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Preparation and *in vivo* investigation of artificial cornea made of nano-hydroxyapatite/poly (vinyl alcohol) hydrogel composite

Xu Fenglan · Li Yubao · Yao Xiaoming · Liao Hongbing · Zhang Li

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Abstract An artificial cornea consisted of a porous nano-hydroxyapatite/poly (vinyl alcohol) hydrogel (n-HA/PVA-H) skirt and a transparent center poly (vinyl alcohol) hydrogel (PVA-H) were prepared. The n-HA/PVA-H skirt was homogeneously porous and these pores were interconnected. Inter-penetrating network was observed along the interface between the core and the skirt. Artificial corneas were implanted in eyes of rabbit. The corneal tissues were evaluated histological. The results displayed that a good biocompatibility and interlocking had happened between artificial cornea and host tissues. This novel cornea prepared here is potential to be used clinically.

1 Introduction

Corneal blindness caused by herpetic disease, acid or alkali burns and various diseases such as rheumatoid arthritis, pemphigoid and Stevens Johnson's syndrome cannot be treated with corneal transplantation. However, the vision can be restored with a clear optic of artificial cornea. Unfortunately, the use of artificial cornea is often associated with a series of complications [1–2]. Most artificial corneas had failed due to the poor compatibility between the artificial implant and host cornea, as well as the poor attachment of the transparent center to the rim [3–6]. These problems drive scientists to search for more suitable materials used as the supporting rim. The rim should be porous, long-term biocompatibility and hydrophilic in the eye to encourage tissue ingrowth and the penetration of body fluids, and thus improve the adhesive strength between the rim and the transparent center.

HA is the main mineral of bone and tooth, and has long-term biocompatibility and favorable interaction with soft tissue and bone [7–10]. León CR et al had firstly used the porous HA as the support of an artificial cornea [11]. The results had shown the artificial cornea had good vascularization, and there had no signs of infection, extrusion and epithelial downgrowth. Because porous HA is brittle and difficult to be prepared and sutured, the use of hybrid materials consisted of HA and polymer may be a good choice.

The research is engaged in developing an improved artificial cornea consisted of a skirt made of porous nano-hydroxyapatite/poly (vinyl alcohol) hydrogel (n-HA/PVA-H) and a transparent center poly (vinyl alcohol) hydrogel (PVA-H). PVA-H has high strength, elasticity and high water content, so it has been employed in some biomedical applications, especially as the core of artificial cornea [12–16]. Porous n-HA/PVA-H skirt is favorable for improving the biocompatibility, hydrophilicity and flexibility. Therefore, it is hopeful to achieve a tight attachment of the skirt to the core through the inter-penetration between them.

2 Materials and methods

Poly (vinyl alcohol) (PVA) was manufactured from Chongqing Beipei Chemical Co. Ltd, P. R. China. (Hydrolysis degree 99%, residual acetate groups content 0.13%, and a degree of polymerization of 1700 ± 50). Calcium nitrate, sodium chloride, ammonium hydroxide and ammonium phosphate were all AR grade and from Chengdu Chemical Agent Co. Ltd, P. R. China.

X. Fenglan · L. Yubao (✉) · L. Hongbing · Z. Li
Research Center for Nano-Biomaterials, Analytical and Testing Center, Sichuan University, 610064 Chengdu, P. R. China
e-mail: nic7504@scu.edu.cn

Y. Xiaoming
Shenzhen Eye Ophthalmic hospital, 518001 Shenzhen, P. R. China

2.1 Preparation of n-HA

$$10\,Ca(NO_3)_2 + 6(NH_4)_3PO_4 + 8\,NH_4OH \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 20\,NH_4NO_3 + 6\,H_2O$$

Calcium nitrate and ammonium phosphate were respectively dissolved in deionized water. Calcium nitrate solution was dropped slowly into the ammonium phosphate solution with continuous stirring and heating at 70°C. The pH value of the solution was kept about 10 adjusted with ammonium hydroxide. The obtained precipitation in water solution was treated in an autoclave at 140°C under 0.3 MPa for 3 h [17]. The n-HA slurry was kept for the following tests.

2.2 Manufacture of artificial cornea

PVA particles and n-HA slurry was added into organic solvent with stirring and heating at 90°C. Then, NaCl crystals were added into the organic solvent. The mixed solution was poured into a mold for crosslinking. The material with NaCl crystals was cut to form a 5.5 mm diameter cylinder in the center. The composite was heated to ensure the penetration of pure PVA solution into the material with NaCl crystals after the PVA solution was injected into the cylinder. Then it was crosslinked. The obtained composite consisting of a circular transparent region (PVA-H) surrounded by a white opaque ring (n-HA/PVA-H) was immersed into distilled water for at least 5 days with daily water exchange to remove the organic solvent and NaCl crystals. The composite hydrogel was frozen and then cut into a thin disk with a size of 10 mm in the diameter, 0.4 mm in thick. The porosity of the disks' skirt was about 50% and the pore size was 80 um. The optic of the disks was 5.0 mm in diameter.

2.3 Morphology observation

The cross-sections of the gold-coated samples, which were dehydrated by the critical point drying technique, were observed by a scanning electron microscopy (SEM) operated at an accelerating voltage of 25 kV. Porosity and average pore size were determined using the software image analysis system of SEM. The porosity was the ratio of pore area to all surface area on SEM photo. The pore size distribution was an average value of different pore size determined by measuring pores' size on the SEM photo.

Transmission electron microscopy (TEM) was employed to detect the morphology of the composite particles in a nano size, in which n-HA crystals were wrapped by PVA.

2.4 Light microscopy

The core-skirt disk was examined and photographed with Diaplan light microscopy (Germany). From the micrograph, the area of the inter-penetration network at the interface between the center of PVA-H core and n-HA/PVA-H skirt was also measured.

2.5 Animal experiments

Ten n-HA/PVA-H artificial corneas were sterilized and implanted into the interlamellar stromal pockets of 10 New Zealand White rabbits. The pure PVA hydrogel was used as a control. These rabbits were generally anaesthetized by intramuscular injections of ketamine (50 mg/kg) and chlorpromazine (25 mg/kg), and incisions were made in a sclera at 0.5 mm distance from the corneal limbus, (11 mm in length, 4/5 thickness of sclera in depth). Then, n-HA/PVA-H artificial cornea was inserted into the interlamellar stromal pockets. The incisions were closed with 10/0 nylon sutures, and injections of 20000u gentamicin and 2.5 mg dexamethasone were applied postoperatively. After that, 0.3% ofloxacin antibiotic ointment was daily applied during the first week. Postoperatively, the rabbits were followed periodically by slit lamp microscopy up to 6 months. The corneal findings were documented and slit lamp photographs were also taken to observe the depth (shallow and normal) and inflammation in the anterior chamber. Two rabbits were sacrificed at 1, 2, 3, 4, 6 months postoperatively. Then the corneas were excised and fixed with 4% polyformaldehyde, buried with paraffin, stained with HE. All tissue samples were evaluated and photographed using light microscope.

3 Results and discussion

3.1 Surface morphology of the n-HA/PVA-H skirt

The porous n-HA/PVA-H skirt was prepared by the salt-leaching method. It could be seen from Fig. 1a and 1b that the skirt prepared without NaCl had a dense surface, while the other had a porous structure. The porosity and pore size depended on the content and the sizes of the added NaCl particles. Fig. 2a showed that the scaffold had 3-dimensional pores and Fig. 2b displayed that there were many nanometer pores in the scaffold. The n-HA/PVA particles shown in Fig. 2c were in nanometer grade. This architecture may provide not only channels for improving mass transportation of host tissues when implanted but also better environments for the distribution, adhesion and growth of stromal fibroblasts.

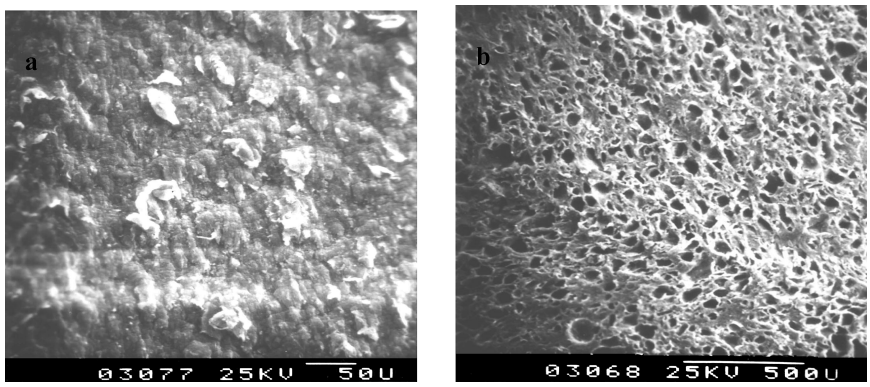

Fig. 1 SEM images of n-HA/PVA-H composite with NaCl 0% (a) and NaCl 50% (b)

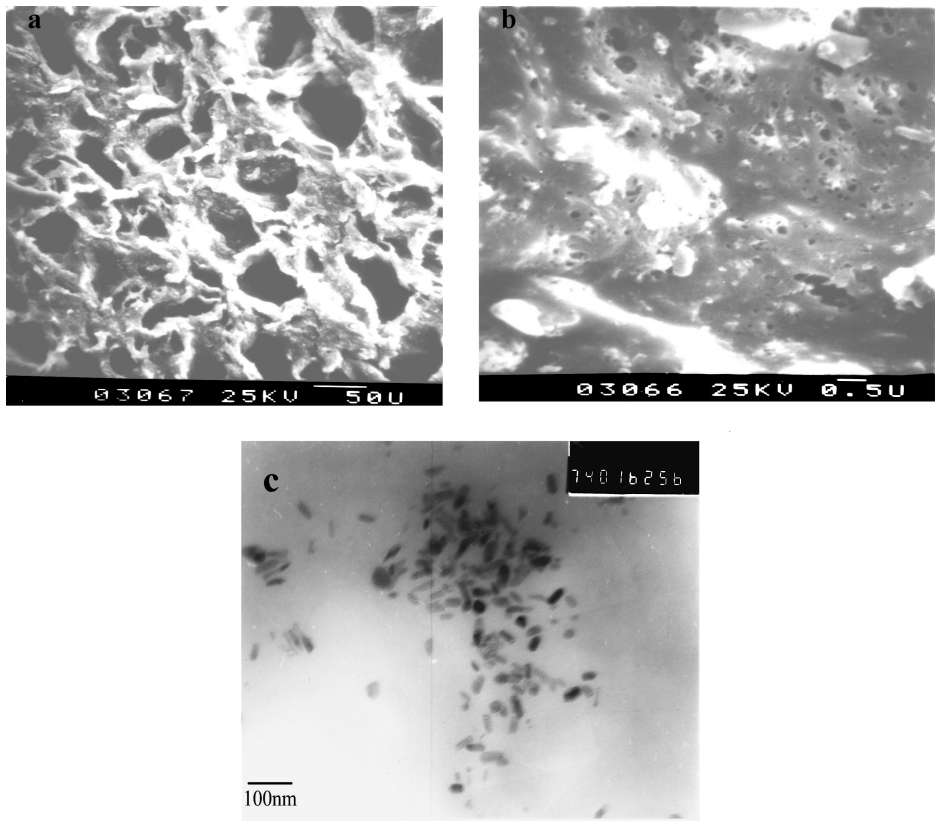

Fig. 2 n-HA/PVA-H composite with NaCl 50% (a) SEM image (b) SEM image (c) TEM image

3.2 Inter-penetrating network between the core and the skirt

Figure 3 showed the interface between PVA-H core and n-HA/PVA-H skirt. The core and the skirt were linked and fused together by the inter-penetrating network. This attachment of the optic to the rim is firmer than screws [18–19], clips [20] and glues [3, 21, 22], which were too weak for long-term stability and safety after being implanted. The interface union was achieved by polymerizing the PVA core within n-HA/PVA-H skirt. It was well known that it is difficult to bond two different polymers because there are many differences in their nature and properties. However, the nature and properties of the PVA-H core is identical to the organic phase of n-HA/PVA-H skirt. Along the boundary, the liquid PVA solution penetrated into the n-HA/PVA-H to a certain distance and insured the formation of an inter-penetrating network. A transition zone about 300–400 μm in distance existed between the opaque skirt and the optic. The inter-penetrating network at the interface of the prosthesis was rigid, which eliminated potential interface problems, such as optic dislocation, leak and prosthesis extrusion.

3.3 Structure of artificial cornea

Many complications resulting from lack of tissue growth into the interaction between the artificial implant and host cornea lead to tissue necrosis and ultimately in the extrusion of the

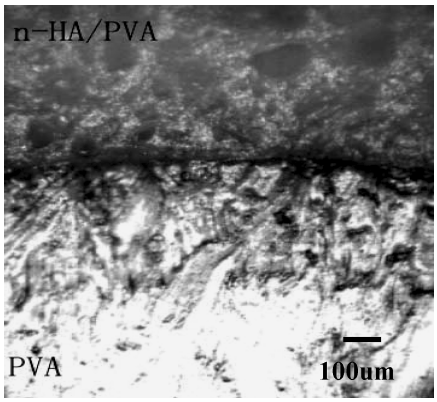

Fig. 3 Photomicrograph of interface between the core and the skirt

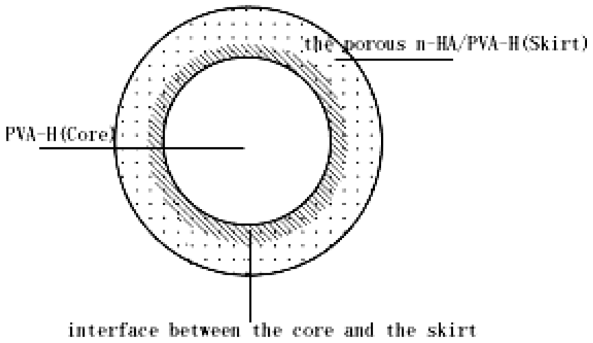

Fig. 4 The sketch of the artificial cornea

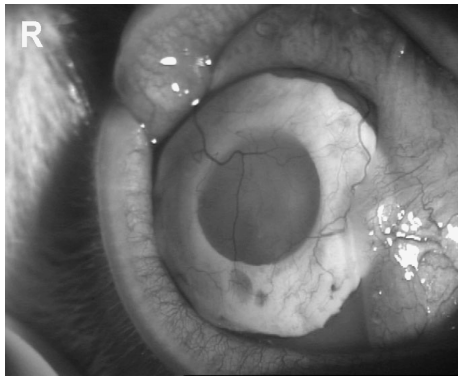

Fig. 5 The new blood vessels were observed within the skirt material of artificial cornea after one month operation (magnification: ×7.5)

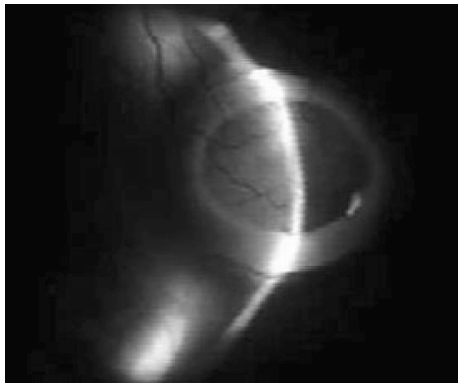

Fig. 6 Normal epithelium and endothelium of cornea structure were seen, and there was no evidence of corneal stroma inflammation after 3 months (magnification: ×5)

artificial cornea [5]. That materials used here allow tissue growth into the supporting rim could be overcome these problems. Many animal tests have demonstrated the long-term biocompatibility of HA and its favorable interaction with soft tissues and bone [7–10]. The introduction of HA may be favorable for the development of the artificial cornea. But HA was brittle. So we developed a novel artificial cornea consisting of a PVA-H optic core and a porous n-HA/PVA-H skirt (Fig. 4). HA in the porous n-HA/PVA-H skirt will activate the cell's adhesion and growth and thus form a favorable interface between the skirt and host tissues. The n-HA/PVA-H skirt was porous, hydrophilic and soft, so that the skirt could avoid mechanical disruption and encourage the penetration of biological fluids, cells and tissues. Furthermore, the formation of chemical bonding between n-HA and PVA, such as hydrogen bonding and/or hydroxyl-calcium-hydroxyl ([HO-]-Ca^{2+}-[-OH]) bonding, allowed the uniform dispersion of n-HA crystals [23] in the porous n-HA/PVA-H skirt, were also helpful to improve the mechanical strength of the sponges, thus avoided tearing the skirt by the surgical sutures.

3.4 Slit lamp microscopic examination and histology examination

The corneas of the rabbits were observed postoperatively for a period of 6 months by slit lamp microscopy. Signs of eyelid swelling, conjunctiva congestion and secretion increase were observed at the first 3 days and then disappeared after one week postoperatively. New blood vessels were observed at the surface layer of cornea limbus on 6th day and the vessel growth reached its climax on the third month, and mostly advanced into stroma layer after 6 months. Fibroblasts and new blood vessels were observed within the skirt material after 1 month (Fig. 5). No gap was seen between the skirt and cornea stroma interface. Normal epithelium and endothelium of cornea structure was observed, and there was no evidence of corneal stroma inflammation after 3 months (Fig. 6). All implanted materials were fixed in initial position without infection, extrusion, epithelial downgrowth or glaucoma. The artificial corneas offered the better interface, and no encapsulation, corneal inflammation and vascularization, which indicated that the porous n-HA/PVA-H skirt has good biocompatibility and favorable interaction with host tissues.

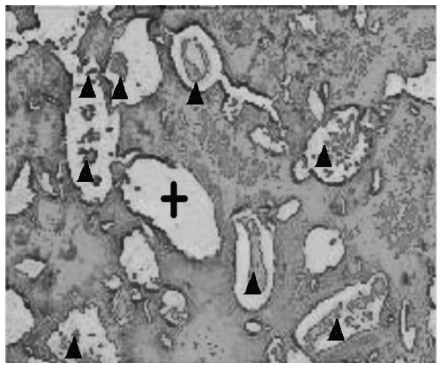

Fig. 7 Histological picture of an eye with artificial cornea after six months operation ((magnification: ×100). Collagen (▲) was observed in the pores (+)

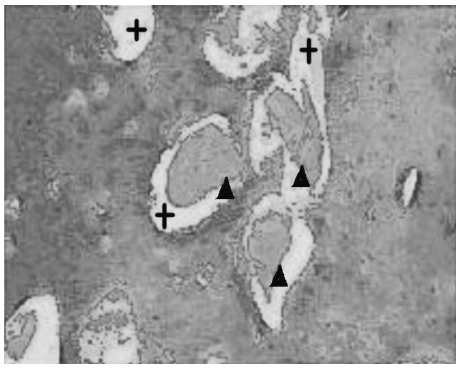

Fig. 8 Histological picture of an eye with artificial cornea after six months operation (magnification: × 200). Collagen (▲) was observed in the pores (+)

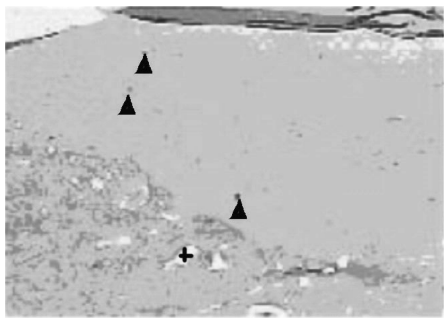

Fig. 9 Histological picture of the interface between the core and the skirt after six months operation. Collagen (▲)was observed in the interface (magnification: ×100, + representative of the pore)

And it proved that the use of hybrid materials consisted of HA and PVA was a good choice to make artificial cornea.

More information on the cellular and biological responses to the porous n-HA/PVA skirt was obtained through a histological study in which artificial corneas were implanted in healthy rabbit corneas and then removed for histological examination. Host tissues ingrowth was observed and no adhesion of epithelia cell and inflammatory cells after 6 months operation happened (Figs. 7 and 8). Collagen and blood vessels were observed at interface between the core and the skirt without adhesion of epithelia cell and inflammatory cells (Fig. 9). These results showed that the porous n-HA/PVA skirt promoted the bio-integration of host tissues into the implanted material and interlockage with the host cornea.

4 Conclusions

A novel artificial cornea consisting of a PVA-H optic core and a porous n-HA/PVA-H skirt was prepared in the research. The PVA-H and n-HA/PVA-H were combined together at the interface by inter-penetrating network between the core and the skirt. So the firm attachment of the porous skirt to the transparent center was obtained. The porous skirt can improve the biocompatibility of the prosthesis with the host tissue and also make it be able to interlock with the host tissue. The design of new artificial cornea may limit the complications resulting from lack of tissue growth into the interaction between the artificial implant and host cornea. So the new artificial cornea will be promising in clinical applications.

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