The Mucinous Layer of Corneal Endothelial Cells

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Abstract

Purpose: The goal of this study was to characterize the morphology of the mucinous layer on rabbit, bovine, owl, and human corneal endothelial cells. Methods: Corneoscleral buttons were fixed using cetylpyridinium chloride to stabilize "mucus" and the tissue was prepared for transmission electron microscopy. Photomicrographs were measured to determine the thickness of the endothelial and epithelial mucinous layer in the central cornea. Results: The endothelial mucinous layer was seen as a nearly uniform, electrondense region on the apical aspect of the endothelium. It was found to be 0.9 µm, 0.9 µm, and 0.5 µm thick in rabbit, bovine, owl, and human, respectively. The owl endothelium had an additional less electrondense layer with a granular appearance and a thickness of about 200 µm. The mucinous layer on the epithelium was similar in appearance to that on the endothelium and across species. Conclusions: The morphologic similarity of the endothelial and epithelial mucinous layers is a serendipitous finding that should prove valuable in experimental design. Ultimately, it is hoped that studies of the posterior corneal surface will deepen our knowledge of endothelial protection.

Key Words: Corneal endothelium, mucinous layer, mucin

INTRODUCTION

The posterior surface of the corneal endothelium is the site where the contents of the anterior chamber interact with the endothelium. Both protective¹ and damaging² interactions at this interface have been reported. An extracellular mucinous layer has been identified on this surface³ which, in theory, should modulate these interactions. Following experimental cataract surgery in rabbits the authors have seen marked variation in the thickness of this layer (Fig. 1). While the biochemical properties of this layer have generated some interest, its morphology has been largely ignored. This report covers work done to characterize the baseline morphology for the mucinous layer of the corneal endothelium (The term

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Fig. 1. Endothelial mucinous layer following surgery. Transmission electron microscopy of a rabbit cornea fixed with CPC (see Methods) following cataract surgery. The mucinous layer is the layer of electron dense material on the apical surface of the endothelial cells. The authors found considerable variation in the thickness of the mucinous layer (arrow) in various surgical specimens (Rabbit corneas; bar=1 µm).
“mucinous” is used in its historic sense to identify this layer and is not meant to identify its composition.

Investigating the mucinous layer on the corneal endothelium in cat, Carrington et al. had shown that the layer was very sensitive to fixation and staining techniques. Using freeze fracture and cetylpyridinium chloride (CPC) based technique, they demonstrated that the posterior surface of the cat cornea is covered by a mucinous layer of up to 1.3 micron in thickness. Schroder and Sperling reported that the human corneal endothelium has a 0.06–0.15 micron polysaccharide coat on its posterior surface, using a ruthenium red technique. The report by Carrington et al. suggested that the CPC technique yields a result more representative of the thickness of the layer in vivo.

The present study is the first to report the morphology of the normal mucinous layer of the corneal endothelium across four species (rabbit, bovine, owl, and human), using a consistent technique. The study is also the first to compare the morphology of the mucinous layer of the corneal epithelium and endothelium within a species. Finally, an extension of the observations of Barany et al. are made regarding the unusual additional material posterior to the endothelium of the owl.

MATERIALS AND METHODS

Sources of corneas

Human eyes, unsuitable for transplantation, were obtained from the Georgia Lions Eye Bank. The corneas came from a donor over the age of 40 and were otherwise in good condition; further information about the donor was unavailable. New Zealand white rabbits (weighing 2.0–2.5 kg) were anesthetized with xylazine and ketamine HCl prior to being killed with pentobarbital. Fixative solution was dropped on the corneas prior to enucleation. Chinese Scops Owls (Otus scops scincus) were obtained through a taxidermist who called when critically injured owls were to be put down. Owls (weighing 0.4–0.7 kg) were anesthetized with xylazine and ketamine HCl and euthanized with pentobarbital. Fixative solution was dropped on the corneas and slowly injected into the anterior chamber prior to enucleation. Bovine eyes were obtained from a local (Seoul) abattoir immediately after slaughter. All animals were cared for in accordance with the ARVO Resolution on the Use of Animals in Research.

Fixation and staining

A corneoscleral button was cut from the globe with a pars plana incision. The lens-iris diaphragm was carefully removed with forceps. Fixation and staining for transmission electron microscopy (TEM) followed a slight modification of the Nichols procedure. The specimens were fixed overnight at room temperature in 2.5% glutaraldehyde with 0.1 M sodium cacodylate HCl (pH 7.4) and 0.5% cetylpyridinium chloride (CPC), a “mucus” stabilizing agent (Sigma Chemical, St. Louis, MO). Specimens were washed in 0.1 M sodium cacodylate HCl (pH 7.3) and fixed again for 1 hour at room temperature in 1% glutaraldehyde in 0.1 M sodium cacodylate HCl with 1% tannic acid (US: Electron Microscopy Science, Fort Washington, PA; Korea: Sigma Chemical). They were washed again in buffer and postfixed with 1% osmium tetroxide in Palade’s buffer for 1 hour at 4°C, dehydrated in ethanol, and embedded in Epon. Specimens were examined in a transmission electron microscope (Atlanta: JEOL 100CX II; Seoul: Hitachi H-500).

Thickness measurements

Thickness measurements of the endothelial mucinous layer were made at multiple locations in the central cornea. As the fixation technique was first described for the epithelial mucinous layer, its thickness was also measured in the central cornea to verify that the specimen was processed properly. Prints of the electron micrographs were measured with a transparent plastic ruler and the actual thickness was calculated from the magnifications of the print and of the negative. The mode (most frequent measurement) of the thickness was estimated for each electron micrograph and the overall estimate of thickness was an average of the estimates from each micrograph.

This technique provided a typical measurement resolution of 0.05 micron or less. As non-perpendicular cuts of a specimen bias the estimate of its thickness (over-estimate), some consideration was given to using the minimum thickness measurement from each micrograph. Because the mucinous layer in most micrographs contained anomalous segments,
some of which were exceptionally thin, we were concerned that using the minimum thickness would create more of a bias than it prevented. Instead, the mode was chosen to minimize bias that might arise from the anomalous segments of the mucinous layer. As to the potential for overestimating the thickness due to non-perpendicular sections through the cornea, it can be shown that the inflation factor is given by the secant of the angular deviation from the perpendicular. Thus, as the final measurements were reported to 0.1 micron, any over-estimation due to sections within 18° of perpendicular are not detectable.

RESULTS

The mucinous layers of the corneal endothelium and epithelium ("mucinous" layer of the tear film) were not visualized by routine fixation and staining procedures for transmission electron microscopy (TEM). Using the Nichols' procedure revealed the mucinous layers on the corneal endothelial and epithelial surfaces in human (Fig. 2), rabbit (Fig. 3), bovine (Fig. 4), and owl (Fig. 5).

A cornea from each of two rabbits, bovine, and owls were studied; two human corneas came from the same donor. Multiple measurements were taken from different locations in the central cornea. The layers were somewhat variable in thickness within each cornea. Morphologically, the mucinous layer of the corneal endothelium was almost uniform in its electrondensity. The epithelial and endothelial layers had a similar appearance, which was preserved across species. The three zone architecture previously reported was not observed. On the posterior surface of the owl cornea, a second, less electron dense layer with a granular appearance was seen immediately adjacent to the first layer (Fig. 5C). The second layer

Fig. 2. Mucinous layers of the human cornea. The mucinous layers of the human corneal epithelium (A) and endothelium (B) were 0.6 μm and 0.5 μm thick, respectively. The mucinous layers appear similar in thickness and electron density (Human cornea, fixed 20 hours after death; TEM; A, B: bar = 1 μm).

Fig. 3. Mucinous layers of the rabbit cornea. The mucinous layers of the rabbit corneal epithelium (A) and endodethelium (B) were 1.1 μm and 0.9 μm thick, respectively. The mucinous layers appear similar in thickness and electron density (Rabbit cornea; TEM; A, B: bar = 1 μm).

Fig. 4. Mucinous layers of the bovine cornea. The mucinous layers of the bovine corneal epithelium (A) and endothelium (B) were 1.1 μm and 0.9 μm thick, respectively. The mucinous layers appear similar in thickness and electron density (Bovine cornea; TEM; A, B: bar=1 μm).

Fig. 5. Mucinous Layers of the owl cornea. The mucinous layers of the owl corneal epithelium (A) and endothelium (B) were 1.0 μm and 0.9 μm thick, respectively. The mucinous layers appear similar in thickness and electron density. Beside the mucinous layer, the TEM of the posterior owl cornea (B) shows an additional, less electron dense, granular appearing layer roughly 200 μm thick. A thick section of the cornea stained with toluidine blue (C) shows the full thickness of the cornea and the extra layer. Higher magnification micrographs show the structure of the extra layer in its middle (D: large square of figure C) and at the posterior margin (E: small square of figure C) (Owl cornea; TEM: A, B, D: bar=1 μm; E: bar=0.5 μm; toluidine blue: C: bar=100 μm).
Table 1. Thickness of Mucinous Layers

<table>
<thead>
<tr>
<th>Animal</th>
<th>Endothelium</th>
<th>&quot;Extra&quot; layer (Endothelium)</th>
<th>Epithelium</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine</td>
<td>0.9</td>
<td>—</td>
<td>1.1</td>
<td>Carrington et al.</td>
</tr>
<tr>
<td>Cat</td>
<td>&lt;= 1.3</td>
<td>—</td>
<td>—</td>
<td>Nichols et al.</td>
</tr>
<tr>
<td>Guinea Pig</td>
<td>—</td>
<td>—</td>
<td>0.8</td>
<td>Schroder and Sperling</td>
</tr>
<tr>
<td>Human</td>
<td>0.5</td>
<td>—</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>Human</td>
<td>0.06 – 0.15</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Owl</td>
<td>0.9</td>
<td>200</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Rabbit</td>
<td>0.9</td>
<td>—</td>
<td>1.1</td>
<td></td>
</tr>
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</table>

Reported thicknesses of the mucinous layer of the anterior and posterior cornea. Entries without references are from this report. All measurements are in μm.

is thought to correspond to a thick gel that could be seen grossly on the posterior of the corneoscleral button. At high magnification, this material is seen to have many long columnar structures with a granular composition (Fig. 5D). At the posterior margin of the extra layer, these structures often have a confluent shell of material of greater electron density (Fig. 5E).

DISCUSSION

This report examines the mucinous layer of the corneal endothelium across four species. Table 1 summarizes the thicknesses of the various corneal mucinous layers reported herein and in previous studies. Prior reports have focused on the mucinous layer in cat or human corneas. The mucinous layers of the rabbit, bovine, and owl are all about one micron in thickness while those of the human are about half a micron. Moreover, the mucinous layer on the endothelium and epithelium is about the same thickness within a species and all have a similar morphologic appearance. Interestingly, this raises the possibility that studies of either layer in one of these species can use the other layer as an internal control.

Prior reports of the thickness of the mucinous layer, though not directly comparable to this data, seem consistent with it except in the case of the human. Our measurements of the mucinous layer in the human showed it to be substantially thinner than that of the other species studied. As both human corneas came from the same subject, we have no basis for speculating about whether this is entirely normal, reflects an unusual mucinous layer thickness in this subject, or is a result of post-mortem degradation of the mucinous layer prior to the start of the experiments (about 20 hours). In contrast, these findings are much thicker than that report by Schroder and Sperling.4 This is probably due to the difference in fixation technique (ruthenium red versus CPC) as suggested by Carrington et al.3 During the excision of the corneoscleral button from the first owl eye, the surgeon (EKK) saw a thick layer of clear material adherent to the posterior cornea. Although the layer on the first eye was disrupted in handling, it was preserved in subsequent eyes. This extra layer appears to be like the layer indirectly observed by Barany et al.5 Besides this layer, the owl cornea also had a mucinous layer that had the same thickness and staining (when fixed with CPC) as those seen in rabbit and bovine corneas.

Characterizing the morphology of the endothelial mucinous layer is an important step toward understanding how it is affected by intraocular manipulations. The morphologic similarity of the endothelial and epithelial mucinous layers is a serendipitous finding that should prove valuable in experimental design. Ultimately, it is hoped that studies of the posterior corneal surface will deepen our knowledge of endothelial protection.

REFERENCES