**Histologic and Optical Coherence Tomographic Correlates in Drusenoid Pigment Epithelium Detachment in Age-Related Macular Degeneration**

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**Purpose:** Drusenoid pigment epithelium detachment (DPED) is a known precursor to geographic atrophy in age-related macular degeneration (AMD). We sought histologic correlates for spectral-domain (SD) optical coherence tomography (OCT) signatures in DPED and determined the frequency and origin of these OCT signatures in a clinical cohort of DPED eyes.

**Design:** Laboratory imaging and histologic comparison, and retrospective, observational cohort study.

**Participants:** Four donor eyes with histopathologic diagnosis of AMD (2 with nonneovascular DPED and 2 with neovascular pigment epithelium detachment [PED]) and 49 eyes of 33 clinic patients with nonneovascular DPED more than 2 mm in diameter.

**Methods:** Donor eyes underwent multimodal ex vivo imaging, including SD OCT, then processing for high-resolution histologic analysis. All clinic patients underwent SD OCT, near-infrared reflectance, and color photography.

**Main Outcome Measures:** Histologic correlates for SD OCT signatures in DPED, estimate of coverage by different retinal pigment epithelium (RPE) phenotypes in the DPED surface; frequency and origin of histologically verified SD OCT signatures in a clinical cohort of DPED eyes, and comparisons of histologic features between neovascular PED and DPED resulting from AMD.

**Results:** Intraretinal and subretinal hyperreflective foci as seen on SD OCT correlated to RPE cells on histologic examination. Hypertransmission of light below the RPE–basal lamina band correlated with dissociated RPE. Subretinal hyperreflective material resulting from acquired vitelliform lesions corresponded to regions of apically expelled RPE organelles. In the clinical cohort, all histologically verified reflectivity signatures were visible and quantifiable. The appearance of intraretinal hyperreflective foci was preceded by thickening of the RPE–basal lamina band. Compared with PEDs associated with neovascular AMD, DPEDs had different crystallization patterns, no lipid-filled cells, and thinner basal laminar deposits.

**Conclusions:** Multiple RPE fates in AMD, including intraretinal cells that are highly prognostic for progression, can be followed and quantified reliably using eye-tracked serial SD OCT. This information may be particularly useful for obtaining an accurate timeline of incipient geographic atrophy in clinic populations and for quantifying anatomic end points and response to therapy in AMD clinical trials. Ophthalmology 2017;124:644-656 © 2017 by the American Academy of Ophthalmology

Supplemental material is available at www.aaojournal.org.

Age-related macular degeneration (AMD) is the leading cause of severe vision loss in older adults in the developed world. In 2014, the worldwide prevalence of AMD was estimated to be 8.7%. It is predicted that there will be 196 million people with AMD in 2020 and 288 million in 2040.1

Geographic atrophy (GA) is an end-stage manifestation of AMD, affecting more than 5% of European descendants older than 90 years.2,3 There are no proven treatments for GA, although trials are in progress. A major challenge in managing GA is determining the therapeutic target(s) for halting the cascade of cellular changes that results in irreversible vision loss. Clarifying the pathways leading to GA is likely to improve our ability to detect disease progression, to prognosticate visual outcomes reliably, and to design targeted strategies to minimize vision loss resulting from AMD.

Although not fully understood, the pathobiology of GA is linked intrinsically to the structure, function, and behavior of retinal pigment epithelium (RPE) cells. Drusenoid pigment epithelial detachments (DPEDs), hallmark features of AMD, are a known precursor of GA.4-7 This lesion can be distinguished from serous and hemorrhage pigment epithelial detachments (PEDs) by clinical appearance and angiography.8 Although associated with better visual prognosis in the short term,8,9 the long-term prognosis of
OCT and Histologic Correlates in DPED

DPED is poorer than that of other types of PED. The Age-Related Eye Disease Study (AREDS) showed in a large sample observed for 5 years or more that 19% of eyes with DPED (defined as >350 μm diameter) progressed to central GA, 57.8% exhibited progressive fundus changes, and 39% lost more than 15 letters of visual acuity.10

High-resolution optical coherence tomography (OCT) is used widely in the clinical management of AMD. In the United States alone, 4.4 million OCT scans of the macula were performed in 2012 (2012 Medicare Provider Utilization and Payment Data; available at: https://www.cms.gov/research-statistics-data-and-systems/statistics-trends-and-reports/medicare-provider-charge-data/physician-and-other-supplier.html). Optical coherence tomography facilitates in vivo evaluation of the retina at a near-cellular level, with the axial resolution of most modern instruments corresponding to approximately 4 μm in retinal tissue. Some of the reported precursors to collapse of DPED and atrophy, as seen on OCT, include intraretinal hyper-reflective spots, hyporeflective areas within the DPED, subretinal hyperreflective material, and increased transmission of OCT signal into the choroid.5,11 The cellular correspondences for these OCT signatures, if identified, may provide critical information about pathophysiologic mechanisms underlying GA.

This study used high-resolution histologic analysis matched to ex vivo OCT B-scans from a postmortem donor eye to define a cellular basis for OCT reflectivity variations in DPED. We built on our recent survey of RPE phenotypes that led to a hypothesis of 2 major pathways of RPE fate in AMD: apoptosis and anterior migration.12–14 The frequencies of various RPE phenotypes in DPED also were determined using a clinical cohort of subjects imaged with spectral-domain (SD) OCT. Together, histologic examination and clinical imaging supported the idea that RPE transdifferentiation to a migratory phenotype is an important antecedent of GA that can be tracked and quantified in vivo.

Methods

The Institutional Review Board at University of Alabama at Birmingham and the Western Institutional Review Board approved the experimental study and retrospective, observational cohort study, respectively. Research complied with the Health Insurance Portability and Accountability Act and adhered to the tenets of the Declaration of Helsinki.

Donor Eyes, Histologic Examination, Photomicrographic Examination, and Analysis

Eyes with AMD were identified through an ex vivo imaging screen of eyes accessioned for research purposes from nondiabetic white donors to the Alabama Eye Bank from 1996 through 2012. Median death-to-preservation time was 3.49 hours (range, 0.40–11.40 hours). Eyes were preserved by immersion in 1% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer after anterior segment excision. After vitreous removal, maculas were photographed in color on a stereomicroscope (SMZ-U; Nikon, Melville, NY).15

Eyes underwent multimodal ex vivo imaging including SD OCT when prepared for histologic analysis (2011–2013). From each globe, an 8-mm diameter full-thickness tissue punch containing the fovea and temporal portion of the optic nerve head was removed with a trephine. This punch was held in a tissue holder mounted on a Spectralis (Heidelberg Engineering, Heidelberg, Germany), as described.15 A 30 × 20° SD OCT volume (143 scans; 30-μm spacing; automatic real-time average, 25) was captured, along with red-free and near-infrared scanning laser ophthalmoscopic images.

The left eye of a 73-year-old female donor preserved 1 hour after death had exceptionally clear imaging of a DPED centered under the fovea and served as the index case. A macular tissue punch was postfixed by osmium tannic acid paraphenylenediamine to accentuate extracellular lipid and was embedded in epoxy resin (PolyBed 812; Polysciences, Warrington PA).16 Submicrometer-thick (0.8-μm) sections at 25- to 30-μm intervals were stained with 1% toluidine blue for polychromatnic, scanned with a ×40 objective, and reviewed and photodocumented with a ×60 oil-immersion objective (numerical aperture, 1.4) and digital camera (XC10; Olympus, Center Valley, PA).

Diameters and heights of the DPED central dome and surrounding drusen were measured from ex vivo OCT scans acquired before histologic processing. B-scans and subsequent histologic sections were matched on the basis of overall tissue contour and patterns of distinctive reflective material. For illustration, B-scans were compressed vertically to reduce the disparity between postmortem edema seen in the B-scan and processing-related shrinkage seen on histologic examination. To estimate the percentage coverage of the DPED by different RPE phenotypes, we recorded RPE morphologic features using the terminology of Zanzottera et al12 and Chen et al14 every 200 μm across each of 28 sections (total, 402 locations) using a custom ImageJ plug-in (available at: http://imagej.nih.gov/ij). Thicknesses of the RPE layer and basal laminar deposit (BLamD) were measured at the same locations.12,13

We compared observations from the index case with 3 published cases of similarly prepared PEDs, for a total of 4 postmortem donor eyes. These included 1 clinically documented DPED17 and 2 clinically undocumented cases of serous and hemorrhagic PED in neovascular AMD.13 New sections were obtained from prior specimens as necessary.

Clinical Cases and In Vivo Imaging

Clinical cases of DPED resulting from nonneovascular AMD were selected retrospectively from a consecutive series of patients with PEDs associated with AMD seen between June 2015 and January 2016 by 2 retina specialists (L.A.Y. and K.B.F.) at the Vitreous, Retina, Macula Consultants of New York, a vitreoretinal referral practice. All patients had been evaluated with color photography and fluorescein angiography or indocyanine green angiography, or all 3 methods using the Topcon TRC-50XF fundus camera (Topcon Medical Systems, Paramus, NJ). All eyes also had been evaluated with fundus autofluorescence imaging using the Spectralis HRA+I-OCT (Heidelberg Engineering, Heidelberg, Germany) or the Topcon TRC-50XF fundus camera (Topcon Medical Systems). All eyes also were imaged with SD OCT using the Spectralis. Eye tracking and image registration functions were enabled for all image acquisitions. The SD OCT scan protocol is summarized in Table S1 (available at www.aaojournal.org).

Exclusion criteria were (1) choroidal neovascularization confirmed with SD OCT and dye angiography; (2) retinal artery or retinal vein occlusion, diabetic retinopathy, previous vitreoretinal surgery, and pathologic myopia; (3) GA at the baseline clinic visit; and (4) prior ocular therapies such as laser photocoagulation or intravitreal therapy.

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Optical Coherence Tomography Nomenclature of Age-Related Macular Degeneration Pathologic Features

Knowledge about correspondences between ex vivo OCT and histologic analysis derived from the index case and previous reports was applied to the in vivo OCT cohort, and the frequency of various OCT signatures was determined. Specifically, the presence or absence and frequency of OCT signatures that corresponded with intraretinal migration of RPE cells, vitelliform material, thickening of the RPE plusbasal laminar (BL)(RPE + BL) band (see below), and outer retinal atrophy were determined. Regarding intraretinal hyperreflective foci, we recorded the retinal layer where foci were seen and whether they formed small (1–4 foci) or large (>5 foci) groups.

In DPED, the RPE is separated from Bruch’s membrane (BM) and also usually includes BLamD, a stereotypic thickening of the RPE basement membrane common in AMD. Therefore, we used the SD OCT nomenclature of Staurenghi et al²² with the substitution of RPE+BL for RPE–BM complex.

Quantifying Pigment Epithelium Detachment Morphometry Using In Vivo Optical Coherence Tomography

Morphometric characteristics of DPEDs, including volume, maximum height, and maximum diameter, were summarized from the clinical cohort at the clinic visit when DPED size was judged to be the greatest. Drusenoid PED volume was determined by applying the Cavalieri principle of stereologic analysis to SD OCT scans, as we have done previously. First, the area between the outer boundary of the RPE+BL band and inner boundary of the BM band for each slice in the volume was calculated using manual planimetry. Second, the volume between 2 consecutive OCT slices (herein called a segment) was determined using the equation:

\[ V = d \left( \frac{A_x + A_{x+1}}{2} \right) \]

where \( d \) is the distance between consecutive slices in micrometers, \( A \) is the area between the RPE+BL and BM in square micrometers, and \( x \) is the OCT slice number. Third, total DPED volume was calculated by summing the volumes of individual segments. The number of segments in the OCT volume was \( n-1 \), where \( n \) is the total number of slices that spanned the DPED. Maximum height (defined as the greatest distance between BM and the outer boundary of the RPE+BL) and maximum diameter (defined as the greatest DPED diameter along BM) also was determined. Although the number of scans through PED varied in the clinical cohort, pilot data for our previous studies did not demonstrate a significant difference in PED volume measurement using OCT volumes from the same eye where the distance between B-scans was 100 to 200 μm or 200 to 300 μm.

Statistical Analysis

Data were analyzed using descriptive statistics. Results are reported as mean ± standard deviation.
Figure 2. Correlations between ex vivo spectral-domain optical coherence tomography (OCT) and high-resolution histologic photomicrographs in drusenoid pigment epithelium detachment (dPED) in the index case. Matched histologic image–OCT pairs taken from indicated scan levels of Figure S1 (available at www.aaojournal.org) are presented (A and B, C and D, and E and F, respectively). A, B, Hypertransmission is associated with atrophy and dissociated retinal pigment epithelium (RPE) (white arrowhead). Two groups of RPE cells are apparent (red arrowhead). C, D, A small cluster (red arrowhead) and multiple clusters (red dashed box) of RPE are apparent. Calcium phosphate nodules are hyperreflective (teal arrowhead). Subretinal drusenoid deposits are moderately reflective (orange arrowhead). E, F, An RPE plume is created by a group of RPE cells tracking among the Henle fibers (red arrow). Hyperreflective material internal to the RPE plus basal laminar band represents a vitelliform lesion (yellow arrowheads). Calcium phosphate nodules are present (teal arrowhead). Ch = choroid; IPL = inner plexiform layer; ONL = outer nuclear layer; OPL = outer plexiform layer.
Results

Ex Vivo Multimodal Imaging

Ex vivo color fundus photography and near-infrared reflectance imaging of the index case are shown in Figure S1 (available at www.aaojournal.org). A hypopigmented lesion with pigment clumps, corresponding to the DPED, is apparent through the thin yellow fovea. Diffuse hyperreflectivity of the central fovea and focal hyperreflectivity of surrounding lesions are seen on infrared reflectance imaging. Supplemental Video 1 (available at www.aaojournal.org) is an ex vivo SD OCT volume demonstrating a prominent central dome (height, 432 μm) surrounded by secondary peaks. These in turn are surrounded by domes of lower elevation representing soft drusen. The base of the entire complex was 3320 ± 3.6 μm. The base of the central dome was 1916 ± 80 μm wide, that is, approximately 60% of the overall width and approximately 36% of its area. Many reflective features are visible within the DPED substance, on the surface, and within the overlying retina.

Pigment Epithelium Detachment Microstructure

Figure 1A confirms that the PED in the index case is a large druse; that is, it is located between BLamD and inner collagenous layer of BM. Soft drusen surrounding the central dome contained lipoprotein-derived debris and pools of lipid (Fig 1B). Subretinal drusenoid deposit, of a finer texture, filled valleys between adjacent soft drusen (Fig 1B). In the central dome (and not in the soft drusen or subretinal drusenoid deposit) were small refractile spherules known to be hydroxypatite and large refractile nodules (Fig 1A). Atop the dome was epithelial RPE with BLamD associated with sloughed RPE cell bodies and vitelliform material consisting of RPE granules mixed with outer segment debris in the subretinal space. Photoreceptors overlying the dome apex were degenerated markedly, with absent outer segments, shortened inner segments, and a thinned outer nuclear layer. Müller cells accounted for the greatest mass of retinal tissue at the point of most severe photoreceptor degeneration in the foveal center (not shown).

Correspondences between Ex Vivo Optical Coherence Tomography Imaging and Histologic Analysis

Figure 2 shows 3 representative pairs of matched B-scans and histologic sections from the index case, with corresponding features indicated by color coding. Hypertransmission (increased penetration of light below the RPE+BL band)23 represents an atrophic area with dissociated RPE and subsidence of the overlying outer nuclear layer (white arrowheads; Fig 2A, B). Subretinal fluid also was found here (not shown). Punctate reflectivities within the DPED represent calcium phosphate nodules (teal arrowheads; Fig 2C–F). A moderate reflectivity in the subretinal space is attributable to subretinal drusenoid deposits (orange arrowheads; Fig 2C, D). Simple (red arrowheads; Fig 2A, B) and complex (dashed box; Fig 2C, D) groups of intraretinal hyperreflective foci represent small and large clusters of RPE cells, respectively. Clumped cell bodies within groups could not be separated readily for counting, but nuclei were discernible. The median number of nuclei in any 1 section was 2 (Q1, 0; Q3, 3; maximum, 7). A distinctive reflective signature reminiscent of a windswept volcanic plume (green arrowheads; Fig 2E, F) represents a group of RPE cells migrating anteriorly and turning nearly 90° to track horizontally among the Henle fibers. Hyperreflective material internal to the RPE+BL band indicates vitelliform change (yellow arrowheads; Fig 2E, F), which can include sloughed RPE as well.

High-magnification views of RPE morphologic features corresponding to hyperreflective foci found throughout the ex vivo SD OCT volume are shown in Figure 3. Reference images from an age-matched eye without DPED also are provided (Fig 3A). Figure 3B shows thin RPE (< 2 μm) in an area where the dominant RPE phenotype is very nonuniform. Figure 3C shows sloughed RPE, that is, a spherical and fully pigmented cell, in the subretinal space. Figure 3D, E shows single pigmented intraretinal RPE cells in the outer nuclear and ganglion cell layers, respectively. Groups of intraretinal RPE in the Henle fiber layer, including several multinucleate cells, appear to swarm (Fig 3F). Five RPE cells were seen to form a ring around an inner retinal capillary (Fig 3G). Figure 3H shows RPE organelles in the extracellular compartment mixed with outer segment debris resulting from photoreceptor degeneration (vitelliform). In an area of hypertransmission and nowhere else, the RPE layer has broken up, and dissociated RPE remains atop BLamD (Fig 3I).

Coverage of the PED surface by different RPE phenotypes is summarized in Table 1 and Figure S2 (available at www.aaojournal.org). Mean thicknesses for RPE, BLamD, and RPE plus BLamD were 10.98 ± 4.63, 3.95 ± 2.22, and 14.93 ± 5.08 μm, respectively. At 402 assessed locations on the PED surface, 42 (10.4%) were associated with RPE scattered throughout layers of the overlying retina (33.3% ganglion cell layer; 7.1% inner plexiform; 11.9% inner nuclear; 7.1%–19% in synaptic and axonal portions of outer plexiform, respectively; and 21.4% outer nuclear). Of locations demonstrating migrated RPE, 38.3% also demonstrated vitelliform material. Shedding (apoptotic) RPE was not found in any evaluated location.

Comparisons between Pigment Epithelium Detachment Subtypes

To determine whether the index case was representative, it was compared with published PED cases. The first comparison case was a 41-year-old white woman who sought treatment for a large subfoveal DPED in 1973, documented clinically with color photography (Fig S3A, available at www.aaojournal.org). At her death, 22 years later, ex vivo imaging revealed a DPED complex (> 350 μm in extent) that was unchanged in appearance since the clinic visit (Fig S3B, available at www.aaojournal.org). Histologic analysis showed that the RPE was well pigmented, continuous, and highly variable in thickness (Fig S3C, available at www.aaojournal.org). The DPED had a finely granular interior with evidence of active remodeling, including cells at the superior aspect (Fig S3D, available at www.aaojournal.org). This clinically stable DPED had no evidence of crystallization, migrated RPE, vitelliform change, or BLamD. Instead, the RPE layer was overall thin, and the shedding (apoptotic) RPE phenotype predominated. Mean thicknesses for RPE, BLamD, and RPE plus BLamD were 8.33 ± 5.14, 0.08 ± 0.30, and 8.41 ± 5.24 μm, respectively.
These DPEDs were compared with serous and hemorrhagic PEDs reported in 2 eyes of 2 older donors with neovascular AMD\textsuperscript{15} (Table 2). These PEDs had varying retinal locations (1 foveal, 1 extrafoveal), cholesterol crystals, intraretinal fluid, anteriorly migrated RPE, intraretinal lipid-filled cells, 100% coverage of the PED surface by BLamD, and no detectable

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**Figure 3.** Histologic correlates of hyperreflective structures seen in ex vivo optical coherence tomography (OCT) of the index case. Panels indicate representative examples matched to reflectivities in the ex vivo spectral-domain OCT volume scan. A, Nonuniform retinal pigment epithelium (RPE). B, Thin, very nonuniform RPE with basal laminar deposit (white arrowheads). C, Fully pigmented sloughed RPE in the subretinal space. D, Fully pigmented intraretinal RPE in the outer nuclear layer (ONL). E, Nucleated RPE in the ganglion cell layer (GCL). F, Swarm of intraretinal RPE. One cell has 3 nuclei (pink arrowheads). G, Five RPE cells surround a vessel in the superficial capillary plexus. H, Vitelliform change, characterized by exploded RPE lipofuscin/melanolipofuscin granules (L/ML), outer segment (OS) debris resulting from photoreceptor degeneration, and intact RPE (not shown here). I, In an atrophic area, individual dissociated RPE cells sit atop a basal laminar deposit (white arrowheads). At the edge of atrophy, the external limiting membrane (black arrowheads) is curved as it descends to Bruch’s membrane. Green arrowheads indicate Bruch’s membrane. HFL = Henle fiber layer; INL = inner nuclear layer; IPL = inner plexiform layer. Scale bar in (A) applies to all. Black arrowheads indicate the course of the external limiting membrane. Red arrowheads indicate a retinal capillary.
vitelliform change. Compared with PEDs associated with neovascular AMD, DPEDs had different crystallization patterns, no lipid-filled cells, and thinner BLamD.

In Vivo Optical Coherence Tomography Image Analysis

Of the 216 eyes with PED that were reviewed, 49 eyes of 33 patients had DPED that met the inclusion criteria. Mean age was 74.64 years (range, 56–91 years). The cohort included 24 women, 9 men, 27 right eyes, and 22 left eyes. Mean duration of follow up was 4.9±2.5 years. As measured using SD OCT and summarized in Table 3, maximum DPED volume, diameter, and height were 1.4±1.5 mm³, 2466.4±1076.8 µm, and 344.0±139.5 µm, respectively.

In vivo OCT images were evaluated for RPE and AMD changes associated with DPED using nomenclature defined using the index case. An illustrative example of a 77-year-old man (similar in age to the index donor case) is provided in Figure 4. Optical coherence tomography signatures corresponding to small and large groups of intraretinal RPE cells, thickening of the RPE+BL band, loss of the ellipsoid zone at the DPED apex, and RPE cells arranged in a ring

Table 1. Frequencies of Retinal Pigment Epithelium Phenotypes in Drusenoid Pigment Epithelial Detachment

<table>
<thead>
<tr>
<th>Retinal Pigment Epithelium Morphologic Features*</th>
<th>Locations at Each Grade Associated with Migrated Retinal Pigment Epithelium</th>
<th>Not Associated with Migrated Retinal Pigment Epithelium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not gradable</td>
<td>2 (0.5)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Uniform</td>
<td>22 (5.5)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Nonuniform</td>
<td>237 (39.0)</td>
<td>2 (0.3)</td>
</tr>
<tr>
<td>Very nonuniform</td>
<td>49 (12.2)</td>
<td>4 (1.0)</td>
</tr>
<tr>
<td>Sloughing</td>
<td>16 (4.0)</td>
<td>1 (0.2)</td>
</tr>
<tr>
<td>Shedding</td>
<td>10 (2.5)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Vacuolated</td>
<td>2 (0.5)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Intraretinal</td>
<td>15 (3.7)</td>
<td>—</td>
</tr>
<tr>
<td>Vitelliform</td>
<td>47 (11.7)</td>
<td>18 (4.5)</td>
</tr>
<tr>
<td>Atrophy with BLamD</td>
<td>1 (0.2)</td>
<td>1 (0.2)</td>
</tr>
<tr>
<td>Dissociated</td>
<td>1 (0.2)</td>
<td>1 (0.2)</td>
</tr>
<tr>
<td>Atrophy without BLamD</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Total</td>
<td>402 (100.0)</td>
<td>42 (10.4)</td>
</tr>
</tbody>
</table>

BLamD = basal laminar deposit.

Data are number (%).

*Phenotypes were assigned originally according to Zanzottera et al. Vitelliform was demonstrated by Chen et al and signifies retinal pigment epithelium organelles exploded into the extracellular space, possibly accompanied by intact retinal pigment epithelium cell bodies.

1The intraretinal morphologic feature is, by definition, migrated retinal pigment epithelium.

Table 2. Comparison of 4 Pigment Epithelium Detachments in Age-Related Macular Degeneration, Characterized with Ex Vivo Imaging and High-Resolution Histologic Analysis

<table>
<thead>
<tr>
<th>Age-Related Macular Degeneration Diagnosis</th>
<th>Index Case (Nonneovascular)</th>
<th>ALARMGS17 (Nonneovascular, Clinically Stable)</th>
<th>Onion 121 (Neovascular)</th>
<th>Onion 221 (Neovascular)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs), gender</td>
<td>73, F</td>
<td>63, F</td>
<td>98, F</td>
<td>91, M</td>
</tr>
<tr>
<td>PED type</td>
<td>Drusenoid</td>
<td>Drusenoid</td>
<td>Hemorrhagic</td>
<td>Senous</td>
</tr>
<tr>
<td>PED location</td>
<td>Foveal</td>
<td>Foveal</td>
<td>Foveal</td>
<td>Extramacular</td>
</tr>
<tr>
<td>Crystalline content</td>
<td>Hydroxyapatite</td>
<td>None</td>
<td>Cholesterol</td>
<td>Cholesterol</td>
</tr>
<tr>
<td>Intraretinal fluid</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Anteriorly migrated RPE</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Vitelliform RPE</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Intraretinal lipid-filled cells</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>No. of assessment locations</td>
<td>402</td>
<td>14</td>
<td>4</td>
<td>15</td>
</tr>
<tr>
<td>Assessment locations with BLamD (%)</td>
<td>93.3</td>
<td>7.1</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>RPE thickness (µm), mean ± SD</td>
<td>10.98±4.63</td>
<td>8.33±5.14</td>
<td>10.77±3.87</td>
<td>8.62±4.88</td>
</tr>
<tr>
<td>BLamD thickness, (µm) mean ± SD</td>
<td>3.95±2.22</td>
<td>0.08±0.30</td>
<td>11.88±9.92</td>
<td>9.77±4.01</td>
</tr>
<tr>
<td>RPE+BLamD thickness (µm), mean ± SD</td>
<td>14.93±5.08</td>
<td>8.41±5.24</td>
<td>22.65±7.12</td>
<td>18.39±5.05</td>
</tr>
</tbody>
</table>

ALARMGS = a disintegrin-like and metalloproteinase with thrombospondin type 1 motif-like 4; BLamD = basal laminar deposit; F = female; M = male; PED = pigment epithelium detachment; RPE = retinal pigment epithelium; SD = standard deviation.

*Onion 2 had 6 PEDs with spectra-domain optical coherence tomography onion sign; all were extramacular.
around an inner retinal vessel were seen in the SD OCT volume. Vitelliform material with and without sloughed RPE cells is visible in Figure 4.

Within this cohort, hyperreflective foci were seen in 44 eyes of 29 patients (90%). Of these, 89.4% and 42.6% of cases demonstrated foci at the level of the outer nuclear and ganglion cell layers, respectively, and 10.9% demonstrated hyperreflective foci ringed around retinal vessels. A swarm comprising 5 or more foci was seen in 70.2% of cases. Photoreceptor atrophy, characterized by loss of the ellipsoid zone and thinning of the outer nuclear layer at the DPED apex, was seen in 45.8% of cases. It was not possible to reliably differentiate thickening of the RPE+BL band from vitelliform material in all cases. Therefore, all thickened, hyperreflective material in the subretinal space was attributed to RPE+BL thickening for analysis. It also was not possible to determine RPE+BL thickening reliably, especially in PEDs with focal areas of RPE+BL thickening. In our cohort, 35 eyes (71%) demonstrated changes corresponding to RPE+BL thickening on OCT. Evaluation of longitudinal imaging data demonstrated that intraretinal hyperreflective foci frequently appeared immediately internal to areas of RPE thickening seen in preceding visits, as demonstrated for 2 patients in Figure 5 strongly supporting an origin from the perturbed RPE layer below.

Discussion

This study demonstrates that RPE behavior can be visualized readily in vivo with optimized structural SD OCT. We defined several correlations between histologic analysis and OCT in DPED: (1) small and large hyperreflective intraretinal foci represent fully pigmented and nucleated RPE cells that migrate anteriorly either singly or in groups, (2) hyperreflectivity internal to the RPE+BL band resembling vitelliform lesions represents subretinal plaques of RPE organelles mixed with outer segment debris and sometimes also RPE cell bodies, (3) hypertransmission represents dissociated RPE cells atop BLamD in an atrophic area, and (4) punctate hyperreflective foci in the DPED interior represent refractile material among the lipid pools. Further, in an in vivo cohort of DPEDs the same size as the index case, 90% of eyes demonstrated OCT signatures consistent with various morphologic features of intraretinal RPE.

Our index case supports and extends a 50-year-old body of literature on DPED by demonstrating contents and associated RPE changes by high-resolution epoxy-resin histologic analysis. Older studies refer to DPED as serous or serogranular detachments, because in paraffin-based histologic analysis, DPED has a smooth internal texture resembling fluid. Klien and later Sarks illustrated lesions with shortened overlying photoreceptors and an intact RPE layer of nonuniform thickness. Sarks also described, but did not illustrate, drusen with RPE exhibiting “proliferative activity… with migration… into the retina.” Green and Key and Bressler et al presented a large DPED containing von Kossa-positive nodules presumed to be calcific and overlaid by subretinal and intraretinal pigmented cells and a DPED approximately 1 mm in diameter with partial contents and intact RPE. Curcio et al illustrated the DPED in Figure S3 (available at www.aaojournal.org). Chen et al showed DPED with hyperreflective RPE layer thickening and intraretinal foci (on ex vivo SD OCT) that were confirmed ultrastructurally as RPE granules within vitelliform material and migrated cells, respectively.

The index case provided histologic evidence for many cellular behaviors visible in both ex vivo and in vivo SD OCT imaging. We observed solitary cells as punctate reflective foci and swarms of multiple intraretinal RPE as large irregular foci. Although most swarms comprised groups of cells, we also noted some large individual cells that were multinucleate and resembled a previously illustrated plume on SD OCT. Experimental studies in mice demonstrated that multinucleate RPE cells in the setting of a disintegrin-like and metalloproteinase with thrombospondin type 1 motif-like 4 (ADAMTSL4) deficiency can be migratory, suggesting a final common pathway to different stresses, whereas others indicate that failed cytokinesis in cell division can cause RPE multinucleation. One interesting group of intraretinal RPE seemed to follow the trajectory of Henle fiber layers, resembling a previously illustrated plume on SD OCT. Migrating RPE cells were seen throughout all retinal layers, even surrounding inner retinal capillaries, as in bone spicule degeneration, a phenomenon noted in 11% of eyes in the in vivo cohort. Acquired vitelliform lesions have several components, and corresponding to ex vivo reflectivity, we found RPE granules, intact RPE cells in transit to the retina, and outer segment debris. We did not observe imaging or histologic evidence of shaggy (e.g., swollen) photoreceptors, possibly because of the minimal subretinal fluid in this case. Finally, we showed for the first time that hypertransmission of light below the RPE+BL band, an SD OCT sign considered diagnostic for GA, can occur in an atrophic area containing dissociated RPE.

We identified important differences between the index case and a previously published DPED case. Numerous migrating RPE cells were seen in the index case (10.4% of the DPED surface), and shedding cells were rare. In contrast, in a morphologically stable DPED, the shedding RPE phenotype predominated and anteriorly migrating RPE cells and vitelliform changes were undetected, as reported elsewhere. Cholesterol crystallization and lipid-filled cells seen in serous and hemorrhagic PEDs were not seen in either DPED, suggesting that these features preferentially accompany neovascularization and exudation. Thus, in this small sample, PEDs that progress to advanced AMD (the index case with atrophy and the 2 neovascular AMD cases) seem to be associated with thicker BLamD and are more likely to demonstrate hyperreflective intraretinal cells than...
Figure 4. Histologically defined features visible in vivo. A large drusenoid pigment epithelial detachment (DPED) in a 77-year-old male demonstrates pigmentary changes on the surface that correlate to (B) sites of increased fundus autofluorescence. Areas of B-scans (I, II, and III) are illustrated in the color image. A range of retinal pigment epithelium (RPE)-related changes is seen on optical coherence tomography scans including intraretinal RPE cells and vitelliform lesions. Note that the ellipsoid zone (red arrowhead) is visible on the surface of the pigment epithelial detachment with the exception of the apex, where it is notably absent. In this case, it was possible to distinguish vitelliform lesions from RPE thickening. However, this distinction was not possible in most cases in this series.
PEDs that do not progress (the clinically stable case). Morphologic heterogeneity should be considered in PED-specific treatment recommendations, as reported previously, because PED lacking neovascularization like the index case may be unresponsive to vascular endothelial growth factor suppression.

Drusenoid PEDs are the largest drusenoid lesions on a continuum, allowing judicious extrapolation from our data on very large DPED to soft drusen in general. Sarks et al noted that GA followed the resolution of large, confluent soft drusen. In the large AREDS data set of color fundus photographs, Klein et al found that atrophy was preceded by a sequence of large drusen, hyperpigmentation and hypopigmentation, and, in some instances, refractile druse contents. In 311 DPED-containing eyes from the AREDS data set, Cukras et al concluded that large soft drusen and DPED evolve along common pathways. This commonality was reinforced by Schlanitz et al, who used automated OCT segmentation technology to classify more than 6000 RPE elevations in patients with intermediate AMD, including approximately 300 DPEDs, using the AREDS size criterion. Ouyang et al emphasized the prognostic value of hyporefractive areas appearing within drusen, the nature of which is uncertain and is a focus of our current research.

A role for intraretinal hyporefractive foci in AMD progression as seen on SD OCT has been demonstrated with increasingly advanced imaging and analysis techniques. Discrete foci with underlying shadowing attached to RPE overlying drusen were attributed to anteriorly migrated RPE by reference to similar foci seen in proliferative vitreoretinopathy, where RPE involvement is known. Foci also were correlated with hyperpigmentation, thus linking to knowledge of AMD progression available from population-based epidemiologic studies. Foci could be found as far inward as the ganglion cell layer, but more often were found in photoreceptor layers. Using eye-tracked SD OCT to track 571 individual drusenoid lesions, Ouyang et al found that risk factors for progression included large lesions, heterogeneous internal reflectivity, and presence of hyporefractive foci at baseline, with inward movement of foci over time conferring the greatest risk (odds ratio, 28.2). Schlanitz et al observed hyporefractive foci over 4.1% of DPEDs, the highest of any lesion size class. The true prognostic value of intraretinal hyporefractive foci suggested by series of very large DPEDs, including ours, will emerge from prospective studies applying objective and automated methods to a wide range of individual lesions.

Authors of imaging studies hypothesized about the identity of hyporefractive foci related to drusen, most calling them migrated RPE, but others also suggesting microglia pigment, macrophages with pigment inside, exudates, lipid, blood, or a degenerative retinal process conferring reflectivity. By pairing the snapshot of histology with the movie of longitudinal clinical imaging, our study substantially narrows the window of uncertainty around intraretinal hyporefractive foci being cells of RPE origin. First, in our index case, all foci were found to correspond to fully pigmented cells. Second, hyporefractive

Figure 5. Optical coherence tomography (OCT) images showing the association of intraretinal hyporefractive foci and thickening of the retinal pigment epithelium (RPE) plus basal laminar (BL) band during the lifecycle of drusenoid pigment epithelial detachments (DPEDs). The natural course of DPED of a 71-year-old woman (case A) and a 68-year-old woman (case B) as seen using in vivo spectral-domain OCT with image registration enabled are presented. At their first visit (0 months), hyporefractive material is seen at the level of the RPE and subretinal space (yellow arrowheads) in both cases. Over time, hyporefractive foci are seen to migrate from the RPE plus BL band into the retina (green arrowheads). The rate of RPE migration and the quantity of cells migrating into the retina were different in the 2 cases. Yellow arrowheads denote the same location in the pigment epithelial detachment in image-registered sections and may signify vitelliform material in addition to RPE cell bodies in transit. The yellow arrow (case A, 10 month) also shows vitelliform material.
foci frequently were seen to appear directly anterior to thickened RPE+BL band or vitelliform lesion on a time scale of a few months. For non-RPE cells to account for these phenomena, they would have to match both the organelle population of epithelial RPE and the spatiotemporal characteristics of appearing in position precisely over and after disturbances of the layer.

An alternate hypothesis for the origin of hyperreflective foci is macrophages or microglia that ingested RPE organelles, thus taking on their reflective properties. This hypothesis is supported by experimental studies in mouse models, in which subretinal microglia are active in aging compared with intermediate disease and are worse in humans. Notably, in human GA, pigmented cells resembling sloughed and intraretinal RPE express an inflammatory marker (CD163, a haptoglobin-hemoglobin scavenger) typical of bone marrow–derived macrophages, suggesting a new range of activities. Transdifferentiation is the changing of a cell from one differentiated state to another. Our data support a role for transdifferentiated RPE in AMD that is unclear but can be studied by in vivo monitoring. These ideas about RPE are not inconsistent with well-established roles of monocyte-derived macrophages and inner retina–derived microglia in advanced AMD. What molecular signals prompt RPE to become migratory and whether signals arise from the sub-RPE space, neurosensory retina, or both is unspecified, although we suspect the retina because of the universally inward movement. Presumably such signals also will confront replacement cells implanted in patients with GA.

Strengths of this study include the complementarity of histologic results and clinical SD OCT, the use of ex vivo tissue imaging as a bridge to in vivo imaging, and a histologic and photomicrographic combination that provided subcellular resolution. Limitations of the histologic analysis are the small number of cases overall and cases with clinical history specifically and the lack of labeling studies to confirm cellular identities. Limitations of the in vivo imaging study include the unreliable differentiation of RPE+BL band thickening from vitelliform material, which may be addressable with RPE-specific detection technology, and the possible underestimate of mean PED height because of maximum heights falling between adjacent B-scans. Despite these shortcomings, our OCT assignments for PED enhance our understanding of GA pathophysiologic characteristics. They also motivate new clinical studies using multimodal longitudinal imaging to determine an accurate timeline of atrophy and designing targeted therapies to mitigate this increasingly common cause of vision loss.

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Footnotes and Financial Disclosures

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Abbreviations and Acronyms:
AMD = age-related macular degeneration; AREDS = Age-Related Eye Disease Study; BL = basal lamina; BLamD = basal laminar deposit; BM = Bruch’s membrane; DPED = drusenoid pigment epithelium detachment; GA = geographic atrophy; OCT = optical coherence tomography; PED = pigment epithelium detachment; RPE = retinal pigment epithelium; RPE+BL = retinal pigment epithelium plus basal lamina; SD = spectral domain.

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Supplementary Figure S1. *Ex vivo* imaging of the *Index* case. Green lines indicate the extent of a drusenoid pigment epithelial detachment (D-PED) studied closely in stepped sections. Arrowheads denote histologically confirmed soft drusen. **A.** Color photograph, with retina on. The D-PED is hypopigmented lesion with pigment clumps, apparent through the thin yellow fovea. Pale stripes are choroidal vessels emptied upon the drop of blood pressure at death. **B.** Near infrared reflectance. The D-PED exhibits intense diffuse and focal reflectivity. Numbers indicate the scan levels shown in Figure 2.

**Supplementary Figure S2. Computer-generated maps of the D-PED surface in the Index case.** The D-PED is represented in blue by a triangulated, piecewise-planar surface. Red dots indicate location of intraretinal RPE. Yellow dots indicate location of vitelliform material. Data gathered using a custom Java plugin for FIJI included tracings of 28 stepped histological sections through the dome of the D-PED, along with locations of interesting features. Features were sampled at pre-determined locations in each section as described in the main text. A separate custom Java program reconstructed a 3D model of the D-PED surface along with markers identifying and locating the features. The complete 3D model was created and viewed using OpenSCAD ([www.openscad.org](http://www.openscad.org)). **A.** Top-down view. **B.** Three-quarter view.
Supplementary Figure S3. Clinicopathologic correlation of a clinically stable drusenoid pigment epithelial detachment in Case 2. A. A large subfoveal druse complex surrounded by soft drusen was first seen in a 42-year-old woman in 1973. B. Ex vivo color imaging with epi- and trans-illumination at death 22 years later indicated that the lesion had been stable. Green lines indicate levels of histologic sections in panels C and D. C. The RPE layer is pigmented, continuous, variable in thickness, and overall thin. The druse interior is variegated in staining and contains cells with sparse melanosomes. Black arrowheads, BrM. D. A granule aggregate is apparent (pink arrowhead). Air bubbles in the epoxy resin artifactually deformed the bacillary layer (asterisk). ONL = outer nuclear layer; IS = inner segments; OS = outer segments; RPE = retinal pigment epithelium; ChC = choriocapillaris

**Supplementary Table S1 – Summary of in vivo SD-OCT protocol for cohort.**

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