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

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ABSTRACT

The purpose of this work was to investigate the relationship between the feed composition of 2-hydroxyethyl methacrylate (HEMA)/acrylic acid (AAc) and hydrogel material compatibility towards ocular anterior segment tissues, particularly the corneal endothelium. 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. Then, the 7-mm-diameter membrane implants made from photopolymerized materials were placed into the ocular anterior chamber for 4 days and assessed by biomicroscopic examinations, corneal thickness measurements, and quantitative real-time reverse transcription polymerase chain reaction analyses. The poly(HEMA-*co*-AAc) implants prepared from the solution mixture containing 0–10 vol.% AAc displayed good biocompatibility. However, with increasing volume ratio of AAc and HEMA from 15:77 to 20:72, the enhanced inflammatory response, decreased endothelial cell density, and increased ocular score and corneal thickness were observed, probably due to the influence of surface charge of copolymer membranes. On the other hand, the ionic pump function of corneal endothelium exposed to photopolymerized membranes was examined by analyzing the Na^+,K^+-ATPase alpha 1 subunit (ATP1A1) expression level. The presence of the implants having higher amount of AAc incorporated in the copolymers (i.e., 15.1 to 24.7 μmol) and zeta potential (i.e., -38.6 to −56.5 mV) may lead to abnormal transmembrane transport. It is concluded that the chemical composition of HEMA/AAc has an important influence on the corneal tissue responses to polymeric biomaterials.

1. Introduction

In the field of ophthalmology, 2-hydroxyethyl methacrylate (HEMA) is one of the most important synthetic materials. It is a neutral hydrophilic monomer containing hydroxyl groups, which can interact with water molecules through hydrogen bonding. Since 1960, poly(HEMA) hydrogels have been extensively investigated for applications as contact lenses and intraocular lenses [1]. In 2001, Chirila showed an overview of the development of HEMA-based keratoprosthesis for implantation and replacement of damaged regions of the cornea [2]. Later, Frucht-Pery et al. evaluated the efficacy of iontophoresis using soft disposable gentamicin-loaded HEMA hydrogel discs for the management of experimental *Pseudomonas keratitis* in the rabbit eyes and found that this method has a potential clinical value in treating corneal infections [3]. A study from Rogers et al. demonstrated that uniform slow-release pellets prepared by mixing angiogenic growth factors with sucralfate and poly(HEMA) could be used for mouse corneal micropocket angiogenesis assays [4]. Recently, Ciolino et al. designed an econazole-eluting contact lens by encapsulating drug-impregnated poly(lactic acid-co-glycolic acid) films in poly(HEMA) for the treatment of fungal infectious diseases [5].

Acrylic acid (AAc) is another key component that has attracted considerable attention as a promising ophthalmic biomaterial. A study from Lehr et al. reported that the polycarbophil (i.e., a mucoadhesive polymer of the poly(AAc) type) could improve the ocular delivery of topically applied gentamicin by increasing drug uptake in the bulbar conjunctiva and enhancing drug penetration into the aqueous humor [6]. In 2008, Ma et al. showed that the ophthalmic drug delivery systems composed of Pluronic F127-*g*-poly(AAc) copolymers were able to prolong the precorneal residence time and improve the bioavailability of the gatifloxacin [7]. More recently, Hui et al. established a molecular imprinting strategy to deliver the ciprofloxacin by using the AAc as the functional monomer [8]. On the other hand, González-Chomón et al. proposed the use of antifouling foldable acrylic intraocular lenses for controlled release of norfloxacin [9]. In order to obtain the thermally detached corneal endothelial cell sheets, the AAc was used to modify the surface of tissue culture plates for further fabrication of thermoresponsive poly(*N*-isopropylacrylamide) coatings [10]. As an alternative to eliminate the risk of corneal endothelial rejection associated with

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penetrating keratoplasty, the design of functional HEMA/AAc copolymer hydrogels was also studied with the aim of application in the anterior lamellar keratoplasty [11]. Our findings suggest that the incorporation of AAc into the polymer network can enhance the water content and oxygen permeability and simultaneously reduce mechanical and suture strength as well as resist protein adsorption and cell adhesion.

Biocompatibility is a prerequisite for the development of potential keratoprosthetic biomaterials [12]. Therefore, it is essential to examine the corneal tissue responses to the poly(HEMA-*co*-AAc) membrane implants. As mentioned in our previous publication [11], the samples synthesized in the presence of 10% AAc display good tissue compatibility without obvious toxicity and inflammation after exposure to the rabbit cornea. However, the ocular biocompatibility of photopolymerized HEMA/AAc hydrogels prepared from different monomer compositions is yet to be tested. More recently, we demonstrated that the chemical composition is critical for regulating the biocompatibility of magnetic thermoresponsive nanohydrogel particles [13]. Based on these earlier findings, this study aimed to examine the relationship between the feed composition of HEMA/AAc and in vivo 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 resulting poly(HEMA-*co*-AAc) samples were cut out to obtain 7-mm-diameter membrane implants. The photopolymerized material implants were placed into the ocular anterior chamber for 4 days and assessed by biomicroscopic examinations, corneal thickness measurements, and pro-inflammatory gene expression studies. In particular, to explore the possible role played by material components in the ionic pump function of corneal endothelium, 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). TRIzol reagent was obtained from Gibco-BRL (Grand Island, NY, USA). 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 [11]. Briefly, 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. After UV irradiation with a light intensity of 11.6 mW/cm^2 for 30 min, the resulting copolymer membranes were immersed in 95% ethanol overnight and washed in double-distilled water for a further 24 h. Using a 7-mm-diameter corneal trephine device, the hydrogel sheets were cut out to obtain membrane implants (~200 μm in thickness). In this study, the hydrogel materials prepared from the solution mixture of HEMA and AAc (82:10, v/v) were designated as H82A10.

The carboxylic acid groups in the poly(HEMA-*co*-AAc) hydrogels were determined by toluidine blue O (TBO) staining method [14]. Each hydrogel membrane (~38.5 mm^2 in area) was immersed in an aqueous solution of 5×10^{-4} M TBO (adjusted to pH 10 with NaOH) for 2 h at room temperature in order to form ionic complexes between the carboxylic acid groups of the AAc chains and cationic dye. Following rinsing with NaOH to remove unbound TBO molecules, the test samples were desorbed with 50 wt.% acetic acid solution. The uptake amounts of TBO were quantified by measuring the absorbance at 633 nm using a UV–visible spectrophotometer (Thermo Scientific, Waltham, MA, USA), and referenced to a standard curve of TBO over a range of known concentrations. The total amount of AAc incorporated in the copolymers was calculated from the optical absorbance of the solution, assuming that 1 mol of TBO had complexed with 1 mol of the carboxylic acid group of AAc. Results were averaged on five independent runs.

2.3. Animals

The in vivo biocompatibility evaluation of the hydrogel implants was conducted according to the methodology described previously [15,16]. All animal procedures were approved by the Institutional Review Board and were performed in accordance with the ARVO (Association for Research in Vision and Ophthalmology) Statement for the Use of Animals in Ophthalmic and Vision Research. Thirty-six adult New Zealand white rabbits (National Laboratory Animal Breeding and Research Center), weighing from 3.0 to 3.5 kg and 16–20 weeks of age, were used for this study. Animals were healthy and free of clinically observable ocular surface disease. Surgical operation was performed in the single eye of animals, with the normal fellow eye. In the five test groups (H92A00, H87A05, H82A10, H77A15, and H72A20) of animals (six rabbits/group), the material implants were inserted in the anterior chamber of the eye. The remaining six rabbits received no implant (only corneal/limbal incision) and served as a control group (Ctrl).

2.4. Surgery

The rabbits were anesthetized intramuscularly with 2.5 mg/kg body weight of tiletamine hydrochloride/zolazepam hydrochloride mixture (Zoletil; Virbac, Carros, France) and 1 mg/kg body weight of xylazine hydrochloride (Rompun; Bayer, Leverkusen, Germany), and topically with two drops of 0.5% proparacaine hydrochloride ophthalmic solution (Alcaine; Alcon-Couvreur, Puurs, Belgium). After disinfection and sterile draping of the operation site, the pupil was dilated with one drop of 1% atropine sulfate ophthalmic solution (Oasis, Taipei, Taiwan, ROC), and a lid speculum was placed. Under the surgical microscope (Carl Zeiss, Oberkochen, Germany), the cornea was penetrated near the limbus by using a slit knife. Then, the corneal/limbal incision was enlarged to 7.5 mm with corneal scissors to allow the insertion of an implant in the anterior chamber [17,18]. The incision site was finally closed with 10–0 nylon sutures.

2.5. Biomicroscopic examinations

To determine the implant–tissue interaction, the rabbits were anesthetized under the same conditions as for surgery. Ophthalmic evaluations were performed before and 4 days after surgical insertion of material implants. The morphology of anterior segment of the eye including corneal and lens clarity, the degree of anterior chamber activity, iris, and implants was analyzed by gross photography (Nikon, Melville, NY, USA) and slit-lamp biomicroscopy (Topcon Optical, Tokyo, Japan) [19,20].

The ocular grading method used for biomicroscopic examinations is shown in Table 1. During clinical assessment, six parameters were recorded from the rabbit eyes and were numerically graded on an increasing severity scale of 0–4. The means of the ocular scores for each parameter were quantitatively calculated to be the sum of the scores