Demonstration of the human ocular surface glycocalyx by transmission electron microscopy

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SUMMARY

The aim of this work was to demonstrate the ultrastructural appearance of the human ocular surface glycocalyx in normal subjects. Conjunctival tissue specimens from 7 normal subjects were obtained by bulbar conjunctival biopsy, and examined by transmission electron microscopy. The free surface of the apical conjunctival epithelial cells was characterized by the presence of microvilli. All specimens showed the presence of an ocular surface glycocalyx, which appeared as a continuous, fine network of filamentous electron-dense material associated with the apical cell membrane. This filamentous layer exhibited a higher density on the tips of the microvilli and extended among the microvilli, forming an extrinsic cell surface coat. Examination of the ultrastructural morphology of the apical conjunctival epithelium by transmission electron microscopy may prove a useful tool in the diagnostic and investigative evaluation of healthy and diseased conjunctivas.

Key words: Conjunctiva – Epithelium – Glycocalyx – Electron microscopy

INTRODUCTION

As an evolutionary response to nonaquatic living, humans have developed a structurally and biochemically complex precorneal tear film (Pflugfelder et al., 1998), which is kept in place by the ocular surface epithelium. The precorneal tear film is classically described as a three layer structure, comprising a mucous layer in contact with the ocular surface, an intermediate aqueous layer, and an anterior lipid layer (Johnson and Murphy, 2004; Bron et al., 2004).

Tear mucous is chiefly composed of mucins and inorganic salts suspended in water (Johnson and Murphy, 2004). Mucins are a family of exceptionally large glycoproteins that have at least half of their mass as O-linked carbohydrate (Argüeso and Gipson, 2001), and they are the major components in all mucous secretions present on wet-surface epithelium. To date, 21 human mucin genes (MUC 1-19, including MUC 3A, 3B, 5AC and 5B) have been identified (Johnson and Murphy, 2004).

Epithelial cells of both the cornea and the conjunctiva synthesize and secrete mucins (Dartt, 2004). There are two major types of mucins, transmembrane and secretory. Secretory mucins are further divided into the gel-forming and soluble types (Watanabe, 2002; Dartt, 2004; Gipson, 2004). The transmembrane mucins that have been detected in the conjunctiva, MUC1, MUC4, MUC13, MUC15, MUC16 and MUC17 (Argüeso and Gipson, 2001; Corrales, 2003), share common

structural features: they contain a hydropho-bic transmembrane domain that anchors the mucin on the apical surface of the apical epithelial cells, a short cytoplasmic tail, and an extracellular domain that reaches into the tear film (Dartt, 2004). These mucins are inserted into the apical cell membrane of the apical conjunctival and corneal epithelial cells, facilitating the formation of the ocular surface glycocalyx (OSG).

The purpose of this study was to demonstrate the ultrustructural morphology of the human OSG, present in conjunctival apical epithelial cells of normal subjects, using transmission electron microscopy.

MATERIALS AND METHODS

Selection Criteria for Conjunctival Specimens

This study followed the tenets of the Declaration of Helsinki. Participation was voluntary and informed consent was obtained after the subjects had been informed about the nature of the study. The study protocol was approved by the University of Thessaly and the University Hospital of Larissa Ethics Committee. Inclusion criteria for subjects to be diagnosed as exhibiting normal aqueous tear production were: a) asymptomatic individuals, b) Schirmer's 1 test > 15 mm in 5 minutes without anesthesia in both eyes, c) negative Rose Bengal staining of the ocular surface, and d) negative serous autoantibodies: antinuclear antibody (ANA), rheumatoid factor (RF), SS-A (Ro) and/or SS-B (La).

The age of the subjects ranged from 33 to 65 years, with a mean age of 50 ± 11.8 years. The information about the normal subjects from whom biopsy specimens were obtained is summarized in Table 1.

Table 1. Donor Information for Biopsy Specimens.

Donor	Age	Gender	Eye	Diagnosis
1	49	F	OD	Normal Subject
2	65	F	OS	Normal Subject
3	56	F	OD	Normal Subject
4	33	M	OS	Normal Subject
5	62	M	OS	Normal Subject
6	39	M	OS	Normal Subject
7	46	F	OD	Normal Subject

Conjunctival Tissue Collection

Superior bulbar conjunctival tissue specimens from 7 eyes of 7 randomly selected asymptomatic normal controls were obtained using topical anesthesia (Alcaine 0.5% drops, Alcon, USA) under slit lamp biomicroscopy.

Transmission Electron Microscopy

The specimens were fixed in 3% glutaraldehyde in phosphate buffer solution (PBS; pH 7.3) for 3 hours. Subsequently, they were post-fixed in 2% osmium tetroxide in PBS for 1.5 hr. After a wash with PBS and double distilled water, the specimens were dehydrated in a graded series of alcohol and were embedded in epoxy resins (SERVA Electrophoresis GmbH). Ultrathin sections (59-90 nm) were studied under a Jeol 2000 FX2 transmission electron microscope (Jeol, Japan) after staining with uranyl acetate and lead citrate solutions.

Images Analysis

For each specimen, 10 to 12 images were acquired on high-quality negative film, which was processed according to the manufacturer's recommendations and printed using a magnification of x 24000. All photos were screened and, for each specimen 5 representative photos were selected for descriptive analysis.

RESULTS

The free surface of the apical conjunctival epithelial cell was characterized by the presence of microvilli. The microvilli form as evaginations of the cell membrane that give rise to straight tubular projections into the conjunctival sac. In all specimens, the microvilli appeared single, elongated, and did not branch. Membrane-bound secretory vesicles were present just below the cell membrane of the apical epithelial cells. These vesicles were also identified in the subapical conjunctival epithelial cells (Fig. 1).

All specimens showed the presence of an OSG, which appeared as a continuous, fine network of filamentous electron-dense material associated with the apical cell membrane. This filamentous layer exhibited a higher density on the tips of the microvilli and extended among the microvilli, forming an extrinsic cell surface coat (Fig. 2).

DISCUSSION

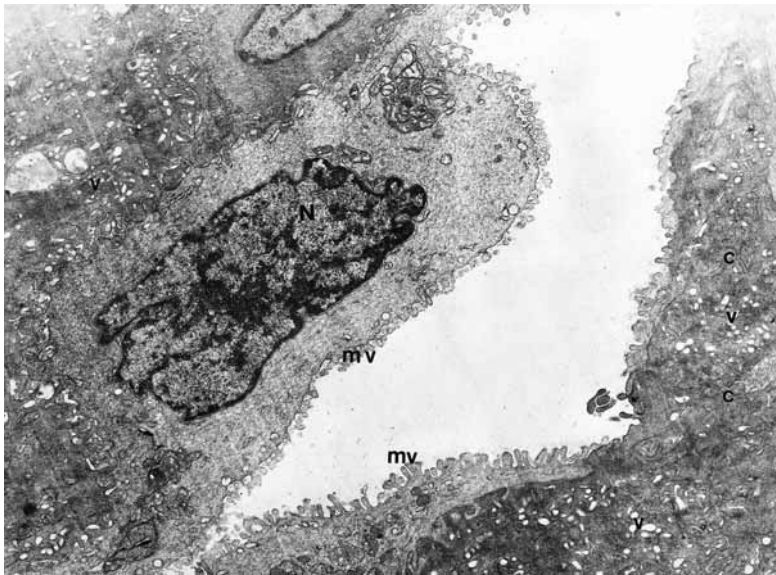The apical conjunctival epithelium is covered with microvilli, which in turn are covered

Figure 1. Electronmicrograph of conjunctival apical epithelial cells from a 56-year-old female normal subject. The free surface of the cells is covered with microvilli (**mv**). Secretory vesicles (**v**) are observed in both apical and subapical epithelial cells. **c** = intercellular junctions, **N** = nucleus. x 14,000.
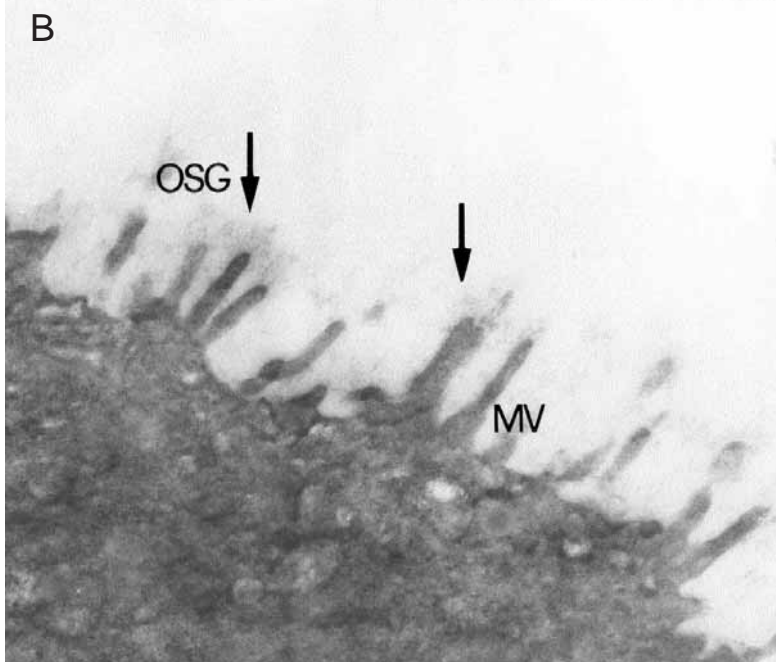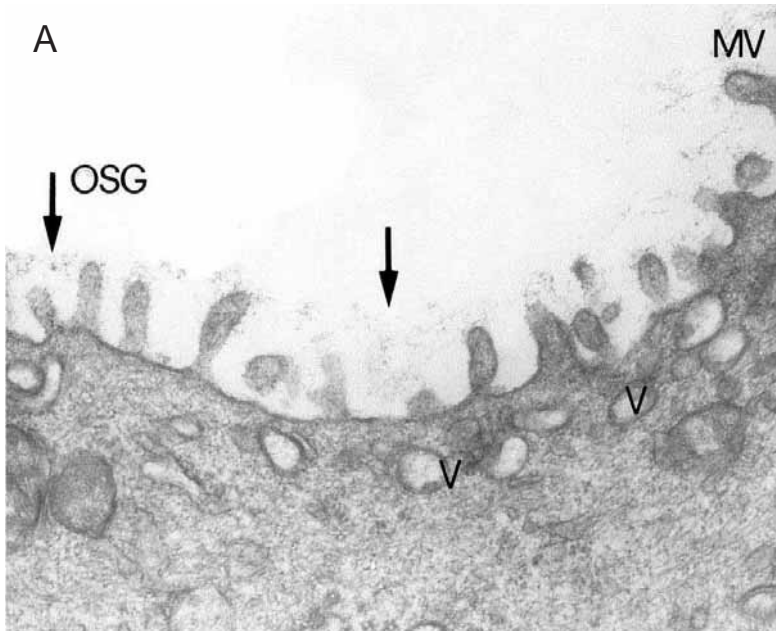
with transmembrane mucins that contribute to the formation of the OSG. The OSG forms a dense filamentous layer at the interface between the tear film and the ocular surface. Several functions have been ascribed to this carbohydrate-rich layer. It protects the ocular surface by providing lubrication, inhibiting bacterial adhesion and reducing friction during blinking. Koufakis et al. (2006) reported an alteration in the ultrastructure of both the microvilli and the OSG in patients with Sjögren's syndrome and suggested that the OSG may play a key role in the maintenance of a healthy ocular surface, possibly by preventing abrasive effects on the apical epithelial cells generated by the lid epithelia during blinking.

Mucins may protect the ocular surface either by binding pathogens before they attach to the corneal epithelium or by inhibiting microbial colonization by competitive blocking of microbial receptors found on the epithelium (Ramphal and Pyle, 1983). Fleiszig and co-workers (1994) showed that removal of corneal mucus increased the adherence of *Pseudomonas aeruginosa* onto rabbit corneas by 3-10-fold.

Additionally, transmembrane mucins, through their hydrophilic carbohydrates, maintain a water-based surface on the cell membrane and at the same time form a repulsive surface for the movement of soluble mucins across the eye during blinking (Gipson, 2004).

Figure 2. Electronmicrographs of conjunctival apical epithelial cells from a 39-year-old male (**A**) and a 46-year-old female normal subject (**B**). The microvilli (**MV**) are single, elongated, and do not branch/bifurcate. Note the numerous secretory vesicles (**V**). The ocular surface glycocalyx (**OSG**) forms an extrinsic cell surface coat of filamentous, electron dense material (**arrows**), which is more prominent on the tips of the microvilli. **A**, **B** = x 24,000.

Furthermore, the OSG has been implicated in cellular signalling. Transmembrane mucins (MUC1) have a short cytoplasmic tail that contains tyrosine residues that can be phosphorylated, interact with SH2 domains on second messenger molecules and hence propagate intracellular signals (Zrihan-Licht et al., 1994). Also, several transmembrane mucins have epidermal growth factor (EGF)-like domains. In MUC4 these enable it to act as a membrane-bound ligand and modulator of the receptor tyrosine kinase ErbB2, a member of the epidermal growth factor receptor family, suggesting that MUC4 may be involved in epithelial growth regulation (Carraway et al., 1999).

With recent advances in understanding of the physiology and structure of the precorneal tear film, new light has been cast on the role of the epithelium in the maintenance of a homeostatic ocular surface environment. Transmission electron microscopy is an effective method to study the ultrastructural morphology of the apical epithelium in healthy and diseased conjunctivas.

Acknowledgements

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