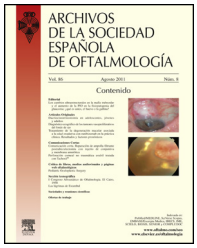
ARCHIVOS DE LA SOCIEDAD ESPAÑOLA DE OFTALMOLOGÍA

www.elsevier.es/oftalmologia



Original article

Characterization of epithelial primary culture from human conjunctiva☆

L. Rivas[a,*], A. Blázquez[b], F.J. Muñoz-Negrete[a], S. López[c], G. Rebolleda[a], F. Domínguez[b], A. Pérez-Esteban[d]

[a] Servicio de Oftalmología, Hospital Universitario Ramón y Cajal, Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Madrid, Spain
[b] Laboratorio de Ingeniería Celular, Hospital Universitario La Paz, Madrid, Spain
[c] Servicio de Oftalmología, Hospital Central de la Cruz Roja, Madrid, Spain
[d] Servicio de Oftalmología, Hospital Universitario La Paz, Madrid, Spain

ARTICLE INFO

Article history:
Received 26 March 2013
Accepted 26 July 2013
Available online 8 April 2014

Keywords:
Primary culture
Cell differentiation
Conjunctival epithelial cells
Human amniotic membrane
Autologous serum

ABSTRACT

Objective: To evaluate primary cultures from human conjunctiva supplemented with fetal bovine serum, autologous serum, and platelet-rich autologous serum, over human amniotic membrane and lens anterior capsules.

Methods: One-hundred and forty-eight human conjunctiva explants were cultured in CnT50® supplemented with 1, 2.5, 5 and 10% fetal bovine serum, autologous serum and platelet-rich autologous serum. Conjunctival samples were incubated at 37 °C, 5% CO_2 and 95% HR, for 3 weeks.

Results: The typical phenotype corresponding to conjunctival epithelial cells was present in all primary cultures. Conjunctival cultures had MUC5AC-positive secretory cells, K19-positive conjunctival cells, and MUC4-positive non-secretory conjunctival cells, but were not corneal phenotype (cytokeratin K3-negative) and fibroblasts (CD90-negative).

Conclusions: Conjunctiva epithelial progenitor cells were preserved in all cultures; thus, a cell culture in CnT50® supplemented with 1–5% autologous serum over human amniotic membrane can provide better information of epithelial cell differentiation for the conjunctival surface reconstruction.

Caracterización del cultivo primario epitelial de conjuntiva humana

Palabras clave:
Cultivo primario
Diferenciación celular

RESUMEN

Objetivo: Evaluar diferentes cultivos primarios de conjuntiva humana enriquecidos con suero fetal bovino, suero autólogo y suero autólogo rico en plaquetas, sobre un soporte de membrana amniótica humana y cápsula anterior del cristalino.

☆ Please cite this article as: Rivas L, Blázquez A, Muñoz-Negrete FJ, López S, Rebolleda G, Domínguez F, et al. Caracterización del cultivo primario epitelial de conjuntiva humana. Arch Soc Esp Oftalmol. 2014;89:10–16.

* *Corresponding author.*
E-mail address: lrivas53@gmail.com (L. Rivas).

2173-5794/$ – see front matter

Células epiteliales conjuntivales
Membrana amniótica humana
Suero autólogo

Métodos: Ciento cuarenta y ocho explantes de conjuntiva humana fueron cultivados en CnT50® enriquecido con 1, 2,5, 5 y 10% en suero fetal bovino, suero autólogo y suero autólogo rico en plaquetas. Las muestras de conjuntivales fueron incubadas a 37 °C, 5% CO_2 y 95% HR, durante 3 semanas.

Resultados: Todos los cultivos primarios tuvieron el fenotipo típico de las células epiteliales de la conjuntiva. Los cultivos mostraron células secretoras MUC5AC-positiva, células conjuntivales CK19-positiva y células conjuntivales no secretoras MUC4-positiva, pero sin fenotipo corneal (CK3-negativa) ni fibroblastos (CD90-negativa).

Conclusiones: Las células epiteliales progenitoras de la conjuntiva se mantuvieron en todos los cultivos, por lo que un cultivo celular en CnT50® enriquecido con suero autólogo entre el 1 y 5% sobre membrana amniótica humana puede proporcionar la mejor información de la diferenciación de las células epiteliales para la reconstrucción de la superficie conjuntival.

Introduction

The conjunctiva as well as the cornea have stratified, squamous and nonkeratinized epithelium with morphological and functional continuity, making up the ocular surface. Even so, there are large epithelial and stromal differences; the conjunctiva, which acts as a physical protection barrier, comprises mucosecretory cells[1]; for this reason, this epithelium is different to that of the cornea due to the genetic expression of various cytokeratin, mucins and glycocalyx.[2]

The conjunctival epithelium is self-renewable; its stem cells turn into epithelial cells throughout life. The location of these cells is controversial although clinical studies have pointed its location in the fornix and the bulbar conjunctiva.[3–5] These stem cells produce non-secretory as well as mucosecretory cells.[6,7]

A reduction in the number of stem cells and cicatrization of donor tissue represents a challenge for reconstructing ocular surface defects. Even though there are numerous surgical methods for recovering the ocular surface in patients with systemic diseases or severe local alterations, we are still far from a definitive solution.[8,9] Recently, autologous conjunctival epithelial cell transplants are opening new perspectives for ocular surface treatment.[10]

The coexistence of non-secretory and mucosecretory cells in the conjunctiva is necessary to maintain normal physiological functions[11] and therefore both types of cells must be maintained in a primary culture, preserving their entire epithelial characteristics.[12]

Amniotic membrane enhances ocular surface cicatrization and faster re-epithelization. It is a basal membrane that stimulates migration and adhesion of epithelial cells while the avascular stromal matrix diminishes inflammation, neovascularization and fibrosis.[13] On the other hand, it is a perfect substrate for restoring the stromal niche of limbal epithelial cells as well as for achieving expansion of these cells in culture.[14]

The above characteristics lead us to set the objective of finding the best conditions for human conjunctiva cell culture. To this end, human conjunctiva epithelial cells must be cultured on various culture media and organic support. Secondly, we must compare the rates and speed of growth and differentiation of the 2 epithelial cell types and finally study the morphology and phenotype of said cultures.

Subjects, materials and methods

The study utilized 148 biopsies of the central upper bulbar area of the conjunctiva, about 10–15 mm from the limbal age, of patients operated on glaucoma filtrating surgery in the Ophthalmology Service of the Ramón y Cajal hospital. The samples came from 67 men and 81 women in the age range between 45 and 67 years. The donations were anonymous after the subjects signed informed consents. The study protocol was approved by the Ethics Committee of the Ramón y Cajal hospital in accordance with the Helsinki Declaration.

The conjunctiva biopsies had a size of approximately 1 mm^2. Every sample was placed in a closed container with PBS supplemented by 2% penicillin-streptomycin for transport to the culture laboratory where, under maximum sterility, the samples were washed in Hank saline tamponated solution with 1% penicillin–streptomycin (three 5-min rinses). Subsequently, the stroma included in the biopsy was partially removed in order to eliminate the highest possible number of fibroblasts, avoiding damages to the epithelium. Every biopsy was divided in 2 parts, totaling 296 explants that took place in the center of 3 cm diameter Falcon® culture plates with the epithelium facing upwards, adding 1.5 ml of CnT50® culture medium (Genycell Biotech and CellnTec, Spain) on its own or enriched with serum. The explants were maintained always within the culture plates because, in the first stage of the study where some were withdrawn, the cells begin to die and disappear entirely after one week.

The cell dispersion proposed by Meller and Tseng was not carried out either because previous experiments did not produce satisfactory results.[2]

CnT50® is a liquid culture medium developed for achieving maximum efficiency in isolation and growth of corneal and conjunctival epithelial cells. It contains amino acids, minerals, vitamins and organic components and is protein-free. In addition, it has a very low concentration of bovine pituitary extract (15 μg/ml) and calcium (0.07 mM).

Overall, 13 culture media were studied: one, CnT50® without supplements, and four supplemented with bovine fetal