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Drug Eluting Intraocular Lens Surface Modification for PCO Prevention

Yuemei Han[a], Xu Xu[a], Yuqin Wang[a], Sihao Liu[a], Xia Zhao[a], Hao Chen[a,b,*], Quankui Lin[a,b,*]

[a] School of Ophthalmology & Optometry, Eye Hospital, Wenzhou Medical University, Wenzhou 325027, China
[b] Wenzhou Institute of Biomaterials and Engineering, Wenzhou 32500, China

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Cataract is the most common eye disease leading to blindness [1]. It is reported that about 47.8% blindness are responsible for cataract and there are about 20 million cataract blind worldwide recently [2]. The cataract is due to the lens opacification in the eye. Clinical treatment for cataract is to remove the opaque lens followed with intraocular lens (IOL) implantation. The vision is obviously improved after IOL application. However, posterior capsular opacification (PCO), a common complication after cataract surgery, will affect the vision recovery seriously. It is reported that the PCO incidence after IOL 5 years' implantation is around 20–40% in adults' cases, and which is as high as 100% in children's cases [3,4]. PCO is originated from the adhesion and proliferation of the residual lens epithelial cells (LEC) in the IOL surface and lens capsule, as which can't be removed completely during the surgery [5]. The only way to treat PCO in clinic is Neodymium-doped Yttrium Aluminium Garnet (Nd:YAG) laser capsulotomy [3]. However, the laser irradiation requires additional high costs, as well as inducing the other complications, such as retinal detachment, or high intraocular pressure, etc.

Many attempts have taken by the ophthalmologists to reduce the PCO incidence. For example, the intra-capsular anti-proliferative drug injection after IOL implantation, or directly immersed IOL in the drug solution before implantation, so as to prevent residual LEC proliferation after IOL implantation [6]. Surface modification of the biomaterials provides a feasible way to improve their biocompatibility [7]. The fast drug release and the accompanied side effects to the adjacent tissues are serious problems and which does not reduce the PCO incidence [8,9]. Scientists have tried the bio-inert or hydrophilic surface modification onto the IOL surface, such as surface immobilization with hydrophilic heparin, polyethylene glycol, and phosphatidylcholine moieties [10–12]. The LEC adhesion on the surface modified IOL materials is inhibited when observed under laboratory conditions, whereas the clinical outcomes do not match as it is expected. The clinical research shows that there is no significant difference in the long run between the pristine IOL and the heparinized IOL, which is the only commercialized surface modified IOL in the world [13]. In previous studies, we have generated enhanced anti-adhesive surface modifications to improve the IOL biocompatibility via Surface Initiated-Reversible Addition-Fragmentation Chain Transfer (SI-RAFT) polymerization and natural polyelectrolyte Layer-by-Layer (LbL) deposition method [14–18]. The enhanced hydrophilic surface modification decreases the posterior capsular hyperplasia in certain extents, whereas the PCO incidence still can't be ignored [14,16,17]. In this study, the antiproliferative drug was loaded into the anti-adhensive hydrophilic natural polysaccharides multilayer. The anti-proliferative drug Doxorubicin (DOX) was firstly condensed into the Chitosan (CHI) nanoparticles via electrostatic complex between the cationic CHI and ionic Sodium Tripolyphosphate (TPP), which resulted in CHI-TPP-DOX nanoparticles. The positively charged CHI-TPP-DOX nanoparticle was used as building block to electrostatic assemble with negatively charged Heparin (HEP) on the IOL material surface, forming HEP/(CHI-TPP-DOX) polyelectrolyte multilayer surface coating. The anti-proliferative drug loaded polysaccharide multilayer modified IOL seems to be better in prevention the PCO incidence after ocular implantation than the hydrophilic polysaccharide multilayer modification only.

The CHI nanoparticle was prepared by the addition of an aqueous solution of TPP (0.2% w/v) to CHI (0.1% w/v) solution in acetic acid (1% v/v). TPP was added dropwise to the CHI solution under stirring at room temperature at a flow rate of 10 mL/min, the mixed solution was incubated for 0.5 h. A series of CHI nanoparticles were prepared under different N/P ratios (N/P ratios refer to the molar ratio of the N element

* Corresponding authors.
E-mail addresses: chenhao@mail.eye.ac.cn (H. Chen), linqk@wmu.edu.cn (Q. Lin).

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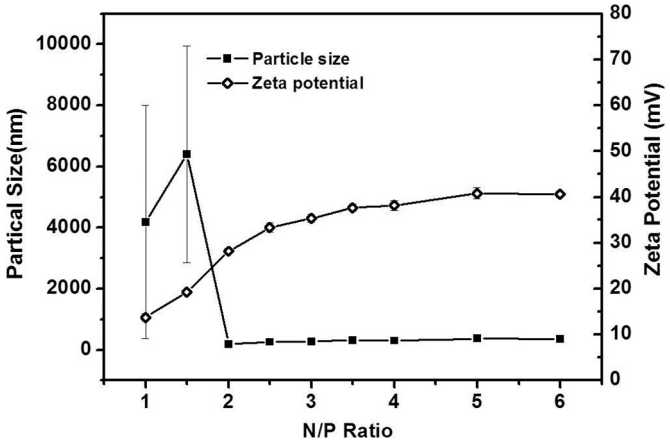

Fig. 1. The chitosan particle sizes and Zeta potentials change with N/P ratios.

in the repeating unit of CHI chain and the P element in the TPP). The particle size and Zeta potential of the obtained nanoparticles were characterized by dynamic light scattering (DLS). As shown in Fig. 1, CHI and TPP form large particles with diverse distribution when the N/P ratios are closing to 1. The diameters of these particles are ranging from 1 to 10 μm. And these particles are not stable, which may precipitate when incubation. The unstability of the CHI-TPP nanoparticle around N/P ratio 1 may due to the charge balance. When N/P ratio increasing, the particle sizes decrease to nanoscale and stabilize in the range of 200–400 nm when the N/P ratio is larger than 2. The Zeta potential of the obtained chitosan nanoparticle is also test soon after its preparation, where the pH of the nanoparticle solution is around 5.5. The Zeta potential slightly increases with the N/P ratio increasing, which stabilize in the range of 30–40 mV when N/P ratio is above 2. As a result, the N/P ratio of 3 was taken in the following investigations. The DOX doped CHI-TPP nanoparticle were obtained by the above described procedure with the DOX doped in the CHI solution (0.05% DOX, w/v). The particle size and Zeta potential of the obtained CHI-TPP-DOX nanoparticle are 381 ± 32 nm and 39.9 ± 4.3 mV, which indicates that the obtained CHI-TPP-DOX nanoparticles are positively charged and are feasible for the subsequent LbL deposition [19,20].

The obtained drug loaded CHI nanoparticles were then used as a building block for the polyelectrolyte multilayer film fabrication on the IOL surface. The procedure of the IOL surface modification with the polyelectrolyte multilayer was similar with the previous publications, excepting the cationic building block was substituted by the freshly prepared cationic CHI-TPP-DOX nanoparticles [20]. Briefly, the IOL material hydrophobic polyester was aminolysized by 3 mg/mL polyethyleneimine solution, obtaining the stable positively charged surface. Then the materials were alternately immersed in 1 mg/mL HEP solution and freshly prepared CHI-TPP-DOX nanoparticle solution, with soaking time of 15 min followed by the acetic buffer rinse. The determined bilayers of HEP/CHI-TPP-DOX multilayer was obtained with different assemble cycles. The multilayer growth was detected by the UV–Vis spectra. The characteristic absorbance of DOX at 254 nm was collected and analyzed. As shown in Fig. 2(a), a typical linear multilayer growth was observed in the HEP/CHI-TPP-DOX multilayer fabrications, which also indicates the successful construction of the drug loaded polysaccharide multilayer. The morphology of multilayer film was also observed by SEM. As indicated in Fig. 2(b), there are plenty of nanoparticles or nanoparticle clusters render on the $(HEP/CHI\text{-}TPP\text{-}DOX)_5$ multilayer surface, which also indicates the successful immobilization of the CHI-TPP-DOX nanoparticles in the multilayer.

The drug release behavior of the constructed $(HEP/CHI\text{-}TPP\text{-}DOX)_5$ multilayer film was investigated. As mentioned above, the PCO was the result of the pathological hyperplasia of the residual lens epithelial cells on the surface of the lens capsule and the intraocular lens surface, both simulant physiological condition (pH = 7.4) and pathological condition (pH = 5.5) were taken in drug release investigation. The surface modified materials were soaked in PBS (pH = 7.4) and acetated buffer (pH = 5.5) and the releasing solution were analyzed by High Performance Liquid Chromatography (HPLC), with the methods according to the Pharmacopoeia of China. As mentioned above, the PCO development is mainly due to the adhesion and proliferation of the residual lens epithelial cells. The cell proliferation under pathological conditions always results in the slightly acidic cellular microenvironment [21]. As a result, the drug release profile in pH = 5.5, which is also the nanoparticle preparation pH condition, is also investigated in this research. As shown in Fig. 3, the drugs incorporated in the polysaccharide multilayer is relatively stable in the physiological conditions, as there is almost no drug eluting was found in the pH = 7.4. Whereas in the pathological conditions (pH = 5.5), the drug in the multilayer renders slowly eluting properties. The accumulative drug release rate in 24 h is around 7.5% in pH = 5.5. It slowly increases to 11% after one week incubation. The environment dependent drug eluting may be the result of the deprotonation of the chitosan. As mentioned above, the antiproliferative drug was incorporated in the chitosan nanoparticles. Chitosan is a linear polysaccharide composed of randomly distributed β-(1 → 4)-linked D-glucosamine (deacetylated unit) and *N*-acetyl-D-glucosamine (acetylated unit). It is the only cationic natural polysaccharide. The amino group in chitosan has a pKa value of ~6.5, which leads to a protonation in acidic to neutral solution with a charge density dependent on pH and the deacetylation value. As a result, when the drug was incorporated in chitosan nanoparticle, the drug may be tightly wrapped by the chitosan molecules in the nanoparticles, due to its insolubility in physiological conditions (pH = 7.4). However, when the drug loaded surface coating was placed in acidic conditions, the chitosan becomes to dissolve, and the wrapped drug in the nanoparticle may be released slowly. This result indicates that the fabricated drug contained surface coating will be stable in the physiological conditions and renders sustained drug release properties in the pathological conditions.

The IOL materials with drug loaded polysaccharide multilayer surface modification were investigated with in vitro cell culture to determine the cell adhesion and apoptosis on the lens epithelial cells. The materials were cut into a 6 mm diameter disc, sterilized by UV irradiation, and then placed in the 96-well cell culture plate. The human lens epithelial cells (HLECs) were seeded with density of 1.0×10^4 cells/well. After 24 h incubation, the cells were treated by live/dead cell staining kit, according to the protocol provided. The cells were stained by the staining dyes, followed with wash and trypsinization. The cell was centrifugally collected and re-suspended. The cell was distributed in the glass substrates for fluorescent microscopy observation. As shown in Fig. 4, the cell stained with red and green cells represent dead and living cells respectively. There is not much cell adhesion density difference between the unmodified and aminolyzed IOL materials. Both of them with plenty cell on their surface. When the surface was modified by the multilayer, the initial cell adhesion is greatly decreased. In our previous studies, we have showed that the Hydrated polysaccharide multilayer coating can resistant the cell and bacteria adhesion after a certain bilayer of the multilayer coatings [14,18]. Herein, even 1 bilayer of the drug loaded polysaccharide multilayer renders remarkable cell number decrease after 24 h' incubation. This may be the combined effect of the hydration and the anti-proliferative drug. The cell apoptosis is much more obvious in the multilayers with higher bilayer numbers. These results indicate that the drug loaded polysaccharide multilayer coatings not only can effectively resist the initial cell adhesion, but also induce the cell apoptosis on the surface.

The drug eluting polysaccharide multilayer coating was then used to surface modify the intraocular lens and implant into the rabbit eyes for in vivo evaluation. The animal experiment of this study was approved by Laboratory Animal Ethics Committee of Wenzhou Medical