of HEMA with AAc may further modify the properties of hydrogel implant, making it promising for future artificial cornea [10].

From the viewpoint of biomedical engineering, the hydrogels have high oxygen and nutrient permeability, which may facilitate cell growth and survival. In 2008, Wu et al. synthesized the hydrogels containing pH-sensitive PAAc chains and biodegradable acryloyl-poly(caprolactone)-HEMA chains and demonstrated that the materials with a lower cross-linking density and a larger pore size exhibit a better performance for cell migration [13]. The findings suggest that the three-dimensional network structure of the hydrogel biomaterials strongly depends on the feed composition of acryloyl-poly(caprolactone)-HEMA/AAc. Later, Fujimoto et al. prepared a

* Corresponding author at: Institute of Biochemical and Biomedical Engineering, Chang Gung University, Taoyuan 33302, Taiwan. Tel.: +886 3 211 8800x3598; fax: +886 3 211 8668.
E-mail address: jylai@mail.cgu.edu.tw.

0928-4931/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.msec.2013.04.059

biodegradable, thermo-responsive hydrogel based on copolymerization of *N*-isopropylacrylamide, AAc, and HEMA-poly(trimethylene carbonate) for the treatment of ischemic cardiomyopathy [14]. The injection of hydrogel at the optimal monomer feed ratio into the rat infarcted myocardium may preserve left ventricular wall thickness and capillary density. According to our earlier observations, the monomer composition is also critical for regulating the physicochemical and biological characteristics of the photopolymerized hydrogel biomaterials [10]. While the introduction of PAAc into the structure of PHEMA can enhance the water content and oxygen permeability, it simultaneously reduces mechanical and suture strength as well as resists protein adsorption and cell adhesion.

Although the surface functionalization of keratoprosthesis with biomolecules promotes the formation of an intact layer of epithelium over the hydrogel device, the biomaterial itself should possess excellent biocompatibility [4]. Given that the hydrogel implants are intended to stay in the cornea, it is essential to examine the cellular responses to the poly(HEMA-*co*-AAc) membranes. After incubation with rabbit corneal epithelial cell line cultures, the samples synthesized in the presence of 10% AAc display good cell viability without obvious cytotoxicity and inflammation [10]. However, the compatibility of these hydrogels with other corneal cells (i.e., stromal and endothelial cells) is yet to be tested. Therefore, the present study aimed to investigate the relationship between the feed composition of HEMA/AAc and in vitro corneal biocompatibility. In the presence of the same amount of initiator (i.e., 4 vol.% 2,2-diethoxyacetophenone (DEAP)) and cross-linker (i.e., 4 vol.% trimethylolpropane trimethacrylate (TMPTMA)), the monomer solutions of HEMA and AAc were mixed at varying volume ratios of 92:0, 87:5, 82:10, 77:15, and 72:20, and were respectively subjected to UV irradiation for 30 min. The surface charge of the resulting poly(HEMA-*co*-AAc) membranes was determined by electrokinetic measurements. Following 4 days of exposure to various hydrogel samples, the primary rabbit corneal stromal and endothelial cell cultures were examined for viability, proliferation, and pro-inflammatory gene expression. In particular, to explore the possible role played by material components in the ionic pump function of corneal endothelial cells, the gene expression of membrane transport protein such as Na^+,K^+-ATPase alpha 1 subunit (ATP1A1) was analyzed by using quantitative real-time reverse transcription polymerase chain reaction (RT-PCR).

2. Materials and methods

2.1. Materials

2-Hydroxyethyl methacrylate (HEMA) supplied from Alfa Aesar (Ward Hill, MA, USA) was purified by distillation under reduced pressure prior to use. Acrylic acid (AAc) was purchased from Merck (Whitehouse Station, NJ, USA) and also distilled under reduced pressure. 2,2,-Diethoxyacetophenone (DEAP) and trimethylolpropane trimethacrylate (TMPTMA) were obtained from Sigma-Aldrich (St. Louis, MO, USA) and used as commercially supplied. Phosphate-buffered saline (PBS, pH 7.4) was acquired from Biochrom AG (Berlin, Germany). Collagenase type II was purchased from Worthington Biochemical (Lakewood, NJ, USA). Dispase type II was obtained from Roche Diagnostics (Indianapolis, IN, USA). Medium 199 (M199), gentamicin, Hanks' balanced salt solution (HBSS, pH 7.4), 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F-12 medium (DMEM/F12), trypsin-ethylenediaminetetraacetic acid (EDTA), and TRIzol reagent were purchased from Gibco-BRL (Grand Island, NY, USA). Fetal bovine serum (FBS) and the antibiotic/antimycotic (A/A) solution (10,000 U/mL penicillin, 10 mg/mL streptomycin and 25 μg/mL amphotericin B) were obtained from Biological Industries (Kibbutz Beit Haemek, Israel). All the other chemicals were of reagent grade and used as received without further purification.

2.2. Preparation of poly(HEMA-co-AAc) hydrogel membranes

The poly(HEMA-*co*-AAc) hydrogels were synthesized by photopolymerization according to the previously published method [10]. In the presence of 4 vol.% DEAP and 4 vol.% TMPTMA, the monomer solutions of HEMA and AAc were mixed at varying volume ratios of 92:0, 87:5, 82:10, 77:15, and 72:20. The solution mixture was then injected between two glass slides separated by a thick 200 μm spacer, and subjected to irradiation using Blak-Ray 160 W model B-100SP high intensity UV lamp (UVP, Upland, CA, USA) with 365 nm. Samples were placed at a distance of 5 cm (light intensity: 11.6 mW/cm^2) from the radiation source and exposed for 30 min. The resulting copolymer membranes were immersed in 95% ethanol overnight to remove unreacted monomers, initiators, and cross-linkers. After a further wash in double-distilled water for 24 h, the samples were dried in vacuo for 48 h. In this study, the hydrogel materials prepared from the solution mixture of HEMA and AAc (82:10, v/v) were designated as H82A10.

2.3. Zeta potential measurements

To determine the surface charge, the zeta potential of various test samples was quantified with the streaming potential method [15]. An electrokinetic analyzer (EKA) (BI-EKA; Anton Paar, Austria) located at the Center for Emerging Material and Advanced Devices at National Taiwan University (Taipei, Taiwan, ROC) was utilized to measure the streaming current. Prior to testing, the poly(HEMA-*co*-AAc) membranes were equilibrated in 1 mM KCl for 2 h. Zeta potential measurements were performed using 1 mM KCl as electrolyte solution. The pH of the solution was adjusted to 7.4 by the addition of 0.1 M KOH. Results were averaged on four independent runs.

2.4. Rabbit corneal stromal and endothelial cell cultures

All animal procedures were approved by the Institutional Review Board and were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Twenty adult New Zealand white rabbits (National Laboratory Animal Breeding and Research Center, Taipei, Taiwan, ROC) weighing 2.5–3.0 kg were used for this study. Primary rabbit corneal stromal and endothelial cells were prepared according to previously published methods [16,17].

Briefly, for the isolation of cells, each cornea was exposed to M199 and 50 μg/mL of gentamicin. Under a dissecting microscope (Leica, Wetzlar, Germany), Descemet's membrane with the attached endothelium was aseptically stripped from the stroma and washed three times with PBS. The Descemet's membrane-corneal endothelium complex was digested using 2 mg/mL collagenase in HBSS for 1 h at 37 °C. Thereafter, the endothelial cells were collected and resuspended in regular culture medium containing M199 as a basal medium, 10% FBS, and 1% A/A solution. The remaining stroma with epithelium was incubated in 5 mg/mL of dispase at 4 °C overnight. Loose epithelial sheets were removed, and stromal discs were cut into small pieces and digested using 4 mg/mL collagenase for 24 h at 37 °C. The stromal cells were mechanically dissociated into single cells and maintained in regular culture medium consisting of DMEM/F12, 10% FBS, and 1% A/A solution. Both cultures were incubated in a humidified atmosphere of 5% CO_2 at 37 °C. Medium was changed every other day. Confluent cell layers were subcultured by treating with trypsin-EDTA and seeded at a 1:4 split ratio. In all experiments, third-passage corneal stromal cells and second-passage corneal endothelial cells were utilized.

2.5. In vitro biocompatibility studies

The in vitro biocompatibility evaluation of the hydrogel membranes was conducted according to the methodology described previously