·Basic Research·

Support of acellular porcine corneal stroma for growth of corneal epithelium and stromal cell *in vitro*

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Foundation item: This study was supported by the Development of High and New Science and Technology (863 Project) of China (No. 2005AA205241)

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Received:2008-12-03 Accepted:2009-04-09

Abstract

• AIM: To determine whether acellular porcine cornea stroma (APCS) could support the growth of the rabbit corneal cells *in vitro*.

• METHODS: APCS was prepared. The rabbit's corneal epithelium and stromal cells were cultured and seeded on APCS *in vitro*. The observation of phase contrast photograph and histological examination were performed.

• RESULTS: Histological examination showed the epithelium grew on the scaffold of APCS in 2-3 layers at 10th day. The stromal cells adhered to the surface of the scaffold after 24 hours and invaded into the interlaminar of the material at 5th day.

• CONCLUSION: These results indicate that APCS can support the growth and proliferation of the corneal epithelium and stromal cells *in vitro*.

• KEYWORDS: acellular porcine cornea stroma; corneal epithelium; corneal stromal cells; tissue engineering

Lin XC, Hui YN, Meng H, Zhang YJ, Jin Y. Support of acellular porcine corneal stroma for growth of corneal epithelium and stromal cell *in vitro*. *Int J Ophthalmol* 2009;2(2):123-125

INTRODUCTION

At present, although the tissue engineering cornea has gained increasing progress, a fully effective prosthetic cornea has not yet been developed [1]. A successful tissue-engineered corneal prosthesis must contain a scaffold that fully supports the growth of corneal cells. In the early application of synthetic scaffolds, the surfaces of scaffolds could not be covered by an intact epithelium *in vivo*, and their applications in tissue engineering was limited [2-5]. Recently, the application of the natural biologic materials in tissue engineering is widened gradually. The natural materials are beneficial to the growth of cells and can remodel more easily *in vivo* because of retaining the natural extracellular matrix (ECM) and original structure. In tissue engineering cornea, it has been reported that the natural corneal stroma containing cells is taken as the substrate for the growth of corneal cells *in vitro* [6,7]. We had prepared acellular porcine corneal stroma (APCS) previously to reduce the antigen and pathogenicity [8]. The aim of the present study was to determine whether APCS could support the growth of the rabbit corneal cells *in vitro*.

MATERIALS AND METHODS

Experimental Animals The pigs in a local abattoir and New Zealand white rabbits were selected. Rabbits (n=6) with body weight ranged from 2 to 2.5kg were provided by the Experiment Animal Center of the Fourth Military Medical University (Xi'an, China). The eyes of the animals were healthy, and the gender was not limited. National Institutes of Health (NIH) guidelines for the care and use of laboratory animals (NIH Publication #85-23 Rev. 1985) have been observed. The porcine corneas were harvested to prepare APCS. The corneas of the rabbits were selected to collect the rabbit corneal cells.

Preparation of APCS The preparation of APCS was in accordance with a previous report [8].

Culture of the Rabbit Corneal Epithelium Cells The procedure of cell culture was as follows: the lamellar limbal tissue (100μm in depth, 3mm in width) was obtained from the rabbit's eyes under an operating microscope. The epithelia were separated from the stroma after a 45-minute incubation in Dispase Ⅱ (2.5mg/mL) at 37℃. The cells were cultured with DMEM/F12 (2∶1) (GIBCO, Grand Island, NY,USA) containing 100mL/L fetal bovine serum (GIBCO), 20μg/L recombinant human EGF (Sigma, St. Louis, MO,

USA),5mg/L insulin (Sigma), 0.18mmol/L adenine (Sigma), 30μg/L cholera toxin (Sigma), 0.5mg/L hydrocortisone (Sigma) and antibiotics (100U/mL penicillin and 100mg/L phytomycin) in a 37℃, 50mL/L CO_2 incubator. The culture medium was replaced every 2 days. The result of immunohistochemical detection of AE5 antibody on the cultured epithelial cells was positive (data not shown).

Stromal cells were isolated from rabbit's corneas with the following methods: The corneas of the rabbit were harvested, and the epithelium and endothelial cells were removed. The stromal layer left was cut into pieces. After digested with collagenase (GIBCO) for 45 minutes at 37℃, the stromal cells were harvested and cultured with DMEM (GIBCO) containing 100mL/L fetal bovine serum (GIBCO). The culture medium was replaced twice per week.

Seeding of the Corneal Cells of Rabbits on APCS

Before the cells being seeded, APCS were soaked in the culture medium for 1 hour and then the medium was blotted. The cell suspension of the third passage epithelium cells was seeded onto the surface adjacent to the epithelium of APCS at a density of 5×10^4 cells/cm^2. Culture medium was not added until the cell-scaffold constructs were kept in a 37℃,50mL/L CO_2 incubator for 3 hours to allow adhesion of the cells to the scaffolds. The cell-scaffold constructs were cultured under media for 1 day and upgraded to air-liquid interface on the second day to continue to be cultured. The culture medium was replaced every 2 days. After being cultured for 10 days, the cell-scaffold constructs were fixed in 40g/L paraformaldehyde, paraffin embedded, then sectioned and followed by staining with H&E.

The third passage stromal cells of the cornea were seeded onto the stroma side of the APCS (the surface adjacent to the epithelium side was down) at a density of 5×10^4 cells/cm^2 with the same protocol mentioned above. The culture medium was DMEM. After culture for 5 days, the cell-scaffold constructs were fixed in 40g/L paraformaldehyde, paraffin embedded, then sectioned and followed by staining with H&E.

RESULTS

Growth of the Corneal Epithelium Cells on APCS *in vitro* The cells adhered to the surface of APCS after 24 hours, elongated and presented polygon mostly at 3 days (Figure 1A) and confluenced like the "slabstone" at 10 days. The section of H&E showed the cells grew on the scaffold of APCS in 2-3 layers at 10 days (Figure 1B).

Growth of the Corneal Stromal Cells on APCS *in vitro* The stromal cells adhered to the surface of the scaffold after 24 hours and elongated at 3 days (Figure 1C). The cells invaded into the interlaminar of material at 5 days (Figure 1D).

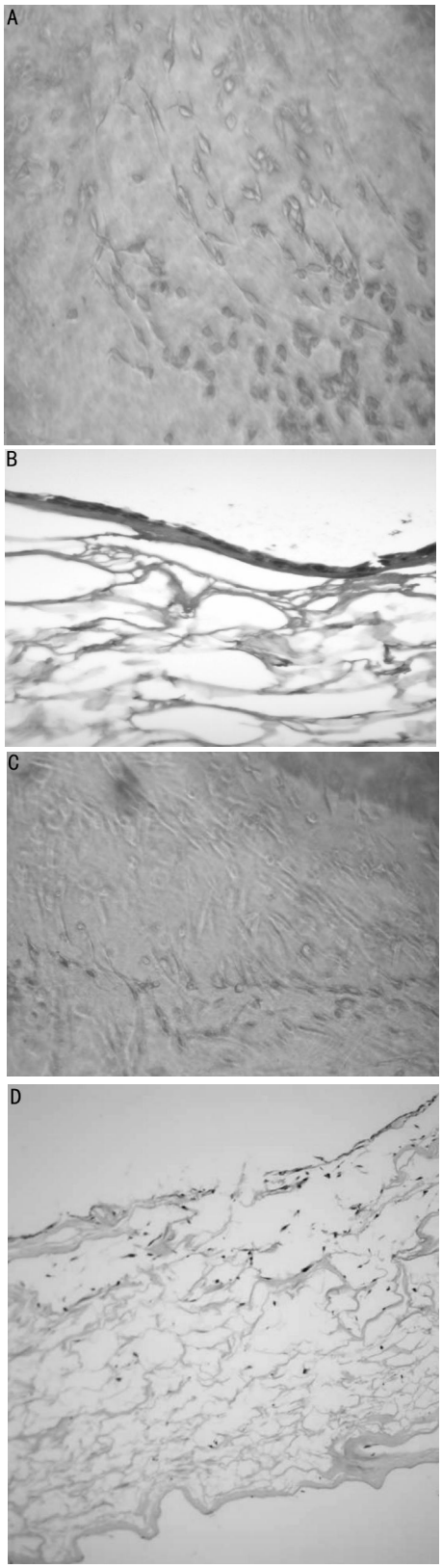

Figure 1 A:Phase contrast photograph shows the growth of the rabbit's corneal epithelium cells on APCS at 3 days. (magnification × 200); B: The section of H&E shows the growth of the rabbit's corneal epithelium cells on APCS in 2-3 layers at 10 days (magnification ×400); C: Phase contrast photograph shows the growth of the rabbit's corneal stroma cells on APCS at 3 days (magnification × 200); D: The section of H&E shows the stroma cells invade into the interlaminar of scaffold at 5 days (magnification ×200)

DISCUSSION

The natural corneal stromas have already been used as the carrier for corneal cells in previous research, but these corneal stroma scaffolds contained cells [6,7,9]. The cell components are the source of the antigens of the major histocompatibility complex [10,11]. The natural corneal stroma containing cells is not fit for the standard of scaffold of tissue engineering cornea because of its antigens. One way to reduce the antigen and pathogenicity of the natural cornea stroma may be to remove the cell compositions in it.

However, decellularization protocols applied on the natural corneal stroma may affect the composition and morphology of the surface of the natural materials. This may affect the cell compatibility of the material.

In this study, to observe the effect of the rabbit's corneal cells growing on APCS, we performed the experiment *in vitro*. The results showed cornea epithelium cells could grow and stratify on the surface of APCS without any coating. The study also suggested that the corneal stromal cells could grow on APCS and invade the interlaminar of the scaffold. This indicates that APCS can support the growth of the rabbit corneal cells *in vitro*. We suppose that APCS prepared by the methods in accordance with previous report [8], like other acellular biologic materials, comprises the natural corneal ECM retaining many types of cytokines and chemical signals [12]. APCS can provide the surface such as the basement membrane for the growth of corneal epithelium and the porous microenvironment for the stromal cells to invade and proliferate [8].

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