Biomaterials 89 (2016) 148–156

Contents lists available at ScienceDirect

Biomaterials

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

A novel capsular tension ring as local sustained-release carrier for preventing posterior capsule opacification

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ARTICLE INFO

Article history:
Received 30 November 2015
Received in revised form
19 February 2016
Accepted 23 February 2016
Available online 26 February 2016

Keywords:
Lens epithelial cells
Capsular tension ring
High internal phase emulsion
Posterior capsule opacification
Docetaxel

ABSTRACT

One of the most important and challenging goals in pharmaceutical prevention for posterior capsule opacification is to preserve an effective drug concentration in capsular bag for a long period without affecting the patients' vision. Here, a novel kind of composite, which was prepared by 2-hydroxyethyl methacrylate (HEMA) and methyl methacrylate (MMA) via a two-step process, was applied for capsular tension ring (CTR) as an implant that could deliver docetaxel (DTX) over a long period of time. The drug release rate of the composite could be controlled by the ethyleneglycol dimethacrylate (EGDMA) content and the ratio of HEMA/MMA as well as the structure of porous PMMA framework. The CTR could continuously release DTX for up to 6 weeks *in vitro* and maintain DTX in effective concentration in the aqueous humor after 42 days. Docetaxel-load capsular tension ring (DTX–CTR) presented strong inhibition on the lens epithelial cells *in-vivo* without obvious damage to normal tissues. These results indicate that the drug sustained-release CTR can provide a promising application in posterior capsule opacification prevention.

1. Introduction

Posterior capsule opacification (PCO), which is also termed secondary cataract, is a common long-term complication after cataract surgery. It is reported that the incidence of PCO ranges from 20% to 40% in 2–5 years after surgery [1,2]. While in children and young adults, PCO is expected in 100% of eyes in 1 year. Following the mechanical injury of surgery, the residual human lens epithelial cells (HLEpiCs) rapidly proliferate and migrate to the posterior capsule, where they approach the central visual axis and cause visual axis obscuration, resulting in progressive loss of vision [3–5]. Thus, how to remove or suppress the proliferation and migration of lens epithelial cells (LEpiCs) is of vital importance for PCO treatment.

At present, the relatively effective treatment of PCO is Nd:YAG laser capsulotomy [6,7], which generates a series of focal ablations in the posterior capsule to create a small circular opening in the visual axis. Though it is widely used, the Nd:YAG laser capsulotomy [8] not only aggravates the economic and emotional burden of patients, but also probably leads to serious complications like retinal detachment and even cystoid macular edema. Therefore, it is necessary to study how to effectively inhibit the proliferation and migration of LEpiCs in order to prevent the occurrence of PCO.

As a convenient solution, pharmaceutical prevention of PCO attracts great interest and is of great potential for clinical use. A variety of drugs such as anti-metabolites and anti-inflammatory drugs have been proved to be of a strong inhibitory effect on HLEpiCs' proliferation. However, the clinical application of these drugs is limited by the following reasons. First of all, effective concentration is hard to achieve by eye drops due to the special location of HLEpiC. What's more, nanocarriers, such as nanoparticles and liposomes, cannot stay in the capsular bag for sufficient time to kill HLEpiC. Last but not least, implants may affect patients' vision. Thus, drug carrier design is the key to the pharmaceutical prevention of PCO.

The commercial capsular tension ring (CTR) [9–11] is an open-ended, flexible, ring-shaped poly(methyl methacrylate) (PMMA) filament with an eyelet at each end. It is implanted into the capsular bag fornix (equator) to stabilize the capsular bag during cataract surgery in eyes at risk of postoperative capsular shrinkage. Ring shaped CTR do not affect patients' vision, making it an ideal drug

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http://dx.doi.org/10.1016/j.biomaterials.2016.02.038
0142-9612/

carrier for prevention of PCO. A capsule device-capsule drug ring (CDR) [12] was developed for sustained drug delivery which showed near-zero order release kinetics after 2 days. However, the membrane-controlled system was at risk of ruptures. Once it happened, large quantity of drugs would burst into the eye, leading to an acute toxicity. The aim of this study is to design a novel drug-loaded CTR with a long inhibitory effect on the HLEpiCs which can be positioned in the lens capsular bag during the cataract surgery. With constant release of the drug without the risk of ruptures, the purposes of prolonging drug reaction time and decreasing drug toxicity can be achieved.

An ideal material for sustained-release CTR preparation should have good controllability on drug release and appropriate mechanical properties as well. In this study, porous open-cell framework of the CTR was designed to improve the drug loading by a high internal phase emulsion template. Among others, porous materials prepared by the polymerization of high internal phase emulsion (HIPE), which is known as polyHIPEs, have many advantages, such as high porosity (typically 74–95 vol%) and open cellular structure [13], which are in favor of high drug loading. However, the mechanical strength of polyHIPEs is very weak. Thus, P(HEMA-co-MMA), copolymer of methyl methacrylate (MMA) and 2-hydroxyethyl methacrylate (HEMA) is introduced to the polyHIPE to form a novel composite. P(HEMA-co-MMA) is transparent, non-toxic and non-immunogenic. It has been widely used in ocular devices, such as contact lens [14–16] and intraocular lens [17–19]. The mechanical strength of CTR and the drug release rate were related to both the pore size of PMMA framework and the density of polymer network. After drug release was completed, the insoluble hydrophilic polymeric material ring could remain permanently in the capsular bag and played the role of capsular tension ring to prevent shrinkage of the intraocular lens dislocation or pocket.

We combined the P(HEMA-co-MMA) with the polyHIPE to form a novel P(HEMA-co-MMA)-PMMA composite. Its mechanical strength and drug release rate could be well controlled. To our best knowledge, we are the first to apply this novel P(HEMA-co-MMA)-PMMA composite as drug-loaded CTR in ophthalmiatrics. This novel drug carrier could offer a more convenient and safer way for prevention of PCO.

2. Materials and methods

2.1. Materials

Methyl methacrylate (MMA), 2-hydroxyethyl methacrylate (HEMA), azodiisobutyronitrile (AIBN) and ethylene glycol dimethacrylate (EGDMA) were purchased from Aladdin Industrial Corporation (China); docetaxel (DTX, purity >99.0%) was purchased from Peking Xinze Technology Co., Ltd (China). Pentobarbital sodium, Dulbecco's modified Eagle's medium (DMEM), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and trypsin-EDTA (with 0.25% trypsin and 0.02% EDTA) solution were purchased from Sigma Chemical Co. (USA). New born calf serum (NBCS) was purchased from Gibco Co. (USA). Human lens epithelial cells (HLEpiCs, SRA01/04) were kindly provided by Zhongshan Ophthalmic Center, Sun Yat-sen University (Guangzhou, China). All other chemicals and solvents were of reagent grade and used without further purification.

2.2. Preparation of the docetaxel-loaded capsular tension ring (DTX-CTR)

2.2.1. Preparation of porous PMMA

Porous PMMA was prepared by high internal phase emulsion template method as shown in Fig. 1A. The mixture of MMA (2 ml), EGDMA (0.2 ml), AIBN (50 mg) and surfactant (Span 80: Cremophor EL = 9:1, w/w) (25–50% based on MMA, w/w) was used as the continuous phase. Then, 18 ml water was added drop-wise to the continuous phase with continuous stir for 10 min. The emulsion was stirred for another 5 min after the addition of water. After a cream-like emulsion was obtained, the emulsion was transferred to the homemade mold and cured at 60 °C for 24 h. After the curing, the resulting polymer was purified by Soxhlet extraction (acetone, for 6 h) and then dried in a vacuum for 24 h at room temperature. Prepared samples were coded as Sx, where the letter "x" stood for the surfactant concentration used in preparing the samples.

2.2.2. Preparation of P(HEMA-co-MMA)-PMMA composites and P(HEMA-co-MMA)

The porous sample was filled with the mixture of monomer (HEMA/MMA = 85:15, 90:10, 95:5), EGDMA (0–2% based on monomer, w/w), AIBN (1% based on monomer, w/w) and DTX (1.5% based on monomer, w/w), which were polymerized at 60 °C for 24 h to obtain P(HEMA-co-MMA)-PMMA composites (Fig. 1B). The products were immersed in water for 24 h to remove the residual monomers. Prepared samples were coded as HxExSx, where the letter "x" stood for the percentage of HEMA, EDGMA and surfactant for preparing the porous sample, in respectively.

P(HEMA-co-MMA) was prepared under the same procedure without porous samples. Prepared samples are coded as HxEx, where the letter "x" stood for the percentage of HEMA and EDGMA.

The prepared P(HEMA-co-MMA)-PMMA composites were then cut into a ring-shaped filaments for *in-vitro* and *in-vivo* studies (Fig. 1C).

2.3. Characterization of porous PMMA framework, P(HEMA-co-MMA) and P(HEMA-co-MMA)-PMMA composites

For observations, the surface of the sample was coated with gold using a sputter coater (model EMSCOPE SC 500). The morphology of the porous PMMA framework, P(HEMA-co-MMA) and P(HEMA-co-MMA)-PMMA composites was examined using scanning electron microscopy (Jeol, JSM-6060).

2.4. Three point bending test

For the three point bending test, long-strip-shaped specimens were prepared. The width and thickness of each sample were measured with a vernier caliper at three evenly spaced locations along its length, and the mean values were used to determine its cross-sectional area. The samples were then mounted in a universal testing machine (CMT 6103, SANS, China) and tested by bending to failure using a crosshead speed of 2 mm/min.

2.5. In-vitro cell proliferation inhibition assay

The *in-vitro* cytotoxicity of different concentrations of DTX was measured on HLEpiCs. Briefly, HLEpiCs were seeded into a 96-well plate at 10^3 cells/well and cultured overnight for cell attachment. DTX-contained media of different concentrations were added to each well, and incubated for further 24 h, 48 h and 72 h. Then, the cell viability was measured by MTT assay (Elx800, BIO-TEK, USA).

2.6. In-vitro release study

To simulate the intraocular environment, phosphate buffer (PBS, pH 7.4) was chosen as the release medium. DTX-CTRs were placed in 3 ml PBS solution at 37 ± 0.5 °C respectively. All the release medium was taken out at fix time intervals, with 3 ml of fresh PBS solution supplied immediately. After filtrated by a 0.22 μm