ULTRASTRUCTURAL STUDY OF RABBIT CONJUNCTIVAL EPITHELIUM BY TRANSMISSION AND SCANNING ELECTRON MICROSCOPY

Somsanguan Ausayakhun, M.D., M.H.Sc.

Department of Ophthalmology, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand

Abstract The surface epithelial cells of the conjunctiva not only perform mechanical functions but also make an important contribution to the physiology of the tear film. The aim of this study is to describe the ultrastructure of the conjunctival epithelium in detail by transmission and scanning electron microscopy, using rabbits as an animal model. The conjunctival epithelium contained the stratified nonkeratinizing squamous cells which were mostly polygonal and were more completely covered with microvilli. The goblet cells had mucin packets which appear electron-lucent. On the basis of ultrastructural findings, especially on the number and kinds of cytoplasmic organelles, different cell types of conjunctival epithelium were identified. These findings possibly reflect the functional complexity within the conjunctival epithelium.

Introduction

The conjunctival epithelium has often been neglected in the past because of its supposed insignificance. Only its goblet cells attracted much scientific interest because of their contribution to the mucin component of the tear film. However, this disinterest has dissipated rapidly with current advances in understanding of the physiology and pathology of the preconal tear film. Many components of the tear film have been identified, e.g. proteolytic and glycolytic enzymes, various antibodies, glycoprotein, glucose, and calcium, which certainly participate in the secretory activity of conjunctival cells and also have their morphological counterparts. The aim of this study is to describe the ultrastructure of the conjunctival epithelium in detail and to identify its morphological characteristics by transmission and scanning electron microscope. This was done with an animal model, a rabbit.

Materials and Methods

 Conjunctival biopsy specimens were taken from both eyes of one rabbit after being injected with a lethal dose of sodium pentobarbital. The specimens were processed according to standard techniques of transmission and scanning electron microscopy.

Results

The conjunctival epithelium contained the stratified nonkeratinizing squamous cells that were unique among any other stratified squamous epithelia in that goblet cells were intercalated between the epithelial cells (Fig.1). The apical cell had numerous organelles within their cytoplasm (Fig.1). The epithelial cellular membranes showed marked
infoldings, with incomplete interdigitation with adjacent cells, and had few desmosomes (Fig. 2).

The characteristic surface details of the conjunctival epithelium appeared in the scanning electron microscope. The conjunctival surface cells were for the most part polygonal and were completely covered with microvilli (Fig. 1, 3A).

Goblet cells were distributed over the epithelial surface, primarily at points where three or four epithelial cells met (Fig. 3A).

**Fig. 1.** Transmission electron microscope (EM) analysis of rabbit conjunctival epithelium showing both goblet cell (GB) and epithelial cells. A. Type V cells are filled by numerous mitochondria (arrows), mostly in the apical part of the cells. Note the electron-dense cytoplasm of the dark-colored epithelial cells. (x 4,000); B. Goblet cell (GB) is intercalated between the epithelial cells with numerous microvilli (arrowheads). (x 5,000)

**Fig. 2.** Transmission EM analysis of surface epithelial cells of rabbit conjunctiva (A and B). Note the highly expandable intercellular spaces with many finger-shaped cellular extensions (stars), and few desmosomes (arrows). (A. x 15,000) (B. x 12,000)

**Fig. 3.** Scanning EM analysis of the surface of rabbit conjunctiva. A. The conjunctival epithelium are polygonal and completely covered with microvilli. Goblet cells are distributed among the epithelial cells (arrows). (x 400); B. Intercellular crypt opening (Henle’s crypt) is demonstrated. (x 600)
They were either empty or contain plugs of mucin. The large opening correspond to the entryway of the epithelial crypts (crypts of Henle) (Fig. 3B).

Based on the number and kind of cytoplasmic organelles, different cell types of conjunctival epithelium were classified.

Type I cells were goblet cells (Fig. 4A). Goblet cells were unicellular, mucin-secreting glands. The mucin packets appeared electronlucent (Fig. 4A).

Type II cells were those with numerous electron-dense granules (Fig. 4B). Granules situated in the apical cytoplasm of the epithelial cells.

Type III cells were particularity rich in Golgi complexes (Fig 5A). Numerous vesicles often collected on the concave or convex side of the Golgi complexes.

Type IV cells contained a large amount of rough endoplasmic reticulum (Fig. 5B). Membranes with thick coatings of ribosome were usually found near the nucleus.

Type V cells were characterized by a high content of mitochondria, typically located in the apical part of the cells (Fig. 1A,B). The cytoplasm of type V cells was more electron-dense than that of neighboring epithelial cells (Fig. 1A).

Discussion

A major difference between cells of the conjunctival epithelium and that of the cornea is an increase of cytoplasmic organelles in their cytoplasm. In older literature only two cells types are described: epithelial cells and goblet cells. There are, however, significantly more cell forms. Greiner et al. were the first to describe a "second mucous secretory system", while Rohen and Steuhl later characterized altogether five different cell forms which appear with a certain constancy in the conjunctival epithelium of cynomolgus monkey.

Goblet cells secrete mainly mucus in daily amounts of about 2.2 μL. There is a regional variation in their distribution and density per unit area. The highest density of the cells is in the medial fornical and palpebral regions near the tear drainage apparatus.

Type II cells are seen as cells from secondary mucous-secretory system since they contained numerous electron-dense granules. These granules contain predominantly acid glycosaminoglycans and glycoproteins.

In the Golgi complex, the finished product in exocytotic vesicles or condensing vacuoles are released outwards through fusion of the vesicle membrane with plasma membrane. It is well known that polysaccharides and proteins combine to form glycoprotein within the Golgi system. This means that the Golgi apparatus is indispensable for synthesis of surface-active substances of the tear film. So, the type III cell also belongs to the functional complex which may be described as "secretion of mucoid substances".

Type IV cells, which contain a large amount of rough endoplasmic reticulum, may produce various proteins in large quantities which normally found within the tear film. Since the conjunctival epithelium is to be regarded as the origin of many of these substances: various antibodies, prostaglandins, acid phosphatase and plasminogen activators, so the type IV cells may be responsible for these defense and self-cleaning functions of the conjunctiva.

Fig. 4. Transmission EM analysis of type I and type II cells of rabbit conjunctiva. A. Type I (goblet) cell contains electron-lucent mucin packets. (x 4,000) B. Type II cell demonstrates numerous electron-dense granules (arrows). (x 8,000)

Fig. 5. Transmission EM analysis of type III and type IV cells of rabbit conjunctiva. A. Type III cell is rich in Golgi complexes (arrows). (x 4,000) B. Type IV cell shows a large number of rough endoplasmic reticulum (arrows) (x 6,000)
Since the mitochondria contain enzymes of the respiratory chain and represent the ATP store, so the type V cells, with many mitochondria (Fig. 1 AB), are cited as a morphological basis for active transport processes, which always requires in the resorption of substances and may also be responsible for maintaining the ionic equilibrium of the tear film. Moreover, by using horseradish peroxidase, Steuhl KP succeeded in showing a certain link between this type V cell and resorption processes in conjunctiva. It was also demonstrated that the microvilli and microplicae were of considerable importance for the enlargement of the resorbing surface.

In summary, based on the ultrastructure by electron microscopic study, three different functional complexes within the rabbit conjunctival epithelium were revealed: the secretion of mucoid substances by type I, type II and type III cells, the defense system by type IV cells, and the resorption by type V cells.

This study will make it possible to identify the morphological basis of various functional complexes of the conjunctival epithelium. With the help of these morphological characteristics, it would be possible to better classify pathological processes in various ocular abnormalities.

Acknowledgments

The author gratefully acknowledges the technical assistance of Dr. Sato and Dr. Hirohata of the WHO Collaborating Center for Research and Training in Diagnostic Electron Microscopy, Nippon Medical School, and Associate Professor N. Yamada, Department of Pathology, and Professor Y. Shimizu, Department of Ophthalmology, Nippon Medical School for encouragement.

References