The Boston type 1 keratoprosthesis (KPro) is an artificial cornea used for the management of corneal diseases not amenable to standard keratoplasty.1 Despite improved outcomes, the Boston KPro continues to carry significant risk for complications. Of these, the most common is retroprosthetic membrane (RPM) formation, which occurs in 25% to 65% of eyes.2-7

Despite their high incidence, RPMs are poorly understood. A histological analysis of 4 RPM specimens by Stacy et al8 showed minimal inflammation in the membranes. However, other investigators have suggested that anterior chamber inflammation is a risk factor for RPM formation and that the use of more biocompatible materials (eg, titanium) in the Boston KPro construct or the administration of perioperative corticosteroids may retard RPM growth.2,9-11

Given recent data that RPM formation may increase the risk of donor corneal melt,12 further investigation of RPMs is warranted. To the best of our knowledge, no dedicated histopathological and immunohistochemical study of melt-associated RPMs has previously been performed. To clarify the role of inflammation in RPMs and to further characterize melt-associated RPMs, we performed a histopathological and immunohistochemical analysis of RPM specimens obtained from Boston KPro devices explanted because of donor corneal melt.
Methods

Study Design
After obtaining institutional review board approval at the University of Illinois at Chicago, a retrospective evaluation of RPM specimens obtained from Boston KPros explanted because of corneal melt was performed. All available RPM pathology specimens obtained between January 1, 2011, and January 1, 2012, at our institution were identified (institutional review board exemption for informed consent was obtained). The clinical history for each patient was reviewed.

Immunohistochemistry was used to characterize the membranes. Anti-CD34 (clone QBEnd/10; Ventana Medical Systems) and α–smooth muscle actin (SMA) stains (clone 1A4; Ventana Medical Systems) were used to distinguish normal stromal keratocytes (CD34 positive) from fibrous keratocytic downgrowth (SMA positive) and to highlight vascularity within the membranes.13 Pankeratin (clone AE1/AE3/PCK36; Ventana Medical Systems) was used to identify epithelium. Anti–cytokeratin 7 (clone OV-TL 12/30; Ventana Medical Systems) was used to identify conjunctival epithelium from corneal epithelium.14 Vimentin (clone V9; Ventana Medical Systems) was used as a negative control for identifying epithelium. All immunohistochemical staining was performed on an automated immunostainer (BenchMark XT; Ventana Medical Systems) and was reviewed by one of us who is a board-certified ocular pathologist (A.Y.L.).

Fluorescence in situ hybridization was then performed using a dual color X/Y (alpha satellite, spectrum orange/satellite III, and spectrum green) centromere enumeration probe (Vysis).15 Results were then scored by a cytogenetics consultant masked to the sex of the recipients and donors to determine the XY-karyotype of the interphase cellular population.

Results
In total, 7 RPM specimens obtained from 7 consecutive eyes following Boston KPro explantation because of donor corneal melt were included in the study. Demographic data for each patient are summarized in the Table. A lymphocytic infiltrate was identified on hematoxylin-eosin staining in 4 of 7 samples (Figure 1). Two had mild inflammation, while 2 others had severe inflammation. Both specimens with severe inflammation were obtained from patients treated for aniridia (Table). A proliferation of nonkeratinizing stratified epithelium was present along the surface of the explanted membrane specimens in 4 of 7 samples (Figure 1). Pankeratin (cytokeratin AE1/3) and cytokeratin 7 showed strong cytoplasmic immunoreactivity suggestive of epithelial ingrowth of conjunctival origin (Figure 1). Epithelial cells were negative for vimentin.

Of 4 patients with histological evidence of epithelial ingrowth, one (case 2 in the Table) had clinical epithelial downgrowth at the time of Boston KPro explantation, with cyclitic membranes, hypotony, and a dense epiretinal membrane. In 2 specimens with no epithelial ingrowth, epithelium was found within stumps of tissue removed from the backplate holes.

In total, 5 of 7 specimens had SMA staining consistent with fibrous stromal (keratocytic) downgrowth. Six of them had significant vascularity on CD34 and SMA staining.

Table. Clinical and Immunohistochemical Characteristics of Melt-Associated Retroprosthetic Membranes

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Diagnosis</th>
<th>Eye</th>
<th>Age at KPro Implantation, y</th>
<th>Sex</th>
<th>Seidel Test at Explantation</th>
<th>Cellular Inflammation</th>
<th>Immunohistochemistry</th>
</tr>
</thead>
<tbody>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Peters anomaly</td>
<td>Right</td>
<td>3</td>
<td>M</td>
<td>−</td>
<td>−</td>
<td>−−−−+−−−−−+−−−−</td>
</tr>
<tr>
<td>2</td>
<td>Aniridia</td>
<td>Left</td>
<td>12</td>
<td>F</td>
<td>−</td>
<td>+++</td>
<td>++++  ++ ++ ++</td>
</tr>
<tr>
<td>3</td>
<td>Aniridia</td>
<td>Left</td>
<td>37</td>
<td>F</td>
<td>−</td>
<td>NT+</td>
<td>++++  ++ ++ ++</td>
</tr>
<tr>
<td>4</td>
<td>Chemical injury</td>
<td>Left</td>
<td>41</td>
<td>M</td>
<td>−</td>
<td>NT+</td>
<td>++ ++ ++ ++</td>
</tr>
<tr>
<td>5</td>
<td>Cicatricular conjunctivitis</td>
<td>Right</td>
<td>65</td>
<td>M</td>
<td>+b</td>
<td>−</td>
<td>++ ++ ++ ++</td>
</tr>
<tr>
<td>6</td>
<td>AKC</td>
<td>Left</td>
<td>83</td>
<td>M</td>
<td>−</td>
<td>+</td>
<td>−−−−+−−−−−+−−−−</td>
</tr>
<tr>
<td>7</td>
<td>Idiopathic LSCD</td>
<td>Left</td>
<td>84</td>
<td>M</td>
<td>−</td>
<td>−</td>
<td>−−−−+−−−−−+−−−−</td>
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</tbody>
</table>

Abbreviations: AKC, atopic keratoconjunctivitis; CK, cytokeratin; F, female; KPro, keratoprosthesis; LSCD, limbal stem cell deficiency; M, male; NT, not tested; SMA, α–smooth muscle actin; −, absent; +, present; ++, higher degrees of one condition that is present, eg, inflammation.

*a Clinical epithelial downgrowth.
*b Seidel positivity with manipulation.

Fluorescence in situ hybridization was then performed using a dual color X/Y (alpha satellite, spectrum orange/satellite III, and spectrum green) centromere enumeration probe (Vysis). Results were then scored by a cytogenetics consultant masked to the sex of the recipients and donors to determine the XY-karyotype of the interphase cellular population.
In total, 4 cases of known donor-recipient sex mismatch were identified (Table). In 3 of 4 cases, 100% of the interphase cells in the RPM specimen showed an XY-karyotype consistent with the recipient’s sex (Figure 2). In the fourth case, which involved a male recipient and a female donor cornea (case 6 in the Table), 69% of the interphase cells were XY, and 31% of the cells were XX (Figure 2). The findings were consistent with mixed contributions to the RPM from both recipient and donor tissues.

Discussion

To our knowledge, this is the first dedicated histological study of RPMs explanted from eyes with a Boston type 1 KPro and sterile corneal melt. The only other histological study of RPMs, by Stacy et al., was based on 4 RPMs (2 were associated with corneal melt). The authors concluded that inflammation was not a likely requisite for the formation of RPMs. In contrast, our study showed chronic inflammatory cells in 4 of 7 RPMs. Therefore, it is likely that inflammation has a role in at least some cases of melt-associated RPMs. Furthermore, therapeutic intervention with local or systemic corticosteroids may benefit a subset of patients with inflammatory RPM components (eg, those with aniridia in our study).

Based on observations of stromal downgrowth through breaks in Descemet membrane at the wound margin, Stacy et al proposed that RPMs are derived from host corneal stroma. However, our FISH analysis of sex-mismatched donor-host pairs suggested that, while most RPMs seem to originate from host tissue, donor tissue may also contribute in some eyes. Based on this, we postulate that stromal downgrowth from donor tissue may occur as well at the wound margin or through the backplate holes.

Another notable finding in this study was the presence of epithelial ingrowth between the backplate and RPM in 4 of 7 of our melt-associated specimens. The isolated presence of epithelium in the backplate holes in 2 of our RPMs suggests that epithelium may localize to the posterior backplate through the holes. Despite the high incidence of epithelial ingrowth between the backplate and RPM in our study (in 4 of 7 cases), clinical evidence of epithelial downgrowth was rare. One explanation for this is that the RPM may provide a physical barrier to invasion of epithelium into the anterior chamber. This conjecture is consistent with the low rates of aqueous leakage on Seidel testing in our patients despite the presence of full-thickness corneal melts.

The major weaknesses of the study are its retrospective nature and the small sample size, which may limit the extrapolation of these findings to all melt-associated RPMs. Because tissue was analyzed retrospectively, the quality of tissue obtained varied.

Conclusions

Melt-associated RPMs seem to be heterogeneous tissues showing varying degrees of inflammation. Retroprosthetic membranes appear to be derived mainly from host cells; however,
contributions from donor tissue seem possible. Further study is needed to better describe the inciting factors for RPM forma-
tion, the role of inflammation, and the relative contribu-
tions of host and donor corneal tissues.

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Administrative, technical, or material support: Hou, Sivaraman, Lin.
Study supervision: de la Cruz, Lin, Cortina.
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REFERENCES
1. Dohlman CH, Schneider HA, Doane MG. Prosthokera-