The preparation and the in-vitro pharmacodynamics study of the intracapsular sustained-release preparations for the prevention of posterior capsule opacification

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ABSTRACT
Docetaxel-loaded sustained-release preparation based on 2-Hydroxyethyl methacrylate (HEMA) and Methyl methacrylate (MMA) cross-linked copolymer (P(HEMA-co-MMA)) was prepared to examine the potential use for preventing posterior capsule opacification (PCO). The preparations were prepared by polymerizing the mixture of HEMA, MMA, cross-linking agent (EGDMA), initiator (AIBN) and docetaxel. The influence factors and mechanism of drug release were studied in the experiments. FT-IR, X-RD and SEM methods were used to characterize the polymer (P(HEMA-co-MMA)) and docetaxel-loaded sustained-release preparations. Biocompatibility of P(HEMA-co-MMA) and in-vitro effect of docetaxel-loaded sustained-release preparations were also evaluated. The results showed that docetaxel could release sustainedly from these preparations prepared by cross-linking polymerization. And the release rate could be accelerated by increasing the MMA ratio or EGDMA ratio of the polymer. Release mechanism of docetaxel fitted the Higuchi model well. The results of IR and X-RD showed that only a hydrogen bond was formed between docetaxel and P(HEMA-co-MMA). Docetaxel dispersed in P(HEMA-co-MMA) in amorphous form. The elution test showed that P(HEMA-co-MMA) had good biocompatibility and the in-vitro pharmacodynamics study proved that docetaxel could release stably from the preparations and inhibit HLECs’ proliferation. The docetaxel-loaded sustained-release preparations proved to be a promising therapy for preventing PCO. These results also lay a theoretical and experimental foundation for the future.

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1. Introduction

By far, cataracts are the most common cause of low visual acuity. It is effectively treated by surgical procedure i.e. Phacoemulsification or Extra-Capsular Cataract Extraction (ECCE). However, the incidence of Posterior Capsule Opacification (PCO) is up to 50% in adults and 100% in children \[1\text{–}3\], and it is a routine complication. It is caused by the proliferation and migration of the residual lens epithelial cells (LECs) left during the cataract surgery. As soon as PCO occurs, Nd:YAG laser must be used to treat the complication. But additional surgery involves additional expenses and mortgages. Moreover, this procedure may also cause rhegmatogenous retinal detachment or endophthalmitis. What's more, it is somewhat difficult to treat dense capsular opacification after operation for congenital cataract by Nd:YAG laser in infancy \[1\]. Thus, it is necessary to develop a non-surgical method to prevent PCO. Pharmacologic agents have been studied to prevent PCO for their inexpensive, convenient and digestible.

Many drugs such as Genipin, Proteasome inhibitors, Lithium, Mitomycin C, 5-FU and Diclofenac \[4\text{–}18\] display a strong inhibitory effect on LECs’ proliferation as well as their serious toxicity to other intraocular structure. In order to reduce the toxicity of pharmacologic agents, some researchers irrigated the capsular bag for a few minutes with pharmacologic agents after phacoemulsification \[12,19\]. Maloof A et al. developed a Sealed Capsule Irrigation (SCI) device \[3,9,20\]. Weidmann A and Xie L et al. developed sustained release preparations such as microspheres, microparticles \[10,21,22\], liposomes \[23,24\] and drug-surface-modified intraocular lenses \[25\text{–}27\]. All of these methods could reduce the incidence of PCO, but they all had their own defects \[28\text{–}30\].

Hydrophilicity acrylic ester made from HEMA and MMA by chemical cross-linking copolymerization is a kind of non-degradation material, which has been widely used in medicine. Due to such properties as non-toxicity, non-antigen and satisfactory biocompatibility, it has been used as the materials of many medical products such as intraocular lenses \[31\text{–}34\], capsular tension rings \[35\text{–}39\], soft contact lenses \[40\] and artificial skin \[41\]. It has also been studied commonly as an ophthalmological carrier of drugs such as pilocarpine \[42\], Mitomycin C \[43,44\] and 5-FU \[45\]. Consequently, HEMA and MMA cross-linked copolymer P(HEMA-co-MMA) was used as a carrier in this research.

In our previous research \[46\], docetaxel was found to be a strong inhibitor of HLECs’ proliferation (IC50: 16.48 ng/ml, 72 h). It could inhibit and delay G2/M phase transition of HLECs, induce HLECs’ early apoptosis, and decrease the expression of bcl-2 protein. Therefore, docetaxel was chosen as the model drug in this research.

P(HEMA-co-MMA) with docetaxel was prepared and shaped into a ring, which could be inserted into ocular when ECCE surgery was carried out. After the drug was released, it could still play a role in supporting the capsular bag and preventing the capsular bag from shrinking.

2. Materials and methods

2.1. Materials

Methyl methacrylate (MMA) and azobis-isobutyronitrile (AIBN) were purchased from Tianjin Damao Chemical Reagent Factory; 2-hydroxyethyl methacrylate (HEMA) was purchased from Guangzhou Zhenlin Trading Co., LTD (China);ethylene glycol dimethacrylate (EGDMA) was provided by Guangzhou Shuangjian Trading Co., LTD (China); docetaxel was purchased from Peking Xinzhe Technology Co., LTD (China); Dulbecco modified Eagle’s medium (DMEM) and trypsin were purchased from Gibco Co. (USA); fetal bovine serum (FBS) was obtained from Hangzhou Sijiqing Biological Engineering Co., LTD (China); and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was purchased from Sigma Chemical Co. (USA). Mouse fibroblast cell line (L-929) was purchased from the Center of Experiment Animal of Sun Yat-sen University (Guangzhou, China); and human lens epithelial cells (HLECs, SRA01/04) were purchased from Cell Resource Center of Peking Union Medical College (Beijing, China). All the other chemicals were of analytical grade.

2.2. Synthesis methods

Docetaxel-loaded sustained-release preparations were synthesized by free radical solution polymerization method. Firstly, the monomer of HEMA, MMA and the cross-linker EGDMA were mixed together. Then the mixture was degassed by bubbling nitrogen for 30 min after AIBN initiator and docetaxel were added to it. Secondly, the solution was poured into a homemade mold. And finally, the polymerization reaction was performed in an oven at 60 °C for 24 h. Docetaxel-loaded sustained-release preparations with different MMA/HEMA ratios (mol/mol = 1:9, 1.5:8.5, 2:8, 2.5:7.5 and 3:7) were prepared respectively. The pure P(HEMA-co-MMA) was synthesized without docetaxel.

2.3. Study on in-vitro drug release

Drug release experiments were conducted as follows: the docetaxel-loaded sustained-release preparations were put into containers containing 3 ml pH 7.4 phosphate buffered saline (PBS). The containers were shaken in a horizontally reciprocating shaker at 37 °C (±0.5 °C). After being shaken for 1 day, the docetaxel-loaded sustained-release preparations were then transferred into other containers containing 3 ml fresh PBS respectively. Transfers were taken at 1d, 2d...16d, 18d...30d and 35d. Samples were detected by high-performance liquid chromatography (HPLC) and the HPLC methods were as follows: 50 μL liquids were injected for analysis. The mobile phase was acetonitrile/water (which equals 55/45), column temperature was 35 °C, flow rate was 1 ml/min, and wavelength was 232 nm.

The drug release behavior of docetaxel-loaded sustained-release preparations with different MMA/HEMA ratios (mol/
mol = 1:9, 1.5:8.5, 2:8, 2.5:7.5 and 3:7) were studied by the same methods described above respectively.

2.4. Swelling ratio

The swelling properties of pure P(HEMA-co-MMA) were determined gravimetrically. Firstly the samples were placed in 5 ml DI water at room temperature. When their weight was stable after removing excess water, the samples were weighed on an electronic balance (92SM-202A, Precisa). And the samples were weighed on an electronic balance again after being dried by a drying vacuum oven at 60 °C for 48 h. The swelling ratio was calculated by the following equation:

Swelling ratio (%) = \( \frac{W_w - W_d}{W_d} \times 100 \),

where \( W_w \) represents the wet weight of the sample; \( W_d \) represents the dry weight of the sample.

2.5. Characterization of docetaxel-loaded sustained-release preparations

The IR spectra of the samples were recorded on an FTIR spectrometer (EQUINOX 55, Bruker, German) using KBr discs. The spectra were recorded from 4000 cm\(^{-1}\) to 500 cm\(^{-1}\).

Powder X-ray diffraction measurements were carried out on D/Max-IIIA (Rigaku, Japan) with Cu, 35 KV and 25 mA. The DS/SS was 1°. The analysis was performed with 2θ varying from 3 to 60° at a scan speed of 12/min.

The microstructure of samples was studied with Scanning Electron Microscopes (JSM-6330F, Jeol, Japan). Samples were cracked in liquid nitrogen with 15 kV accelerating voltages.

2.6. Biocompatibility studies of P(HEMA-co-MMA)

According to the national standard GB/T 16886, the cytotoxicity of one-month leaching liquor of homemade P(HEMA-co-MMA) \( (MMA_{mol}/HEMA_{mol} = 9:1, 8.5:1.5, 8:2, 7.5:2.5, 7:3) \) was evaluated on L-929 cells. Phenol (6.3%) and culture medium (DMEM with 20% FBS, DMEM (20)) were used as positive and negative control groups respectively. Cells with samples were incubated together in a 96-well plate for 48 h. Then the samples were removed and 20 \( \mu l \) MTT solution (5 mg/ml, final concentration) was added to the 96-well plate and the cells were incubated continually for 4 h. After the MTT solution was removed, MTT formazan precipitate was dissolved in 750 \( \mu l \) DMSO. The absorbance of each well was measured at 490 nm with an ELISA Reader (Elx800, BIO-TEK, USA). The cell viability was calculated by the absorbance ratios of the sample group to the control group.

2.7. Studies on in-vitro pharmacodynamics

HLECs were cultured in a 24-well plate (\( 1\times10^5 \) cells/ml) at 37 °C for 24 h, then the medium was changed and the docetaxel-loaded sustained-release preparations were hung in the well of the plate by a stainless steel wire in order that the preparations could be immersed in the culture solution without touching the cells. Then after incubated at 37 °C for 2 days, the docetaxel-loaded sustained-release preparations were transferred into another 24-well plate containing HLECs. Transfers were taken at 2d, 4d, ..., 20d, respectively. The concentration of docetaxel in DMEM (20) was then determined by HPLC. HPLC methods were the same as above. The in-vitro effect of inhibiting HLECs proliferation by docetaxel-loaded sustained-release preparations was evaluated through MTT assay in this part. Firstly, 200 \( \mu l \) MTT solution (5 mg/ml, final concentration) was added to the 96-well plate for each well and the cells were incubated continually for 4 h. After the MTT solution was removed, MTT formazan precipitate was dissolved in 750 \( \mu l \) DMSO. The absorbance of each well was measured at 490 nm with an ELISA Reader (Elx800, BIO-TEK, USA). The cell viability was calculated by the absorbance ratios of the sample group to the control group.

3. Results and discussion

3.1. Synthesis methods

The docetaxel-loaded sustained-release preparations and the pure P(HEMA-co-MMA) were successfully synthesized in cross-linking polymerization procedure. HEMA and MMA are monomers used to synthesize pHEMA, pMMA and P(HEMA-co-MMA). EGDMA is commonly used as a cross-linking agent in synthesizing P(HEMA-co-MMA).

There are always two methods for loading drugs into P(HEMA-co-MMA). One is that the drug solution is absorbed into the pure polymer due to different distribution coefficients between polymer and water [47,48]. The other is dissolving or suspending the drug into the mixture of monomer at the beginning of the polymerization procedure [42,49]. Drug usually releases very fast from the preparations made by the first method [48], while temperature-sensitive drugs are not suitable to be used by the second method. For the purpose of obtaining sustained-release preparations, the second method was employed in our research.

3.2. Release mechanism investigation

With a constant amount of cross-linker, the release rate of docetaxel could be retarded by increasing the ratio of MMA/HEMA. The Effects of different MMA/HEMA ratios on in-vitro docetaxel release (0.3% EGDMA) \((n = 3)\) are shown in Fig. 1. The HPLC methods were the same as above. The in-vitro effect of inhibiting HLECs proliferation by docetaxel-loaded sustained-release preparations was evaluated through MTT assay in this part. Firstly, 200 \( \mu l \) MTT solution (5 mg/ml, final concentration) was added to the 96-well plate for each well and the cells were incubated continually for 4 h. After the MTT solution was removed, MTT formazan precipitate was dissolved in 750 \( \mu l \) DMSO. The absorbance of each well was measured at 490 nm with an ELISA Reader (Elx800, BIO-TEK, USA). The cell viability was calculated by the absorbance ratios of the sample group to the control group.

![Fig. 1](image-url) - Effects of different MMA/HEMA ratios on in-vitro docetaxel release (0.3% EGDMA) \((n = 3)\).
HEMA (Fig. 1). On the first day, the release percentage of docetaxel decreased from 38.5% to 2.0% as the MMA/HEMA ratio increased from 1:9 to 3:7. On the twentieth day, there were about three-quarters of docetaxel released from the sustained-release preparations with an MMA/HEMA ratio of 1:9, but less than 10% docetaxel was released from the sustained-release preparations with an MMA/HEMA ratio of 3:7 (Fig. 2).

Zero-order kinetics, first-order kinetics, the Higuchi model and Peppas equation were used to investigate docetaxel release mechanism from the sustained-release preparations. According to the correlation coefficient (r²), the fitting results showed that the release curves of docetaxel-loaded sustained-release preparations with MMA/HEMA ratios of 1:9, 1.5:8.5 and 2.5:7.5 fitted the Higuchi model (Table 1a, b, d) very well. Both the Higuchi model and Peppas equation fitted the release curves of the preparations with MMA/HEMA ratios of 2:8 and 3:7 (Table 1c, e) and the statistical analysis results indicated that there were no statistical significance (p > 0.1) of the y calculation in the two models. The drug release rate constant (k) calculated from the Higuchi model showed a linear relationship with MMA/HEMA ratio (R), The linear equation was k = -0.4457r + 0.1976, r² = 0.9452 (p < 0.01), which indicated a negative correlation between k and R.

The release behavior of docetaxel-loaded sustained-release preparations in PBS (pH 7.4) was shown in Fig. 3. The release rate gradually slowed down with an initial burst release in the first five days. The concentration of docetaxel on the first day was 3 times higher than that on the thirtieth day especially. What’s more, the concentrations of docetaxel in PBS (pH 7.4) was shown in Fig. 3. The negative correlation between 

\[ \frac{y}{x} = \frac{k}{C_0} \]

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with EGDMA ratio (W). The linear equation was 

\[ k = -0.5225 W + 0.1462, r^2 = 0.8640 (p < 0.01) \]

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Studies on in-vitro drug release could not reflect the in-vivo drug release characteristics directly. But until now, there is no practicable method specially used for intraocular drug release studies. Therefore, according to the pertinent literature [49], pH (about 7.4) and flow rate (2 ~ 3 μl/min) of the aqueous humor, 3 ml PBS (pH 7.4) was adopted as a release medium and the effects of different MMA/HEMA ratios and EGDMA content on in-vitro release behavior of docetaxel from the sustained-release preparations were studied.

The Higuchi model, which was the best fitting model, suggested that the pure diffusion procedure was the main drug release mechanism from these sustained-release preparations [50,51]. According to the correlation equations

Table 1 – Fitting equations for docetaxel release profiles from P(HEMA-co-MMA) with different MMA/HEMA ratios.

<table>
<thead>
<tr>
<th>No.</th>
<th>MMA/HEMA</th>
<th>Equation</th>
<th>Fitting equation</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>1:9</td>
<td>Zero-order kinetics</td>
<td>y = -0.0209x + 0.2737</td>
<td>0.8333</td>
</tr>
<tr>
<td></td>
<td></td>
<td>First-order kinetics</td>
<td>y = -0.0209x - 0.1037</td>
<td>0.9492</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Higuchi model</td>
<td>y = 0.1522x + 0.0207</td>
<td>0.9500</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Peppas equation</td>
<td>y = 0.5979x - 0.9117</td>
<td>0.9415</td>
</tr>
<tr>
<td>b</td>
<td>2.5:8.5</td>
<td>Zero-order kinetics</td>
<td>y = 0.0172x + 0.226</td>
<td>0.8553</td>
</tr>
<tr>
<td></td>
<td></td>
<td>First-order kinetics</td>
<td>y = -0.0144x - 0.0933</td>
<td>0.9367</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Higuchi model</td>
<td>y = 0.1296x + 0.0123</td>
<td>0.9618</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Peppas equation</td>
<td>y = 0.5795x - 0.9654</td>
<td>0.9605</td>
</tr>
<tr>
<td>c</td>
<td>2:8</td>
<td>Zero-order kinetics</td>
<td>y = 0.0099x + 0.1313</td>
<td>0.9176</td>
</tr>
<tr>
<td></td>
<td></td>
<td>First-order kinetics</td>
<td>y = -0.0056x - 0.0591</td>
<td>0.9430</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Higuchi model</td>
<td>y = 0.0628x + 0.0432</td>
<td>0.9858</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Peppas equation</td>
<td>y = 0.4225x - 1.0348</td>
<td>0.9802</td>
</tr>
<tr>
<td>d</td>
<td>2.5:7.5</td>
<td>Zero-order kinetics</td>
<td>y = 0.0068x + 0.09</td>
<td>0.8853</td>
</tr>
<tr>
<td></td>
<td></td>
<td>First-order kinetics</td>
<td>y = -0.0036x - 0.0397</td>
<td>0.9090</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Higuchi model</td>
<td>y = 0.0509x + 0.0069</td>
<td>0.9774</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Peppas equation</td>
<td>y = 0.5493x - 1.3136</td>
<td>0.9719</td>
</tr>
<tr>
<td>e</td>
<td>3:7</td>
<td>Zero-order kinetics</td>
<td>y = 0.0021x + 0.0368</td>
<td>0.8522</td>
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<td></td>
<td></td>
<td>First-order kinetics</td>
<td>y = -0.001x + 0.0163</td>
<td>0.8593</td>
</tr>
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<td></td>
<td></td>
<td>Higuchi model</td>
<td>y = 0.0133x + 0.0179</td>
<td>0.9383</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Peppas equation</td>
<td>y = 0.374x - 1.5906</td>
<td>0.9330</td>
</tr>
</tbody>
</table>

Fig. 2 – In-vitro release of docetaxel-loaded sustained-release preparations of different MMA/HEMA ratios at the same time (n = 3). (MMAmol/HEMAmol = 1:9, 1.5:8.5, 2:8, 2.5:7.5 and 3:7).

Fig. 3 – The concentration diagram of in-vitro release of docetaxel-loaded sustained-release preparations (n = 3).
between $k$ and $R$ and the correlation equations $k$ and $W$, specific preparations with expectable release of docetaxel could be designed and made. These correlation equations provided a theoretical basis for designing and preparing the docetaxel-loaded sustained-release preparations in the future.

### 3.3. Swelling ratio

The swelling ratios decreased as the MMA/HEMA ratios increased. The swelling ratio of P(HEMA-co-MMA) was 55% when the MMA/HEMA ratio was 1:9; but when the MMA/HEMA ratio increased to 3:7, the swelling ratio was only 33% (Fig. 4). At the same time, the release of docetaxel from the sustained-release preparations also decreased obviously. The reason was that there were many hydrophilic groups (–OH) in HEMA, so when the MMA ratio in P(HEMA-co-MMA) increased, the hydrophilicity decreased, which caused the drug release rate to slow down. Cross-linker EGDMA could enhance the density of P(HEMA-co-MMA), which also slowed down the drug release rate [42].

### 3.4. Characterization of docetaxel-loaded sustained-release preparations

The FT-IR spectrum (4000–500 cm$^{-1}$) was employed to analyze the form of docetaxel entrapped in P(HEMA-co-MMA). The stretching vibration of N–H at 3383 cm$^{-1}$ and 3335 cm$^{-1}$ and the out-plane vibration of N–H at 708 cm$^{-1}$ (Amide V band) were the characteristic absorption band of docetaxel. However, these N–H absorption bands disappeared in P(HEMA-co-MMA).
The characteristic absorption band of N–H existed in both docetaxel (Fig. 5a) and the mixture of docetaxel and P(HEMA-co-MMA) (Fig. 5c), but it also disappeared in FT-IR spectra of docetaxel-loaded sustained-release preparations (Fig. 5d). These results indicated that a hydrogen bonding was formed by the interaction of the N–H of docetaxel and the –OH or C=O groups of P(HEMA-co-MMA).

The hydrogen bond between docetaxel and P(HEMA-co-MMA) could improve the compatibility between drug and polymer. No other chemical bond was formed between them, which suggested that the activity of docetaxel did not change.

The X-ray diffraction was employed to measure the crystalline property of docetaxel entrapped in P(HEMA-co-MMA). The characteristic diffraction peaks of docetaxel were 2θ = 5.2°, 9.8°, 13.7°, 15.7°, 20.2°. These characteristic diffraction peaks existed in both docetaxel and the mixture of docetaxel and P(HEMA-co-MMA), but disappeared in docetaxel-loaded sustained-release preparations (Fig. 6).

These results indicated that docetaxel dispersed in P(HEMA-co-MMA) in the form of amorphism or molecule.

Scanning electron micrographs showed that there was no gross difference between pure P(HEMA-co-MMA) and docetaxel-loaded P(HEMA-co-MMA) (Fig. 7), which suggested that the internal structure of P(HEMA-co-MMA) did not change when loaded with docetaxel. The SEM results also showed that there was no poriform structure on the surface of the sustained-release preparations after release (Fig. 8), thus it wouldn’t affect the integrality and morphology of the preparations. So it could still support the capsular bag and prevent the capsular bag from shrinking after the drug was released completely.

3.5. Biocompatibility studies of P(HEMA-co-MMA)

The normal L-929 cells grew adherently and did not show significant morphological changes when cultured with test groups, and the viability of L-929 cells was more than 90%.

Fig. 7 – Scanning electron micrographs of the surface of P(HEMA-co-MMA). (a) Blank P(HEMA-co-MMA), MMA/HEMA = 1.5:8.5 (b) P(HEMA-co-MMA) with docetaxel, MMA/HEMA = 1.5:8.5 (c) Blank P(HEMA-co-MMA), MMA/HEMA = 3:7 (d) P(HEMA-co-MMA) with docetaxel, MMA/HEMA = 3:7.

Fig. 8 – Scanning electron micrographs of docetaxel-loaded sustained-release preparations before and after release. (MMA_mol/HEMA_mol = 3:7). (a) Before release (b) After release.
While cultured with phenol, the cells floated and died and the cell viability was only 9.11% (Table 3).

P(HEMA-co-MMA) is a kind of non-degradation material, which has been widely used in medicine, but it is still necessary to investigate the biocompatibility of P(HEMA-co-MMA) made by ourselves. Results showed that the test groups had a reactivity grade of zero or one, and the positive control group’s reactivity grade was four (Table 4). These indicated that the homemade P(HEMA-co-MMA) had good biocompatibility.

### 3.6. Studies on in-vitro pharmacodynamics

HLECs were cultured to evaluate the inhibitory effect of the docetaxel-loaded sustained-release preparations. When treated with drug-loaded preparations, the viabilities of HLECs were less than 20% within 20 days, especially in the first eight days, and the viabilities of HLECs were less than 10% (Fig. 9). These results proved that docetaxel-loaded sustained-release preparations could inhibit HLECs proliferation for at least 20 days. And docetaxel could be released for at least one month from these preparations in DMEM (20).

The release results of docetaxel in DMEM (20) and pH 7.4 PBS showed that the release rate of docetaxel in PBS had a good liner correlation with that in DMEM (20). The regression equations was $y = 1.2165x - 15.241$ (MMA/HEMA = 1:9, $r = 0.9727$ ($p < 0.01$)), which indicated a positive correlation between them.

In the in-vitro pharmacodynamics studies, the docetaxel-loaded sustained-release preparations were immersed in the culture solution without touching the cells. In the biocompatibility studies of P(HEMA-co-MMA), the results had already shown that the polymer was non-toxic, so the anti-proliferative agent must have been released effectively from the polymer [44]. In the in-vitro pharmacodynamics studies, the inhibitory effect of the docetaxel-loaded sustained-release preparations proved to be constant and effective, especially on the second, fourth, eighth and tenth day.

In the in-vitro drug release studies, the concentrations of docetaxel in release media were much higher than its IC50 in

### Table 3 – The growth rate of L-929 cells treated with one-month leaching liquor of P(HEMA-co-MMA) for 2 days.

<table>
<thead>
<tr>
<th>MMA/HEMA (mol:mol)</th>
<th>Growth rate after 2d (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:9</td>
<td>97.84</td>
</tr>
<tr>
<td>1.5:8.5</td>
<td>97.18</td>
</tr>
<tr>
<td>2:8</td>
<td>97.24</td>
</tr>
<tr>
<td>2.5:8.5</td>
<td>93.29</td>
</tr>
<tr>
<td>3:7</td>
<td>97.01</td>
</tr>
<tr>
<td>Phenol</td>
<td>9.11</td>
</tr>
</tbody>
</table>

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### Table 4 – Reactivity grades for elution test.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Reactivity</th>
<th>Conditions of all cultures</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>None</td>
<td>Discrete intracytoplasmic granules; no cell lysis</td>
</tr>
<tr>
<td>1</td>
<td>Slight</td>
<td>Not more than 20% of the cells are round, loosely attached, and without intracytoplasmic granules; occasional lysed cells are present</td>
</tr>
<tr>
<td>2</td>
<td>Mild</td>
<td>Not more than 50% of the cells are round and devoid of intracytoplasmic granules; no extensive cell lysis and empty areas between cells</td>
</tr>
<tr>
<td>3</td>
<td>Moderate</td>
<td>Not more than 70% of the cell layers contain round cells or are lysed</td>
</tr>
<tr>
<td>4</td>
<td>Severe</td>
<td>Nearly complete destruction of the cell layers</td>
</tr>
</tbody>
</table>

According to USP, the sample will meet the requirements of the test if the response to the Sample Preparation is not greater than grade 2 (mildly reactive).

![Fig. 9](image-url) – MTT analysis of cell viability after treated with docetaxel-loaded sustained-release preparations of different MMA/HEMA ratios ($n = 3$).
the first five days and were always higher than the IC₅₀
(16.48 ng/ml, 72 h) of docetaxel at all times. The drug could be released from the docetaxel-loaded sustained-release prepar-
rations stably in DMEM (20) and the release rate of docetaxel in
DMEM (20) had a positive correlation with that in PBS (pH 7.4).

According to the pathogenesis and clinical experiences, a higher inhibitor concentration is necessary to clear LECs at
the initial stages after surgery, when the proliferation and
migration of LECs is extremely active. So it is necessary that
the drug should be quickly released from the docetaxel-loaded
sustained-release preparations and has a high inhibition rate
during the first ten days. Furthermore, PCO is unlikely to occur
if proliferation and transplant of the rudimental LECs don’t
occur. Thus, the initial burst release is beneficial for the pre-
vention of PCO. However, in-vivo pharmacodynamics studies
are still needed to prove whether the concentration of drug
can reach therapeutic levels and whether it is toxic to other
intraocular tissues.

4. Conclusion

In our research, the in-vitro pharmacodynamics experiments
indicated that HLECs were sensitive to docetaxel and the
docetaxel-loaded sustained-release preparations could
maintain an effective drug concentration for up to 20 days.
Owing to the limitations that in the present studies, only in-
vitro experiments were conducted for the proposed prepara-
tion, further experiments would be conducted to study the
relation between the in-vitro experiments and the in-vivo
experiments. This system and the preparations had very
high potential for future clinical application.

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