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New strategy for design and fabrication of polymer hydrogel with tunable porosity as artificial corneal skirt

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ABSTRACT

In order to obtain an ideal material using for artificial corneal skirt, a porous polymer hydrogel containing 2-hydroxyethyl methacrylate (HEMA), trimethylolpropane triacrylate (TMPTA) and butyl acrylate was prepared through one-step radical polymerization method and the usage of $CaCO_3$ whisker as porogen. The physical-chemical properties of the fabricated polymer hydrogel can be adjusted by $CaCO_3$ whisker content, such as pore size, porosity, water content of materials and surface topography. Then a series of cell biology experiments of human corneal fibroblasts (HCFs) were carried out to evaluate its properties as an artificial corneal skirt, such as the adhesion of cells on the materials with different pore size and porosity, the apoptosis on materials with different characteristics, the distribution of the cells on the material surface. The results revealed that high porosity not only could improve water content of hydrogel, but also strengthen the adhesion of HCFs on hydrogel. In addition, high porosity hydrogel with the whisker shape of pores showed much elongate spindle-like morphology than those low porosity hydrogels. MTT assay certified that the resulted polymer hydrogel material possessed excellent biocompatibility and was suitable for HCFs growing, making it promising for being developed as artificial corneal skirt.

1. Introduction

Cornea, as the anterior wall of eyeball, is a transparent membrane and plays an important role in our dioptric system. The lesion of cornea will lead to visual acuity decrease and even blindness, which need to undergo corneal transplant [1]. There are about 10 million people suffering from blindness caused by the corneal disease in the world [2]. The traditional method for the treatment of corneal disease is human donor cornea transplant. However, corneal transplants were not always possible in many parts of the world due to the limitation of storage and high cost. In recent decades, many researchers devoted themselves to develop artificial cornea based on chemical or physical synthesis materials [3–5], processing from glass to plastics, to subsequent soft rubbers. And most recently, hydrogels have been extensively studied and used in biomedical device, such as Poly(2-hydroxyethyl methacrylate) (PHEMA), Polymethyl methacrylate (PMMA), Polyvinyl alcohol (PVA), Polyacrylate and so on [6]. Among them, has received a great deal of attention for their applications in various fields, such as tissue engineering [7], drug delivery vehicle [8] and ophthalmic application [9] owing to its excellent biocompatibility, adequate mechanical strength and non-cytotoxic characteristic etc. Therefore, PHEMA has been considered to be a potential substitute for cornea [10].

On the other hand, the keratoprosthesis extrusion is a big challenge for artificial cornea which usually lead to the failure of transplantation. In order to achieve the high stability and biocompatibility of the artificial corneal skirt, mimicry of pore structure under the limbal region of the eye is usually carried out for cell binding, migration and adhesion [11,12], which could offer channels for sufficient nutrients and waste transference, further facilitating cells proliferate into the artificial corneal skirt to settle the extrusion. Up to date, a variety of techniques for fabricating materials with porous structures have been developed, such as freeze-drying [13], gas foaming [14], phase separation [15], and electrospinning [16,17]. However, these methods usually suffer the obstacle of large pore size. Besides that, the pores of porous materials prepared by these strategies are usually spherical or long fibrous pores [18–21]. According to "contact guidance" [22], cells are prone to adhere on the surface of artificial material whose structure is similar with the corneal surface because the similar structure is in favor of cell identification. In consideration of that the human corneal fibroblasts (HCFs) are fusiform, spherical or long fibrous pores are unfavorable for cell immobilization, leading to keratoprosthesis extrusion.

Herein, we developed a new strategy to fabricate fusiform porous hydrogel material with tunable porosity as an artificial corneal skirt. As shown in Schemes 1 and 2, HEMA was chosen as the main synthetic raw material and moderate trimethylolpropane triacrylate (TMPTA) and butyl acrylate (BA) were copolymerized with HEMA by radical polymerization to improve the mechanical property of the as-prepared hydrogel material. The fusiform $CaCO_3$ whisker was chosen as the porogen to make fusiform porous structure inside the hydrogel material due to its similar shape with HCFs. And the porosity could be controlled by the content of $CaCO_3$ whisker added into the polymer. Owing to the similar

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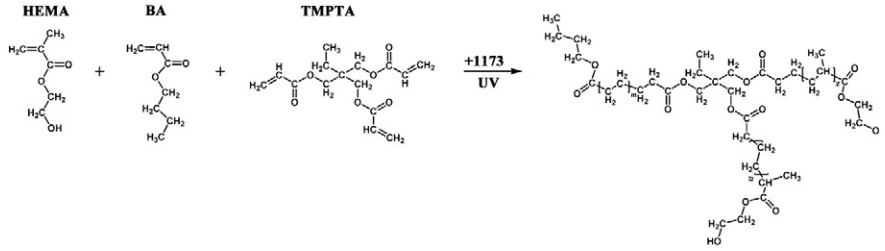


Scheme 1. Schematic representation of radical polymerization of the HEMA, BA and TMPTA.

structure between HCFs and the pores of as-prepared hydrogel material, "contact guidance" can promote the firm adhesion and elongation of HCFs along the hydrogel material, effectively preventing keratoprosthesis extrusion. In addition, the physical-chemical characterizations of the fabricated material were analyzed, such as pore size, porosity, water content, surface topography. What's more, a series of cell biology experiments of HCFs were carried out, such as the adhesion of cells on the materials with different pore size and porosity, the apoptosis on materials with different characteristics and the distribution of cells on the material surface. In a word, the purpose of our research is to develop an ideal corneal substitute with reasonable environment for cells to adhere and extend.

2. Materials and methods

2.1. Materials

2-hydroxyethyl methacrylate (HEMA), trimethylolpropane triacrylate (TMPTA), butyl acrylate (BA) were purchased from Aladdin. 2-phenyl-hydroxy-2-methyl-1-1-acetone (photoinitiator 1173) was provided by Bangsheng electronic material company (Guangdong, China). $CaCO_3$ whisker (Shanghai, China), Hydrochloric Acid (HCl), Anhydrous ethanol solution were purchased from China National Pharmaceutical Group Corporation (China). Ham's F12, Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), Phosphate buffered saline (PBS), formaldehyde solution were purchased from Hyclone. Hematoxylin, Eosin, neutral balsam, MTT cell proliferation and cytotoxicity assay kit were purchased from Sigma-Aldrich. All chemicals were of analytical grade and used as received without further purification.

2.2. Preparation of hydrogel

As shown in Schemes 1, 2-hydroxyethyl methacrylate (HEMA), trimethylolpropane triacrylate (TMPTA), butyl acrylate (BA) were mixed in a beaker at predetermined ratio (20:1:2) to make a homogenous blend prior to polymerization. Then $CaCO_3$ whisker and photoinitiator 1173 (5 wt% monomers) were added into the solution. For producing an ideal polymer sheets, we designed a mould consisting of two pieces of 5 mm thick glass plates covered with PET film and the plates were separated with a 2 mm thick rectangular rubber pads (Scheme 2). The compound solution was poured into the mould and irradiated with 2000 W UV lamp for free radical polymerization (5 min each side). Thereafter, the resultant polymer sheets were soaked in the deionized water for 7 days, then immersed in gradient hydrochloric acid to etch the $CaCO_3$ whisker. Finally, the polymer sheets were infused in 30% ethanol solution for 7 days to clear away any unreacted monomers and the uncross-linked oligomer. The control group was prepared under the same procedure as described above except no $CaCO_3$ whisker. In order to regulate the porosity of the polymer hydrogel, 8 samples were prepared with different $CaCO_3$ whisker content, as shown in Table 1. The polymerization efficiency were measured and expressed by gel fraction method through the ratio of polymer hydrogel weight from ethyl alcohol and the initial monomer weight [23].

2.3. Porosity measurement

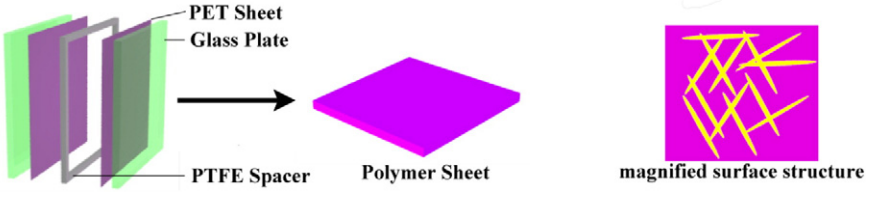
Porosity was determined according to the Archimedes' principle, the equation was as follows [24,25]:

$$\%\text{Porosity} = 100(m_2 - m_1)/(m_2 - m_3)$$

where m_1 represents the mass of dry hydrogel, m_2 is the mass of swollen hydrogel in the air, and m_3 represents the mass of swollen hydrogel suspended in water. The measurement was performed in triplicate for each type of sample.

2.4. Swelling measurement

The water content (WC) of the polymer hydrogels were evaluated in terms of the ratio of swollen-weight to dry-weight. The experimental procedure was as follows: the dry hydrogels were weighed and immersed in water to swell. Then the swollen hydrogels were lifted at regular intervals, patted to dry the surface water by filter paper, weighed



Scheme 2. The surface structure of hydrogel material. The yellow pattern is the fusiform pores made by $CaCO_3$ whisker.

Table 1
$CaCO_3$ whiskers content added in blend and polymerization efficiency of monomers.

Sample	Control	Hydrogel 1	Hydrogel 2	Hydrogel 3	Hydrogel 4	Hydrogel 5	Hydrogel 6	Hydrogel 7	Hydrogel 8
$CaCO_3$ content (g/10 g solution)	0	5	6	7	8	9	10	11	12
Gel fraction (%)	99.40	98.77	98.51	98.26	97.85	97.78	97.73	97.68	97.59