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A rabbit anterior cornea replacement derived from acellular porcine cornea matrix, epithelial cells and keratocytes

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ABSTRACT

The aim of this study was to construct a rabbit anterior cornea replacement with an acellular porcine cornea matrix (APCM) as a scaffold. The scaffold was prepared from fresh porcine corneas which were treated with 0.5% (wt./vol.) sodium dodecyl sulfate (SDS) solution and stirred for 24 h in a 4 °C refrigeration chamber. The complete removal of corneal cells was confirmed by H&E and DAPI staining. The stroma structure and mechanical properties were well preserved. The extracts had no cytotoxicity to rabbit corneal keratocytes, epithelial and endothelial cells as determined by MTT assay. Moreover, there was no sign that an immune reaction occurred in or around the transplanted disks within 6 months of animal implantation. To construct a rabbit anterior cornea replacement, keratocytes were injected into APCM and cultured for 7 days in a dynamic culturing system, followed by culturing corneal epithelial cells on the stroma construct surface for another 7 days. The phenotype of the construct was similar to normal rabbit corneas, with high expression of cytokeratin 3 in the epithelial cell layer and expression of vimentin in the stromal cells. These results suggested that the APCM developed by using SDS might be a suitable scaffold for cornea tissue engineering.

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1. Introduction

The healthy cornea is major element in the optical pathway of the eye and also serves as a physical barrier to the outside environment [1]. More than 10 million individuals worldwide [2] suffer from corneal blindness due to disease and trauma [3]. Up to now, the only widely accepted treatment is corneal transplantation using human donor tissue. However, there is an increasing need for human donor corneal tissue and a severe shortage of suitable cornea donors in many countries with an increase of the old-aged population and increased use of corrective laser surgery [4]. Therefore, many groups have tried to fabricate cornea substitutes including keratoprosthesis and tissue-engineered corneal equivalents that would overcome the present disadvantages of allografts. Early corneal replacements, mainly keratoprosthesis (KPro) [5,6], have been on-going for over a century. However, none of the current KPros used clinically combines all the desirable features within one model and usually have severe complications due to the poor biocompatibility and inadequate interaction of the artificial implant with the host cornea [7,8].

Tissue-engineered corneal equivalents have experienced major progress during the past few years. Many researchers have constructed full thickness corneal substitutes similar to the native cornea by using natural or synthetic polymers with corneal cells, but these corneal replacements have been limited to in vitro applications [1,9–12].

Due to the cornea special biological characteristics, an ideal scaffold for a tissue-engineered cornea should have good biocompatibility, high optical clarity, toughness to withstand surgical procedures, and non-immunogenicity properties [13]. To date, three-dimensional scaffolds for corneal equivalents were most often fabricated by collagen [9,13,14] and fibrin [15], as data showed that materials based upon native extracellular matrix (ECM) macromolecules could enhance biocompatibility [13,16–18].

Recently, a new scaffold based on native tissues for tissue engineering has become more attractive. Acellular ECM of porcine origin from a variety of tissues, including heart, heart valves, blood vessels, skin, small intestinal submucosa and urinary bladder have been studied for tissue engineering and regenerative medical applications [19–27]. As reported, a collagen scaffold fabricated with porcine collagen appeared to be well tolerated after

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heterogeneous implantation [28], and the porcine cornea has anatomic similarity to the human cornea [29] as well as its rich resources. Hence, the native porcine cornea ECM without the hereditary materials might be an alternative corneal scaffold. Some researchers had tried different methods to decellularize porcine corneas, and obtained results [30–33]. However, whether their acellular cornea matrix could be used for cornea replacement construction has not yet been proved. Additionally, some hereditary materials were not completely removed, which may have the potential to transmit infectious agents. Fu at al [34] recently constructed a tissue-engineered cornea with a porcine acellular corneal matrix developed by Triton X-100, but if the scaffold had enough toughness to withstand surgical procedures for transplantation was unknown. Moreover, the keratocytes were sparsely and non-uniformly distributed in their construct.

In this study, an acellular porcine cornea matrix (APCM) was developed by using SDS which is an anionic surfactant usually used for cell lysis. The residual cellular components including lipids, proteins, and nucleic acids were removed by a washing process using sterile PBS. The key properties of the cornea including high optical clarity and the toughness to withstand surgical procedures were well preserved, and favorable biocompatibility was confirmed. Subsequently, a rabbit anterior cornea replacement containing epithelium and part of the stroma was constructed for lamellar keratoplasty (LKP).

2. Materials and methods

2.1. Animals

Adult porcine eyes were obtained from a local slaughterhouse (Jinan Welcome Food Co., Ltd, Jinan, China) within 3 h postmortem, and subjected to decellularization procedure within 1 h of acquisition. Twenty New Zealand white rabbits (Center for New Drugs Evaluation of Shandong University, Jinan, China) weighing 1.5 ~ 2.0 kg were used for animal transplantation and cell culture. All animals were treated in accordance to the ARVO (Association for Research in Vision and Ophthalmology) Statement for the Use of Animals in Ophthalmic and Vision Research. All animal experiments were approved by the Medical Ethics Committee of Shandong University, China.

2.2. Corneal cells culture

Before cell dissection, the rabbit corneal tissues were rinsed three times with DMEM (Invitrogen) containing 100 U/ml penicillin and 100 U/ml streptomycin (Shandong Lukang Pharmaceutical co., Ltd, Jining, China).

After careful removal of excessive sclera, iris, corneal endothelium, conjunctiva and Tenon's capsule, the limbal rings were incubated at 37 °C for 1 h with 2.4 U/ml Dispase II (Roche, Basel, Switzerland). The limbal epithelial sheets were separated from the residual corneal stroma under a dissecting microscope using two fine forceps by means of gentle horizontal movements, and further digested with 0.25% Trypsin/0.02% EDTA (Sigma) at 37 °C for 5 min to isolate single cells.

Corneal endothelial cell sheet were mechanically isolated from the rabbit cornea Descemet's layer under a dissecting microscope. And then, the cell sheets were planted on 60-mm culture dishes for tissue culture.

To obtain keratocytes, the corneal stroma deprived of epithelial and endothelial cells was cut into small pieces, and 2 mm × 2 mm explants were attached to 60-mm culture dishes to allow corneal keratocytes to grow.

A 1:1 mixture of Dulbecco's minimal essential medium and Ham's F12 medium (Invitrogen) supplemented with 10% fetal bovine serum (Invitrogen), 100 U/ml penicillin and 100 U/ml streptomycin was used as a growth medium for all the cells. All cultures were maintained under standard conditions at 37 °C in a humidified atmosphere containing 5% CO_2. The growth medium was replaced every two days. All cells used in our experiments were at passages 1–4.

2.3. Preparation of scaffold

Before the decellularization process, fresh porcine corneas were washed 3 times in sterile phosphate buffered saline (PBS, Beyotime, Nantong, China), and then trimmed to 10 mm diameter sections by a pair of curved scissors. To remove the hereditary materials, the cornea discs were immersed in a 0.5% (wt./vol.) SDS (Sigma) solution with a solvent/tissue mass ratio of 20:1 (vol./wt.). The samples were placed on an orbital shaker for 24 h in a 4 °C refrigeration chamber and then rinsed 8 times in sterile PBS for 16 h. Subsequently, the APCM was washed 3 times in sterile PBS supplemented with 200 U/ml penicillin and 200 U/ml streptomycin for 3 h. Finally, the scaffolds were freeze-dried at −20 °C for 12 h, air-dried at room temperature for 3 h in a biological safety cabinet, and stored at −20 °C before use. All steps were conducted under sterile conditions.

2.4. Mechanical properties

According to a previously reported method [35], the tensile strength, elastic modulus and elongation at break of the APCM were measured by using an Instron electromechanical universal tester (Instron, Canton, MA) equipped with Origin 7.0 software. The specimens were kept wet using PBS and cut into 10 mm × 3 mm rectangular strips. The load range was set at 0–500 g, and the crosshead speed was 10 mm min^{-1}. Native porcine corneas were a control. Statistical analysis was performed using a one-way analysis of variance (ANOVA). The statistical significance was set at $P < 0.05 (n = 4)$.

2.5. Cytotoxicity of extractable materials

To determine whether the extracts from APCM would cause cytotoxicity, each scaffold (10 mm diameter) was extracted using 5 ml 1:1 mixture of Dulbecco's minimal essential medium and Ham's F12 medium containing 10% fetal bovine serum at 37 °C for 48 h. Rabbit corneal keratocytes (2×10^3), epithelial (3×10^3) and endothelial (3×10^3) cells were seeded into each well of 96-well plates and cultured with extracts (the experimental group, $n = 6$) or normal medium (control group, $n = 6$). The proliferation activity of the cells was quantitatively determined at 1, 3, 5, and 7 days by a MTT assay. The optical density (OD) value of absorbance at 490 nm was measured by a microplate reader (InTec Reader 2010, USA). Differences in the OD value between experimental and control groups were analyzed statistically using a one-way ANOVA. Statistical significance was set at $P < 0.05$.

2.6. Implantation

APCMs were implanted into the right corneas of 10 New Zealand White rabbits to evaluate scaffold biocompatibility. First, ten APCM lamellas (150 μm, 6 mm diameter) were made using a microkeratome. Subsequently, 6.5 mm diameter pockets in the cornea of rabbits were made by a 2 cm incision under general anesthesia using an iris repositor, and then the cornea lamellas were implanted into the pockets. The incision was closed by using 10–0 nylon sutures. The non-operated, contralateral eye was used as a positive control. Subconjunctival injection of gentamicin and dexamethasone was used over the first post-operative week. Follow-up clinical examinations included slit-lamp examination to assess corneal optical clarity, neo-vascularization and degradation of grafts. After post-operative 2,4,12 and 24 weeks, 2 rabbits were euthanized respectively and the corneal specimens were examined by H&E staining. The remaining 2 rabbits were used for long-term observation.

2.7. Construction of corneal anterior lamellar with APCM

Before being freeze-dried during the decellularization process of the scaffold, a 1 mm thickness, 8 mm diameter APCM lamella containing Bowman's membrane was prepared using trephine and a scalpel under a dissecting microscope. The thin APCM was soaked in culture medium at 37 °C for 24 h before cell seeding. Cultured rabbit corneal keratocytes from passage 3 were trypsinized, and re-suspended at a final concentration of 5×10^5 cells/ml. Parallel to the surface of the scaffold, 1 ml of cell suspension was gently injected into the prepared APCM at eight different sector regions using a 1 ml insulin syringe. The cell scaffold constructs were kept in an incubator for 24 h to allow complete adhesion of the cells to the scaffold, and then they were transferred onto an orbital shaker and cultured for the next 7 days, at a rotation rate of 15 ~ 20 rpm. After 7 days culture on the orbital shaker, 2.5×10^5 cultured rabbit corneal epithelial cells from passage 1 for each scaffold ($5 \times 10^3/mm^2$) were gently seeded onto the surface of the reconstructed stroma and cultured for 7 days. All constructs were cultured at 37 °C in a humidified atmosphere containing 5% CO_2. The culture medium was exchanged every two days.

2.8. Microscopy and histology

For light microscopy, fresh porcine corneas, APCMs and constructed corneal anterior lamellas ($n = 3$, respectively) were fixed in 4% formaldehyde, and embedded in paraffin. 4 μm sections were stained with H&E, and viewed under a light microscope.

Samples for scanning electron microscopy were fixed in cacodylate- buffered 3% glutaraldehyde and post-fixed for 1 h in 1% osmium tetroxide. Then samples were gradually dehydrated by ethanol (50%, 70%, 95%, 100%), critical- point dried, and gold sputter-coated according to routine procedures [36]. Subsequently, specimens were examined in a scanning electron microscope (SEM, S-570, Hitachi, Japan).

For transmission scanning microscopy, samples were fixed, post-fixed, and dehydrated as described for scanning electron microscope and were infiltrated with Spurr's resin through a resin:ethanol series of 1:2, 1:1, 2:1, 100% Spurr's with continuous mixing on a rotator throughout the infiltration process, and cut into

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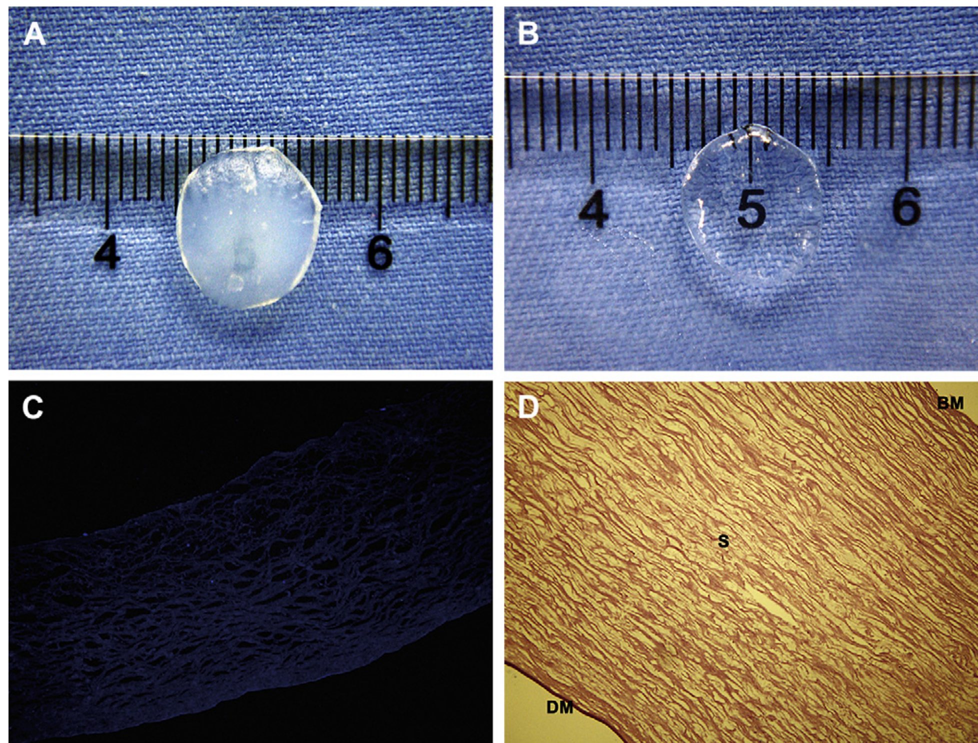

Fig. 1. Representative images and histological characteristics of the APCM. The scaffold was opaque due to the decellularized process (A), and transparency was restored when soaked in 100% sterile glycerol (B). H&E (C) and DAPI staining (D) showed that the cells or cell nuclear material were completely removed.

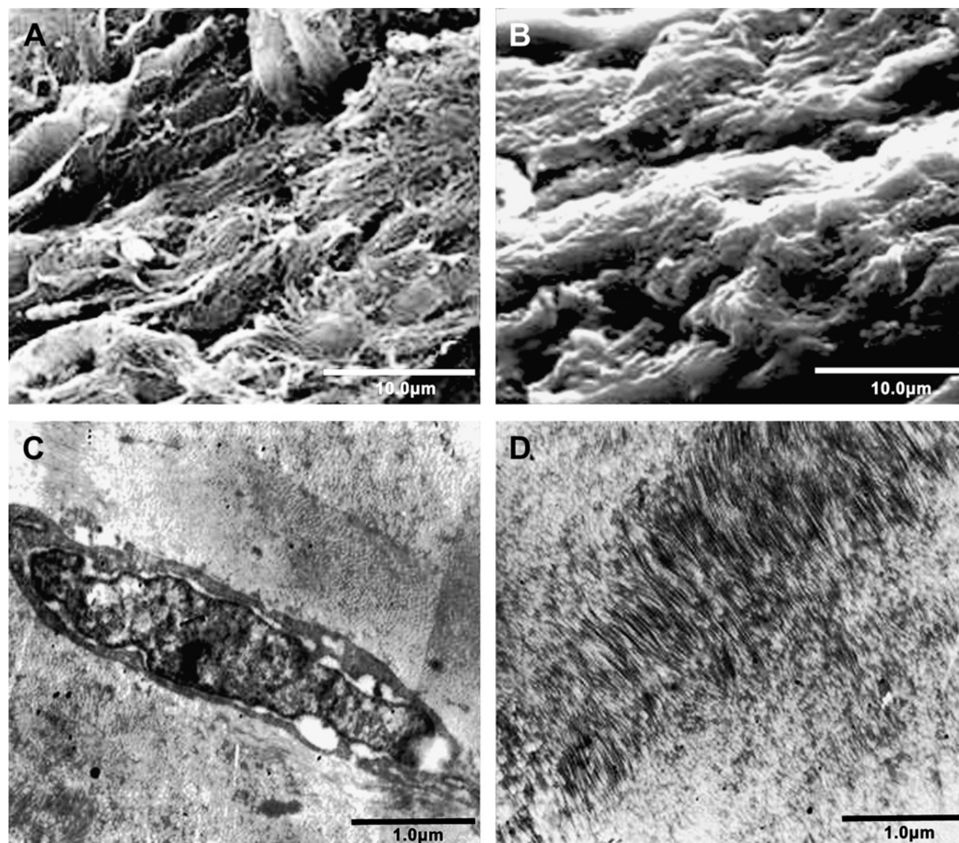

Fig. 2. Ultrastructure of the native porcine cornea (A, SEM and C, TEM) and APCM (B, SEM and D, TEM). The collagen of the acellular scaffold was regularly arranged with no cells among them (B, D). As a control, there was an intact keratocyte in native porcine cornea (C).

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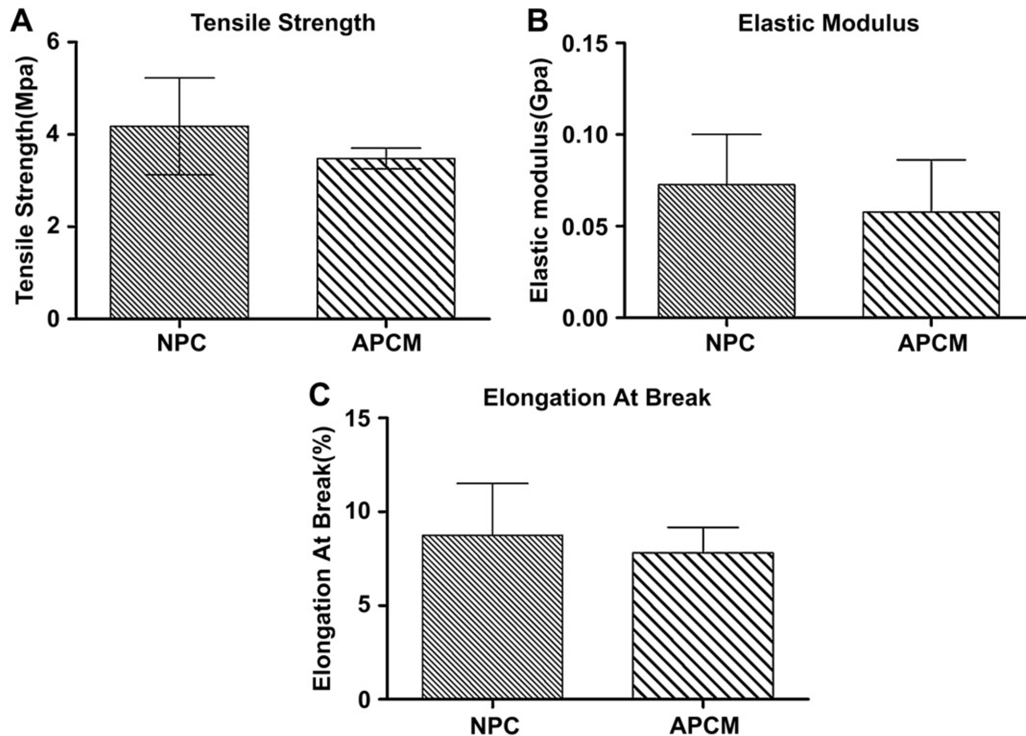

Fig. 3. Mechanical properties of the APCM and native porcine cornea (NPC). Values are expressed as the mean ± standard deviation ($n = 4$). Statistical analysis was performed using a one-way analysis of variance (ANOVA). The statistical significance was set at $P < 0.05$.

ultrathin sections with an ultramicrotome. Finally, the sections were stained with aqueous uranyl acetate and lead citrate and then viewed with a transmission electron microscope (TEM, H-7000FA, Hitachi, Japan).

2.9. Immunofluorescent staining

Immunofluorescence was performed as in a previously reported method [37,38]. In brief, the mouse monoclonal antibody for either anti-cytokeratin 3 (CK3, Abcam, San Francisco, CA), a marker of corneal epithelial cells [39], or anti-vimentin (Abcam, San Francisco, CA), a marker of keratocytes [40,41], was placed on the sections and primary corneal cell cultures fixed with 4% paraformaldehyde and incubated at 4 °C overnight (PBS as negative control). The final working concentrations for anti-CK3 or anti-vimentin were used at 1:200 and 1:100 dilutions, respectively. The secondary antibody, fluorescein-conjugated affinipure goat anti-mouse IgG (Zhongshan Goldbridge Biotechnology Co., LTD, Beijing, China), was applied for 30 min in a dark incubation chamber at room temperature. After washing in PBS, the specimens were mounted with 4′,6-diamidino-2-phenylindole (DAPI) nuclear staining and examined under a fluorescence microscope.

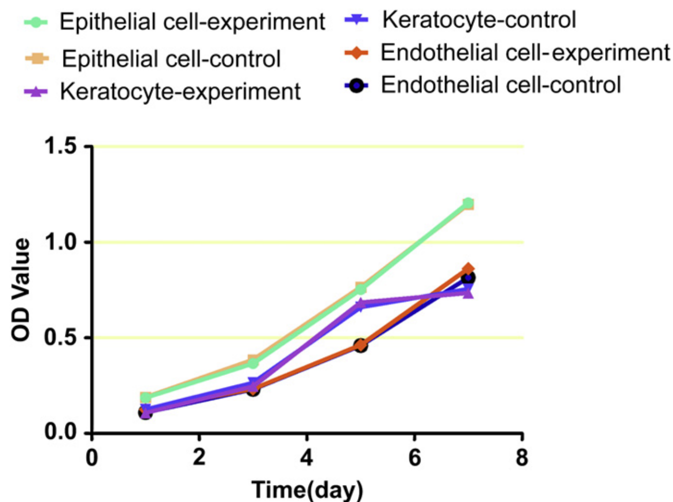

Fig. 4. MTT assay for cytotoxicity determination of the extracts from APCM.

2.10. Immunohistochemistry staining

Immunohistochemical staining was performed according to a previously reported method [42] to evaluate certain markers including CK3 and vimentin. After paraffin removal and microwave treated antigen retrieval, the sections were treated with 0.3% H_2O_2 in PBS to quench endogenous peroxidase activity and then incubated with 5% goat serum to block the non-specific sites. CK3 (1:200) or vimentin (1:100) mAb was applied and incubated at 4 °C overnight (PBS as the negative control), followed by incubation with peroxidase-conjugated affinipure goat anti-mouse secondary antibody (Zhongshan Goldbridge Biotechnology Co., LTD, Beijing, China) at 37 °C for 30 min. The specimens were finally incubated with 3,3′-diaminobenzidine (DAB) peroxidase substrate to give a brown stain and counterstained with hematoxylin.

3. Results

3.1. Characterization of the APCM

3.1.1. Microscopy and histology

The APCM was opaque after the decellularizied process on gross observation (Fig. 1 A). But after being soaked in sterile glycerol for 30 min, the scaffold transparency was restored (Fig. 1 B). No visible cells or cell nuclear material were observed in the APCM by H&E (Fig. 1D) and DAPI staining (Fig. 1C), and no cellular debris was left by TEM images (Fig. 2D). Additionally, TEM and SEM images both demonstrated that the collagen fibrils of the APCM were regular, with an increased collagen fibril spacing compared to the native porcine cornea matrix (Fig. 2). There was no sign of disruption or degradation of collagen fibril.

3.1.2. Mechanical properties

The tensile strength (Mpa) of APCM was 3.4775 ± 0.1584, close to that of native porcine corneas (4.1750 ± 0.7438, $n = 4$, $P > 0.05$), and the elastic modulus (Gpa) was 0.0575 ± 0.0287,

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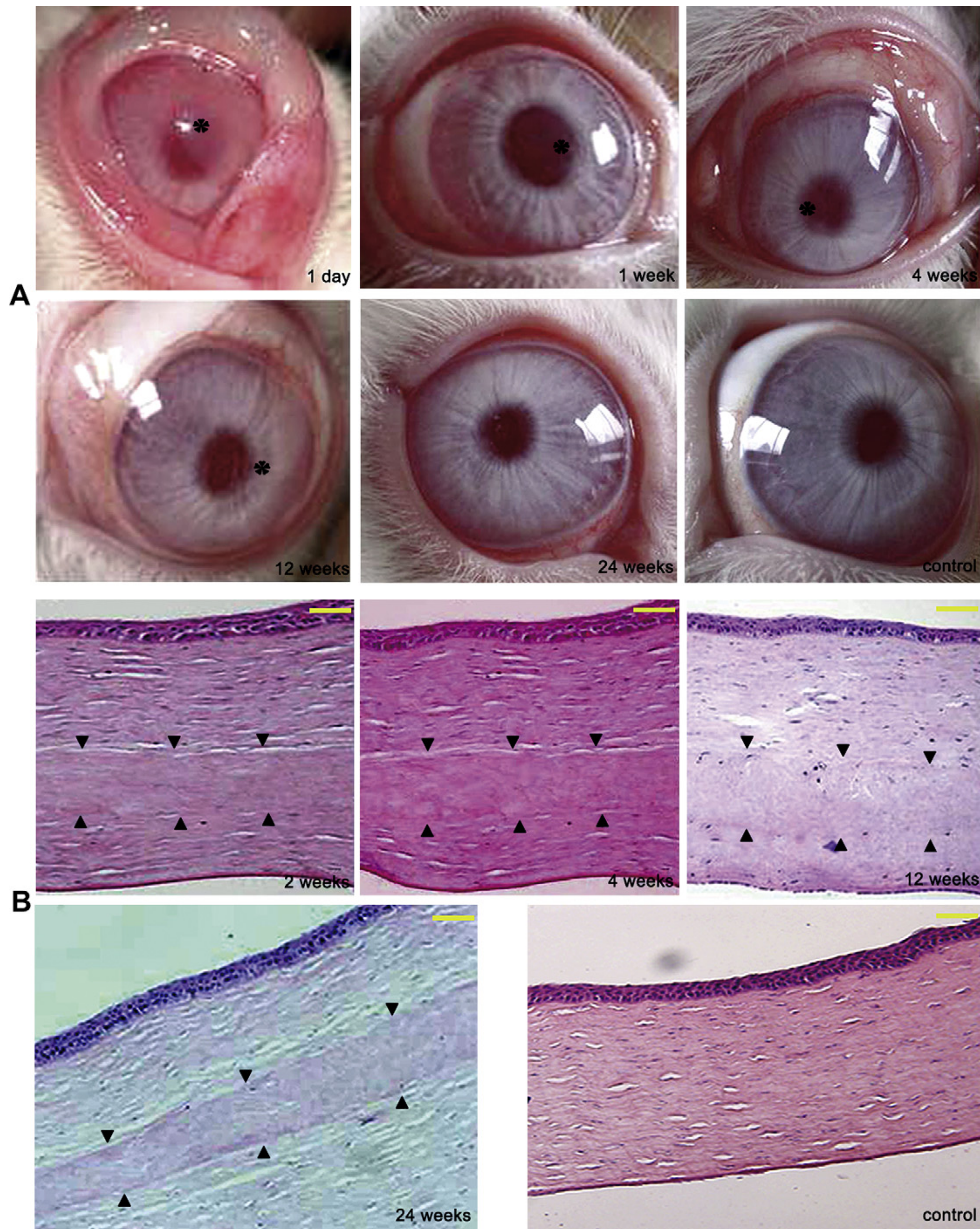

Fig. 5. Representative images and histological sections of APCM post-operative 24 weeks. (A) The process of restoration of transparency. (B) H&E staining showed implanted APCM (black arrow) and few keratocytes infiltrated into them after 12 and 24 weeks post operation. Scale bar: 50 μm.

0.0725 ± 0.0275, respectively ($n = 4$, $P > 0.05$). The elongation at break (%) of APCM was 7.8150 ± 1.3591, similar to that of native porcine corneas (8.7475 ± 2.7525, $n = 4$, $P > 0.05$). There was no significant difference between these two groups (Fig. 3).

3.1.3. *Biocompatibility*

There were no significant differences in the proliferation of rabbit corneal epithelial and endothelial cells as well as keratocytes between the experimental and control groups, as determined by MTT assay ($n = 6$, $P > 0.05$) (Fig. 4). After implantation of the APCM, all animals survived without infections or other complications during the follow-up period. Sutures were removed at 4 weeks after surgery. Although a mild haze was initially observed, the transplanted APCM could be recognized in the rabbit corneas at 12 weeks after the operation, with no neo-vascularization and inflammation or any other rejection signs in or around the transplanted disks (Fig. 5 A), as demonstrated by H&E staining (Fig. 5B). At 24 weeks, the transplanted corneas were as absolutely clear as the control cornea. H&E staining showed that the implants were well integrated within the host corneas, with some cells infiltrating the transplanted disks (Fig. 5B).

3.2. *Corneal cells*

Rabbit corneal epithelial cells, keratocytes and endothelial cells were well achieved by the method described in Materials and

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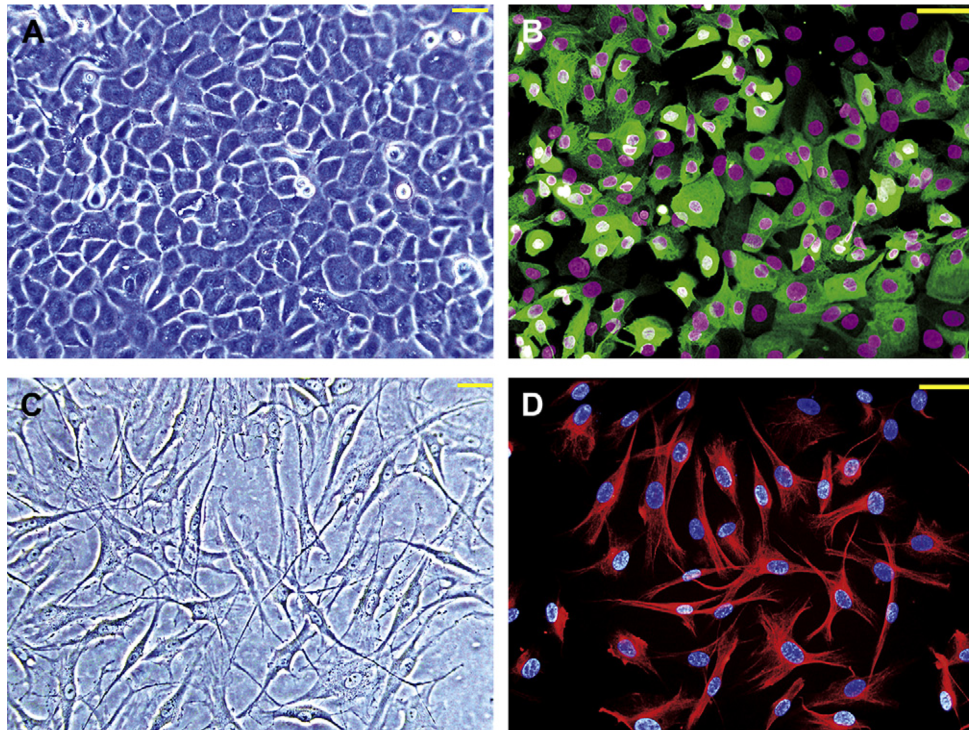

Fig. 6. Phase appearance and marker protein expression of cultivated rabbit corneal limbal epithelial cells and keratocytes. The corneal epithelial cells exhibited typical cobblestone morphology (A) and high expression of marker cytokeratin 3 (B, green). The keratocytes displayed a spindle-like shape (C) and showed high expression of vimentin (D, red). Scale bar: 50 μm.

Methods. Cultured corneal epithelial cells exhibited typical cobblestone morphology (Fig. 6A). Small colonies composed of 5 ~ 10 cells were formed two days after inoculation. The colonies extended very fast thereafter, began to fuse by 6 ~ 7 days, and cells covered the whole culture plate (Fig. 6A). As previously reported [39,40,43,44], corneal epithelial cells showed high expression of CK3 as demonstrated by immunofluorescent staining (Fig. 6B). Different to epithelial cells, keratocytes displayed a spindle-like shape (Fig. 6C) and showed a good growth rate in culture. The cells began to grow out from the explants in 4 days, and reached confluence by 10 days. Immunofluorescent staining showed that vimentin was abundantly distributed in the keratocytes cell plasma (Fig. 6D).

Corneal endothelial cells exhibited outgrowth from the corneal endothelial sheet placed on 60 mm culture dishes in three days, and reached confluence within two weeks. In culture, the morphology of these cells was similar to native corneal endothelial cells in vivo, but tended to display a polygonal, elongated shape rather than the typical hexagonal shape as previously reported [15] (data not shown).

3.3. Characterization of the constructed corneal anterior lamellar

The rabbit corneal anterior lamellar equivalents were efficiently constructed with the scaffold of APCM. Rabbit keratocytes showed good survival and rapid proliferation with a relative uniform cell distribution in the matrix, as Fig. 7 A exhibited that many keratocytes grew out from the seventh day corneal stroma construct when it was reseeded on a new culture dish for five days. After the epithelial cells were seeded on top of the stromal substitutes, two or three layers of cells were observed in 7 days of submerged culture but without the column-shaped cells at the bottom. The phenotype of the construct was similar to normal rabbit corneas, with high expression of CK3 in the epithelial cell layer and expression of vimentin in the stromal cells, as demonstrated by immunohistochemical and immunofluorescent staining (Fig. 8).

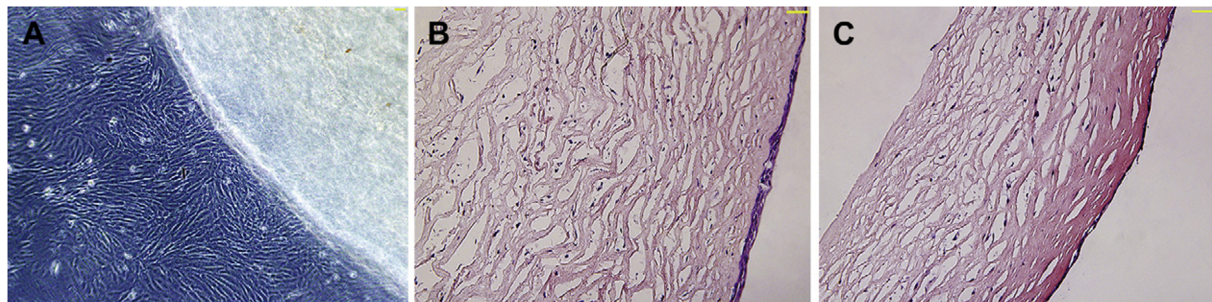

Fig. 7. Phase appearance and histological sections of constructed rabbit anterior cornea replacement containing epithelium and part of the stroma with APCM. (A) Keratocytes grew out from the seventh day cornea stroma construct. (B) H&E staining showed two or three layers of cells formed on the scaffold with a distribution of keratocytes in the stroma. (C) H&E staining of the construct soaked in 100% sterile glycerol for 5 min. Scale bar: 50 μm.

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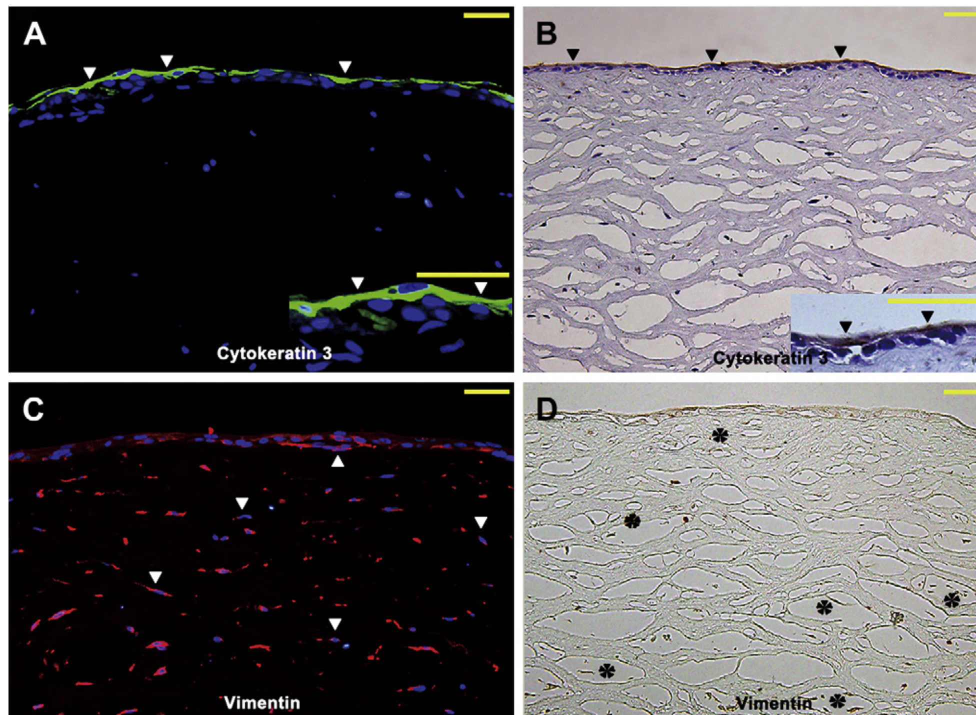

Fig. 8. Immunostaining for cytokeratin 3(A, B) and vimentin (C, D) in constructed rabbit anterior cornea replacement. (A) Immunofluorescent staining for cytokeratin 3 (green, white arrow). (B) Immunohistochemistry staining for cytokeratin 3(brown, black arrow) counterstained with hematoxylin (blue). (C) Immunofluorescent staining for vimentin (red). (D) Immunohistochemistry staining for vimentin (brown, asterisk) without hematoxylin staining. DAPI displayed blue fluorescence in (A) and (C) for cell nuclear staining. Scale bar: 50 μm.

Although the initial constructed tissue was translucent and highly swollen with large fibril spacing (Figs. Fig.7B and Fig.8) due to the long-time immersion in culture medium, it quickly became transparent with an architecture close to native rabbit corneas when soaked in sterile glycerol for 5 min (Fig. 7C).

4. Discussion

An ideal scaffold for tissue-engineered corneas should have good biocompatibility, high optical clarity, toughness to withstand surgical procedures, and non-immunogenicity properties [13]. The native cornea itself has a unique ECM organization which provides high transparency and significant mechanical properties as well as innate biological cues which help to direct and organize cell function, including cell differentiation, proliferation and migration [32]. For this reason, the ECM of the porcine cornea is an ideal choice for tissue engineering due to its similar anatomic properties to human corneas [29] and low immunoreactions [28]. Before application for cornea tissue engineering, the heterogeneous components of porcine corneas such as cellular and nuclear materials should be efficiently removed. Hence, an APCM was developed by using SDS which was an anionic surfactant usually used for cell lysis. The whole decellularization process was initiated at a low temperature, to avoid ECM destruction induced by innate cell lyase.

In preliminary studies, several different concentrations of SDS were tried to remove the cellular and nuclear materials while did not destroy the cornea native structure (data not shown), and it was found that 0.5% (wt./vol.) SDS was good, possibly due to 24 h-long incubation time at 4 °C. No visible cells or cell nuclear material were observed in APCM by H&E and DAPI staining, and no cellular debris was left in the corneal stroma as observed by TEM images. The APCM was somewhat cloudy after the decelluarized process due to hydration of the proteoglycans, which resulted in increased collagen fibril spacing and scaffold swelling. The collagen structure in the corneal stroma may have been slightly changed by dealing with SDS. When the corneal structure is irreversibly changed, the cornea transparency will never fully recover [33]. However, after it was soaked in 100% sterile glycerol, the scaffold quickly became transparent, which suggested that the corneal stroma structure was not altered during the decellularized process, as displayed by TEM and SEM images.

Although the quantitative detection of the residual SDS was not taken out after efficient washing, the leachables extracted from APCM had no cytotoxicity against rabbit corneal keratocytes, epithelial and endothelial cells as determined by MTT assay, and no chronic inflammation or persistent epithelial defects happened after six months of animals' implantation. All these data demonstrated that the scaffold or the residual SDS did not adversely affect the physiological functions of corneal cells.

Most scaffolds developed for cornea tissue engineering previously reported could not be applied for implantation due to their low toughness to withstand surgical procedures. But in this study, an APCM was developed with mechanical properties similar to native corneas. This is probably because the stroma structure of corneas decellularized by SDS was well preserved. That means that the scaffold had sufficient mechanical strength to be sutured and applied for implantation.

Additionally, the APCM has good biocompatibility and transparency in corneal transplantation. It allowed in-growth of host corneal cells and was well integrated within the host cornea with no neo-vascularization and inflammation or any other rejection signs in or around the transplanted disks, as demonstrated by slit-lamp examination and H&E staining. Therefore, all these data mentioned above strongly give a conclusion that the APCM prepared by SDS is an ideal scaffold for cornea tissue engineering.

In clinics, deep lamellar keratoplasty (DLK) has been found to be a safe alternative to penetrating keratoplasty (PK) for common

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diseases such as keratoconus, because the best corrected visual acuity, refractive results, and complication rates are similar after DLK and PK [45], and the risk of endothelial rejection can be avoided [46]. To achieve this goal, we tried to develop a rabbit anterior cornea containing epithelium and part of the stroma with APCM. The phenotype of the construct was similar to normal rabbit corneas, with high expression of CK3 in the epithelial cell layer and expression of vimentin in the stromal cells. The survival of keratocytes injected into the matrix was identified by many keratocytes growing out from the seventh day corneal stroma construct when it was reseeded on a new culture dish. Although it was swollen with increasing collagen fibril spacing in the stroma after a long-time in vitro culture, the construct transparency was quickly restored by immersion in 100% sterile glycerol, which suggested that the APCM structure was well preserved during the period of the reconstruction process.

In this work, we had developed an APCM which possessed the critical features of cornea, especially the mechanical properties, by using SDS in a low temperature, and successfully constructed a rabbit anterior cornea replacement for lamellar keratoplasty, with more keratocytes distributed in the construct than Fu's models [34]. However, the epithelium of the construct was thinner than native corneas and no columnar basal cells were obtained. Therefore, as a step toward clinical testing, further study on construction technique with stem cells and reconstructed anterior cornea equivalents in animals with diseased corneas will next be evaluated.

5. Conclusions

An APCM was successfully developed by using SDS. The heterogeneous components of porcine corneas such as cellular and nuclear materials had been completely removed, and the stroma structure was well preserved. The scaffold possessed the key features of the cornea such as optical clarity and the toughness to withstand surgical procedures, and also had good biocompatibility. In addition, a rabbit anterior cornea replacement was also constructed containing epithelium and part of the stroma with APCM. The phenotype of the construct was similar to normal rabbit corneas. These results suggest that the APCM developed by using SDS might be a suitable scaffold for cornea tissue engineering and have future clinical applications as a substitute for cornea repair or other soft tissue repair.

Acknowledgments

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Appendix

Figures with essential color discrimination. Figs. 1, 4–8 in this article are difficult to interpret in black and white. The full color images can be found in the online version, at doi:10.1016/j.biomaterials.2010.05.066.

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