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Research article

Comparison of chorioretinal layers in rhesus macaques using spectral-domain optical coherence tomography and high-resolution histological sections

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ABSTRACT

Nonhuman primates are important preclinical models of retinal diseases because they uniquely possess a macula similar to humans. Ocular imaging technologies such as spectral-domain optical coherence tomography (SD-OCT) allow noninvasive, in vivo measurements of chorioretinal layers with near-histological resolution. However, the boundaries are based on differences in reflectivity, and detailed correlations with histological tissue layers have not been explored in rhesus macaques, which are widely used for biomedical research. Here, we compare the macular anatomy and thickness measurements of chorioretinal layers in rhesus macaque eyes using SD-OCT and high-resolution histological sections. Images were obtained from methylmethacrylate-embedded histological sections of 6 healthy adult rhesus macaques, and compared with SD-OCT images from 6 age-matched animals. Thicknesses of chorioretinal layers were measured across the central 3 mm macular region using custom semi-automated or manual software segmentation, and compared between the two modalities. We found that histological sections provide better distinction between the ganglion cell layer (GCL) and inner plexiform layer (IPL) than SD-OCT imaging. The first hyperreflective band between the external limiting membrane (ELM) and retinal pigment epithelium (RPE) appears wider on SD-OCT than the junction between photoreceptor inner and outer segments seen on histology. SD-OCT poorly distinguishes Henle nerve fibers from the outer nuclear layer (ONL), while histology correctly identifies these fibers as part of the outer plexiform layer (OPL). Overall, the GCL, inner nuclear layer (INL), and OPL are significantly thicker on histology, especially at the fovea; while the ONL, choriocapillaris (CC), and outer choroid (OC) are thicker on SD-OCT. Our results show that both SD-OCT and high-resolution histological sections allow reliable measurements of chorioretinal layers in rhesus macaques, with distinct advantages for different sublayers. These findings demonstrate the effects of tissue processing on chorioretinal anatomy, and provide normative values for chorioretinal thickness measurements on SD-OCT for future studies of disease models in these nonhuman primates.

1. Introduction

Nonhuman primates are the only mammals to possess a true macula similar to that of humans, making them ideal animal models for studying foveal development and macular diseases (Cornish et al., 2005; Dawson et al., 1989b; Engel et al., 1988; Francis et al., 2008; Hendrickson and Zhang, 2017; Hope et al., 1992; Sandercoe et al., 2003; Yuodelis and Hendrickson, 1986). Laser-induced choroidal neovascularization may be created to simulate exudative age-related macular degeneration (AMD) (Miller et al., 1990; Miller and Sparrow, 1948; Ryan, 1979), and both rhesus and cynomolgus macaques (*Macaca mulatta* and *Macaca fascicularis*), spontaneously develop drusenoid lesions with some features that are clinically similar to those seen in AMD patients (Bellhorn et al., 1981; Dawson et al., 1989a; El-Mofty et al., 1978; Hope et al., 1992; Umeda et al., 2005; Yiu et al., 2017), although nonhuman correlates of more advanced forms of AMD such as choroidal neovascularization and geographic atrophy have never been described. Nonhuman primates also serve as important pre-clinical animal models

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for novel therapies and drug testing in ophthalmology.

In recent years, spectral-domain optical coherence tomography (SD-OCT) has been increasingly employed as a non-invasive imaging modality to evaluate retinal and choroidal anatomy in human clinical practice and ocular research. Based on low-coherence interferometry using near-infrared light, SD-OCT provides high-resolution cross-sectional images of the retina and choroid due to differential light scattering from individual chorioretinal layers. The differences in reflectivity allow in vivo distinction of the retinal nerve fiber layer (NFL), ganglion cell layer (GCL), inner plexiform layer (IPL), inner nuclear layer (INL), outer plexiform layer (OPL), outer nuclear layer (ONL), external limiting membrane (ELM), as well as the inner and outer segments (IS + OS) of photoreceptors in the retina. Various organelles including melanosomes, lipofuscin, melanolipofuscin granules, and mitochondria account for differential reflectivity of various layers due to the principle of Mie scattering (Wilson et al., 2007; Wilson and Foster, 2007). Melanin granules also accounts partly for the hyperreflectivity of the retinal pigment epithelium (RPE), and may contribute to the reflectance pattern of the underlying choriocapillaris (CC) and outer choroid (OC) as well (Yiu et al., 2016).

The histological correlates of these chorioretinal layers as seen on SD-OCT have been extensively characterized in human subjects, and their normative thickness values have been previously described (Bagci et al., 2008; Chan et al., 2006; Curcio et al., 2011; Grover et al., 2009, 2010; Liu et al., 2011; Sull et al., 2010). Similar studies have also been conducted in laboratory animals including mice and cynomolgus macaques (Anger et al., 2004; Ferguson et al., 2014), but not in rhesus macaques – the most widely-used nonhuman primate in biomedical research. Here, we provide a comprehensive comparison of chorioretinal layers seen on SD-OCT images and high-resolution histological sections in healthy adult rhesus macaques, with the goal of providing a framework for future studies employing this species as a model for retinal or choroidal diseases.

2. Material and methods

2.1. Subject & eye selection

Healthy adult rhesus macaques greater than 6 years of age (roughly equivalent to 18 human years) representing a spectrum of ages through early, middle, and late adult life were selected from animals undergoing routine semiannual physical examinations at the California National Primate Research Center (CNPRC). The CNPRC is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International. Our studies using rhesus macaques followed the guidelines of the Association for Research in Vision and Ophthalmology (ARVO) statement for the Use of Animals in Ophthalmic and Vision Research, complied with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals, and were approved by the University of California, Davis Institutional Animal Care and Use Committee (IACUC). All subjects underwent complete ophthalmic examination including slit-lamp biomicroscopy and dilated fundus biomicroscopy. Animals that showed any retinal or choroidal lesions on clinical examination, such as drusenoid lesions, were excluded from the study.

For histological analysis, cadaveric eyes were collected from rhesus macaques undergoing necropsy for nonocular health issues, with a death-to-preservation time of several minutes (no more than 10 min for any eye), and only eyes that had well-preserved chorioretinal anatomy after tissue processing were included. For SD-OCT, age-matched animals with normal ophthalmic examinations were selected to undergo ocular imaging. Only one eye was selected from each animal for analysis, based on the quality of the histological section or SD-OCT image relative to the fellow eye.

For histologic evaluation, the mean age of animals was 16.3 ± 8.7 years (range, 10–27 years), with 4 females and 2 males. 4 were right eyes, and 2 were left eyes. For SD-OCT analysis, the mean age of the selected animals was 18.6 ± 6.5 years (range, 9–28 years), with 4 females and 2 males. Three were right eyes, and 3 were left eyes. The animals selected for SD-OCT were age-matched to those from which histological sections were taken, with no significant difference in mean age ($P = .61$) and sex ($P = 1.00$) of animals, or laterality of eyes selected ($P = .56$).

2.2. Histological processing

For high-resolution histological sections, entire globes were fixed in 2% paraformaldehyde and 0.5% glutaraldehyde, with a small slit made through the pars plana with a scalpel blade to facilitate penetration of the fixative. Tissues were dehydrated using increasing concentrations of ethanol from 50% to 100%, pre-infiltrated with a 50:50 mixture of ethanol and methylmethacrylate (Technovit®, Kulzer) for 2–3 h, then infiltrated with methylmethacrylate for a minimum of 24 h. Serial sections were cut at 1.5 μm thickness through the foveal region using a Leica EM UC6 ultramicrotome, with a horizontal orientation along the optic disc-foveal axis matching the SD-OCT line-scans. The slides were stained with 1% toluidine blue O for 30 s, rinsed, dried, and coverslipped (Permount, Fisher). Histological sections were imaged using a 40× objective lens on a Virtual Slide Microscope (VS120-S6-W, Olympus, Tokyo, Japan), at a resolution of 172.51 nm per pixel. For each eye, we performed layer segmentation on images of the histological section closest to the fovea centralis, defined as the center of the foveal depression with lateral displacement of the inner retinal layers, and greatest elongation of the outer segments.

2.3. Spectral domain OCT imaging

SD-OCT imaging was performed using the Spectralis SD-OCT device (Heidelberg Engineering, Heidelberg, Germany), which has been modified with a flat chin-rest to allow the rhesus monkeys' head to be positioned. We used a wire lid speculum to keep the eyes opened, and applied artificial tears to maintain the tear film on the ocular surface. Animals were monitored by a trained technician and a CNPRC veterinarian while imaging took place. We captured 7 horizontal line scans in a 30° x 5° region centered on the fovea with mean line spacing of 218.7 ± 6.0 μm, in high-resolution mode with 1536 A-scans per B-scan and axial resolution of 3.87 μm per pixel, and using enhanced depth imaging (EDI) mode to optimize visualization of the choroid. The transverse resolution was calibrated based on average corneal curvatures measured for each animal (mean 6.48 ± 0.03 mm) using a corneal topography device (Pentacam HR, Oculus). Twenty-five images were averaged for each B-scan using the Automatic Real-Time (ART) eye-tracking option of the Heidelberg Explorer software (version 1.8.6.0, Heidelberg Engineering). For each eye, we performed image segmentation of the line scan closest to the foveal center, defined as the center of the foveal pit with the greatest separation between the IS-OS (EZ) (Jonnal et al., 2014, 2017; Meadway et al., 2013; Staurenghi et al., 2014) and the RPE-Bruchs membrane complex.

2.4. Image segmentation

Digital images of histological sections and SD-OCT images were segmented using the Duke Optical Coherence Tomography Retinal Analysis Program (DOCTRAP, version 62.0), a custom image analysis software designed using MATLAB (Mathworks) (Chiu et al., 2010). For histological sections, layer segmentation was performed manually by two independent graders based on careful evaluation of the chorioretinal structures and anatomic boundaries of digital images on a 27″ LCD computer display, viewed at an effective magnification of 500× (Fig. 2A). For SD-OCT images, segmentation boundaries were automatically calculated by DOCTRAP using graph-based determination of reflectivity profiles, then manually refined by the two graders (Fig. 2B).