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Original Article

Optical coherence tomography and histologic measurements of retinal and choroidal thicknesses in guinea pig eyes

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Abstract: Background: The purpose of this study was to evaluate optical coherence tomography and histologic measurements of retinal and choroidal thicknesses in guinea pig eyes, and assess the relationships between those thicknesses with spherical equivalent and axial length. Methods: Twelve tri-colored guinea pigs, aged from 5 to 6 weeks, were collected. Spherical equivalent (SE) was measured with a streak retinoscope. Axial length was determined by A-scan ultrasonography. Retinal and choroidal thicknesses were measured by OCT and histology, respectively. Results: The Bland-Altman analysis showed that there were significant differences for retinal and choroidal thicknesses between the OCT and histology. Furthermore, choroidal thicknesses were correlated with SE and axial length, while there were no significant correlations between retinal thicknesses with SE and axial length. Retinal and choroidal thicknesses measured by OCT were positively correlated with histology. Conclusion: Retinal and choroidal thicknesses could be well measured from OCT images and histological sections with reasonable agreement. The degree of myopia and elongation of the globe are associated with thinning of choroidal thickness, while not with retinal thickness.

Keywords: Retinal thickness, choroidal thickness, OCT, histology

Introduction

Optical coherence tomography (OCT) is a noninvasive technology capable of producing high-resolution optical cross-sections of retina and choroid [1, 2]. Clinically, OCT is used in diagnosis of retinal and choroidal diseases and to monitor structural changes of the retina and choroid, both post-treatment and during disease progression [3-5]. Numerous studies have been conducted to evaluate the relationship between OCT and anatomic features in order to gain a better understanding and interpretation of OCT images [6-10]. In vitro reflectivity profiles of retinal and choroidal structure could differ from in vivo reflectivity profiles because the effects of fixation and perfusion of blood vessels may alter the optical properties of the retina and choroid. Furthermore, OCT instruments in different studies have different axial resolutions and more bands of reflectivity are apparent in the OCT signal from higher resolution instruments.

There is still debate regarding the precise relationship between the OCT and the retinal thickness due to the subjective nature and the differences in design of the previous studies [4, 11]. Fourier-domain OCT has been established, enabling high-resolution (less than 5 μm) volumetric images of the retina without eye movement artifacts [12]. The guinea pigs are a promising alternative to other mammals for experimental myopia, as they are born with a well-developed visual system [13, 14]. The purpose of the present study was to compare Fourier-domain OCT and histologic measurements of retinal and choroidal thicknesses in guinea pig eyes, and evaluate the relationships between those thickness with spherical equivalent and axial length.

Methods

Animals

Twelve tri-colored guinea pigs, aged from 5 to 6 weeks, were obtained from the breeding room

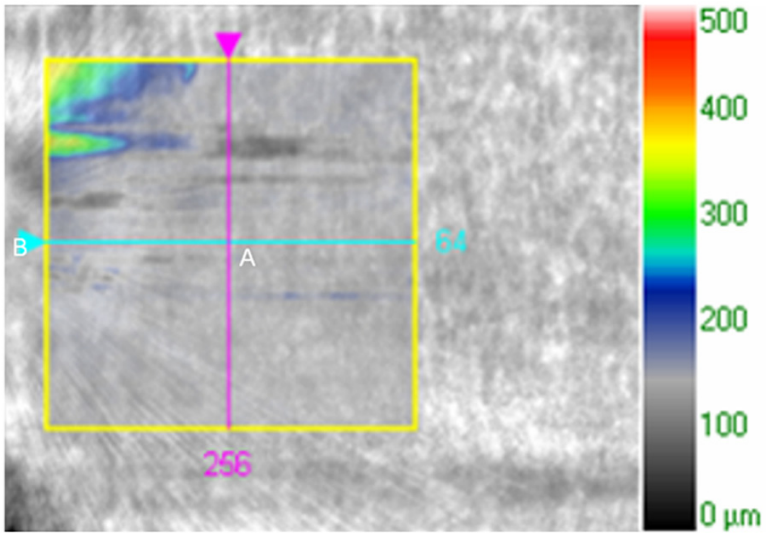

Figure 1. OCT image of a guinea pig eye. A: A single measuring point locating 3 mm away from optical nerve head. B: Optical nerve head.

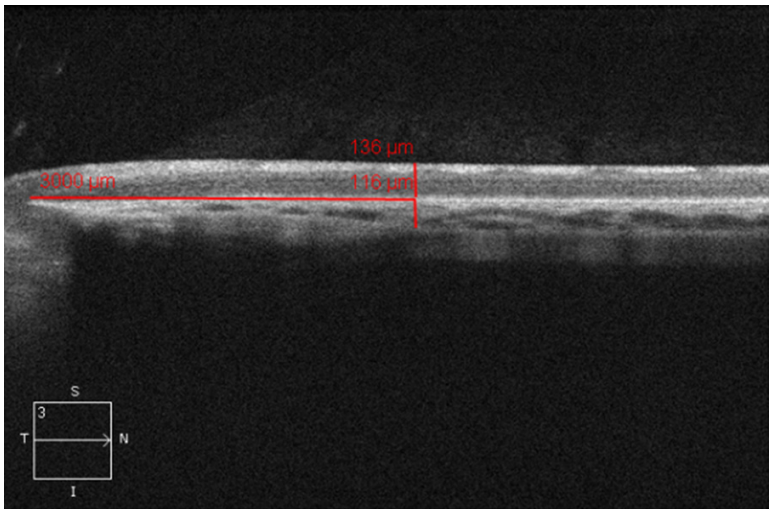

Figure 2. OCT image of a guinea pig eye. The retinal and choroidal thicknesses at a single measuring point are 3000 μm away from optical nerve head. The retinal and choroidal thicknesses are 136 μm and 116 μm, respectively.

in Thai town, Fengxian District, Shanghai City, China. Guinea pigs were reared at 25°C with a 12-12 h light-dark cycle. The animal research was approved by the Animal Care and Ethics Committee at Jinshan Hospital of Fudan University, Shanghai, China. The treatment and care of the animals were conducted according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision research.

Spherical equivalent and axial length

Biometric measures were performed in vivo to verify normal ocular development. Cycloplegia and dilation of the pupil was induced by 4 drops of tropicamide 0.5%, and 30 minutes later spherical equivalent (SE) was measured with a streak retinoscope. Axial length was determined by A-scan ultrasonography (10-MHz focused transducer; SUPER SW1000; Suoer electronic technology Co., Ltd., Tianjin, China). Further details of the biometric methodology have been described else where [13]. Ten repeated measurements were taken and the mean was recorded for analysis.

Optical coherence tomography

Macular thickness scannings in right eyes of guinea pigs were performed by Cirrus-HD OCT 4000 (Carl Zeiss Meditec, Inc., Dublin, USA), with HD 5 line raster protocol. This protocol consists of 5 individual line scans regularly arranged in a radial pattern, with a default scan length of 6 mm. Each line scan was composed of 128 single A-scans. When scanning in this study, the middle scan line connected with the midline of optical nerve head, while the left end of scan line remains tangent to the right side of optical nerve head. The retina centered on the live scanning-laser image was captured with signal strengths of at least seven out of ten. We measured the thickness at the central point of the middle scan line located 3 mm away from the optical nerve head (**Figure 1**). The refractive power of each eye was compensated for by adjusting the focus knob to a value closest to the spherical value of the eye examined.

Retinal thickness at the central point was determined as the distance between the vitreoretinal interface and the boundary corresponding to the photoreceptor inner-outer segment junction, using the Cirrus linear (**Figure 2**). Choroidal thickness was measured from the outer portion

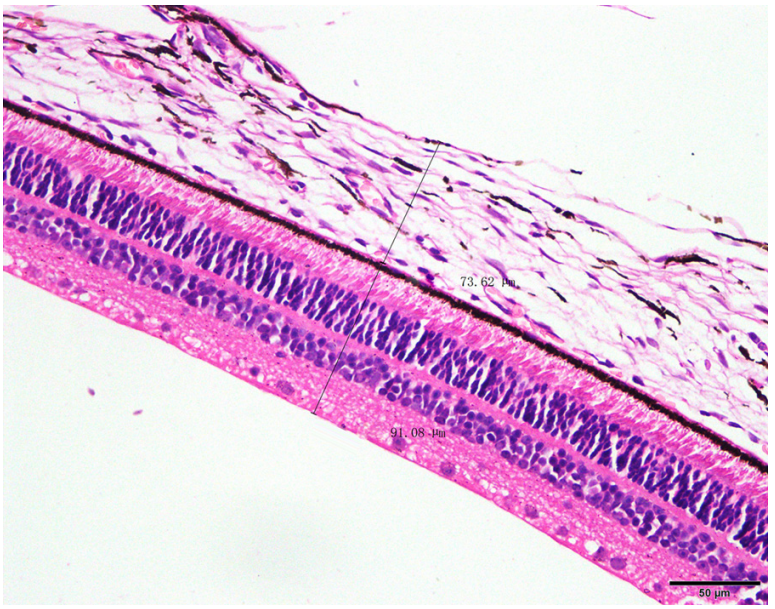

Figure 3. Histologic section of a guinea pig eye. The retinal and choroidal thicknesses are the same tissue location measured in the OCT. The retinal and choroidal thicknesses are 91.08 μm and 73.62 μm, respectively. Scale bars, 50 μm.

of the hyperreflective line corresponding to the retinal pigment epithelium to the inner surface of the sclera at the central point, using the Cirrus linear (**Figure 2**). All measurements were made by the same examiner who was masked to the SE.

Histological processing

Full details of the histological processing protocol have been previously described. Briefly, the eyes were enucleated and the posterior eyecup was immersion fixed in 2.5% glutaraldehyde and 1% paraformaldehyde in 0.1 M phosphate buffer, pH 7.2. Serial vertical semithin (1 μm) sections were cut on a microtome, stained with toluidine blue and micrographs were taken with an Olympus microscope. Retinal and choroidal thicknesses in micrometers were measured at ×400 magnification on a digital micrograph by manually identifying the position of the retinal and choroidal boundaries across the section, then measuring the distances between the boundaries (**Figure 3**). We measured the thickness at the same point located 3 mm away from the optical nerve head with OCT measurement.

Statistical analysis

SE was defined as spherical power plus half-negative cylinder power. All data are expressed as the mean ± SD, and were analyzed using SPSS version 17.0 software (SPSS Inc., Chicago, IL, USA). The agreement between OCT and histological thickness values were evaluated using Bland-Altman plot analysis. The 95% limits of agreement (LoA) were calculated as the mean difference ± 1.96 SD. Furthermore, linear regression analysis was applied to assess the relationships between retinal and choroidal thickness measured by OCT and histology. In addition, the Spearman correlation analysis was used to assess the relationships between (1) SE and retinal thickness measured by OCT and histology; (2) SE and choroidal thickness measured by OCT and histology; (3) Axial length and retinal thickness measured by OCT and histology; and (4) Axial length and choroidal thickness measured by OCT and histology. $P < 0.05$ was considered statistically significant.

Results

The mean SE refractive error (± SD) was + 0.44 ± 2.65 D and the mean axial length (± SD) was 8.31 ± 0.06 mm. The mean, SD and range of the retinal and choroidal thicknesses assessed by each modality are presented in **Table 1**. The Bland-Altman plots showed that the differences of both parameters evenly spread around the mean difference without any specific trends (**Figures 4** and **5**). The range and 95% LoA were significant for retinal and choroidal thicknesses between the OCT and histology, showing in reasonable agreement. Retinal and choroidal thicknesses measured by OCT were positively correlated with histology ($r^2 = 0.47$, $P = 0.015$, and $r^2 = 0.77$, $P < 0.001$; **Figure 6A** and **6B**).

There were no significant correlations between SE and retinal thickness measured by OCT or histology ($r^2 = 0.24$, $P = 0.11$, and $r^2 = 0.01$, $P = 0.79$; **Figure 7A** and **7B**). Furthermore, no significant correlations were found between axial length and retinal thickness measured by OCT or histology ($r^2 = 0.23$, $P = 0.11$, and $r^2 = 0.01$, $P = 0.80$; **Figure 8A** and **8B**). Choroidal thick-

Table 1. Characteristics of measurement data with statistical analysis (n = 12)

	Modality	Mean ± SD	Minimum	Maximum	Mean difference ± SD
Retinal thickness	OC	142.17 ± 10.46	124	157	7.96 ± 11.88
	Histology	134.20 ± 16.22	104.74	150.93	
Choroidal thickness	OCT	111.42 ± 18.77	76	142	2.55 ± 9.46
	Histology	108.87 ± 13.83	84.66	130.18	

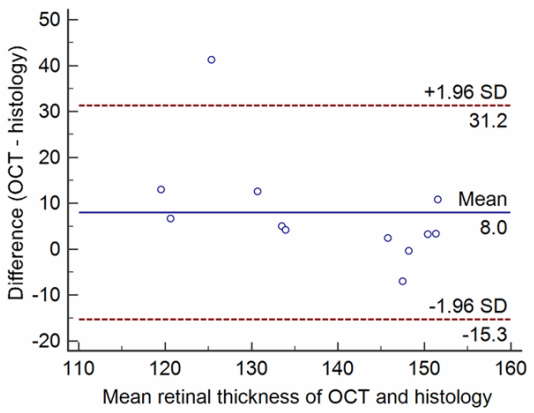

Figure 4. Mean retinal thickness of OCT and histology (μm) versus difference.

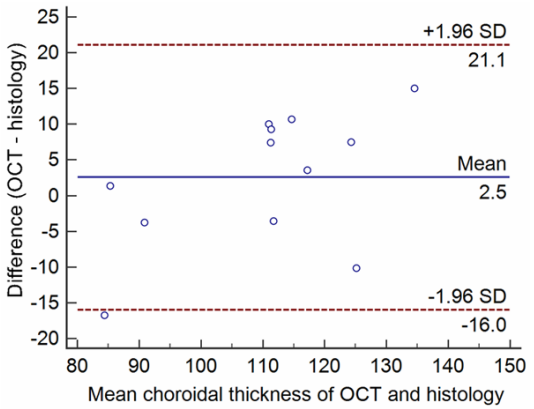

Figure 5. Mean choroidal thickness of OCT and histology (μm) versus difference.

nesses, whether measured by either OCT or histology were positively correlated with SE (r^2 = 0.68, P = 0.001, and r^2 = 0.58, P = 0.004; **Figure 7C** and **7D**), but negatively with axial length (r^2 = 0.68, P < 0.001, and r^2 = 0.56, P = 0.005; **Figure 8C** and **8D**).

Discussion

The present study measured retinal and choroidal thicknesses from OCT images and histological sections. We verified that reasonable agreement between OCT and histological thickness occurred using Bland-Altman plot analysis. In addition, we observed that choroidal thicknesses were significantly correlated with SE and axial length, in agreement with the finding of Marcus et al [15], which demonstrated a positive correlation between choroid thickness and refractive error in guinea pigs. However, retinal thicknesses were not correlated with SE and axial length.

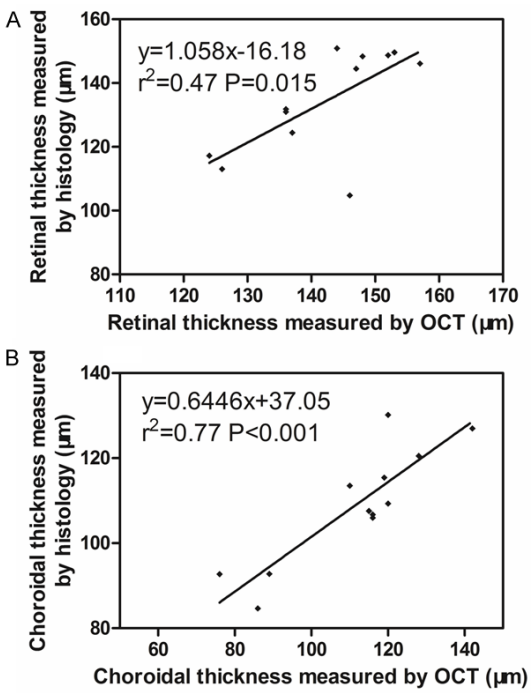

Figure 6. Retinal thickness measured by OCT and histology (A); choroidal thickness measured by OCT and histology (B). Retinal and choroidal thicknesses measured by OCT are positively correlated with histology (r^2 = 0.47, P = 0.015, and r^2 = 0.77, P < 0.001).

OCT has been used in many experimental animals (e.g., guinea pig [16], chicken [17], swine [9, 18], tree rat [19], mouse [8], and monkey [20, 21]). Anatomically, the optic disc of guinea pig is located in the temporal region of the fundus, without macula and fovea [16]. In this

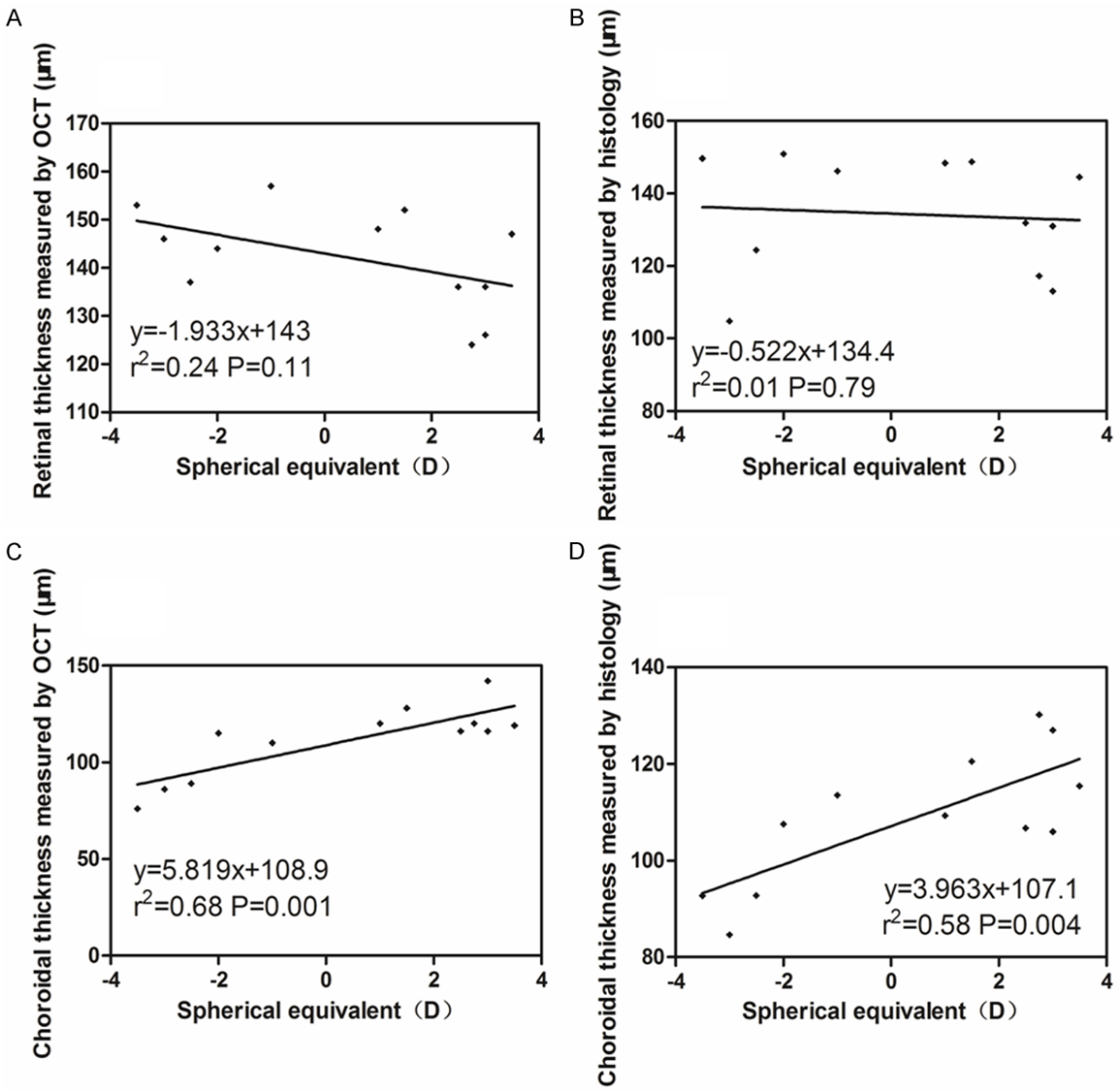

Figure 7. Spherical equivalent and retinal thickness measured by OCT (A) and histology (B); spherical equivalent and choroidal thickness measured by OCT (C) and histology (D). Significant correlations are seen between spherical equivalent and choroidal thickness measured by OCT (r^2 = 0.68, *P* = 0.001) and histology (r^2 = 0.58, *P* =0.004). In contrast, there are no significant correlations between spherical equivalent and retinal thickness measured by OCT (r^2 = 0.24, *P* = 0.11) and histology (r^2 = 0.01, *P* = 0.79).

study, we ensure that scan's horizontal midline of OCT connected with the midline of optical nerve head, while the left end of scan line remains tangent to the right side of optical nerve head. With optical nerve head as physiological structure markers, this modality makes OCT can measure the same site of the retinal and choroidal thickness in guinea pigs with histological section.

Since Huang et al [6] firstly found that OCT retinal thickness corresponded to the retinal thickness measured histologically, the OCT technique has been widely used in clinical and experimental properties. Abbott et al [19] found that the similarity between the results from OCT and histology methods validates OCT as a useful tool for in vivo measuring and monitoring of retinal thickness changes in myopia of tree shrews. The average thickness of the retina in vivo could be similar to that of tissue morphology after calibration [19, 20, 22]. However, the previous studies [19, 20, 22] showing good agreement, had small sample (n = 3) and did

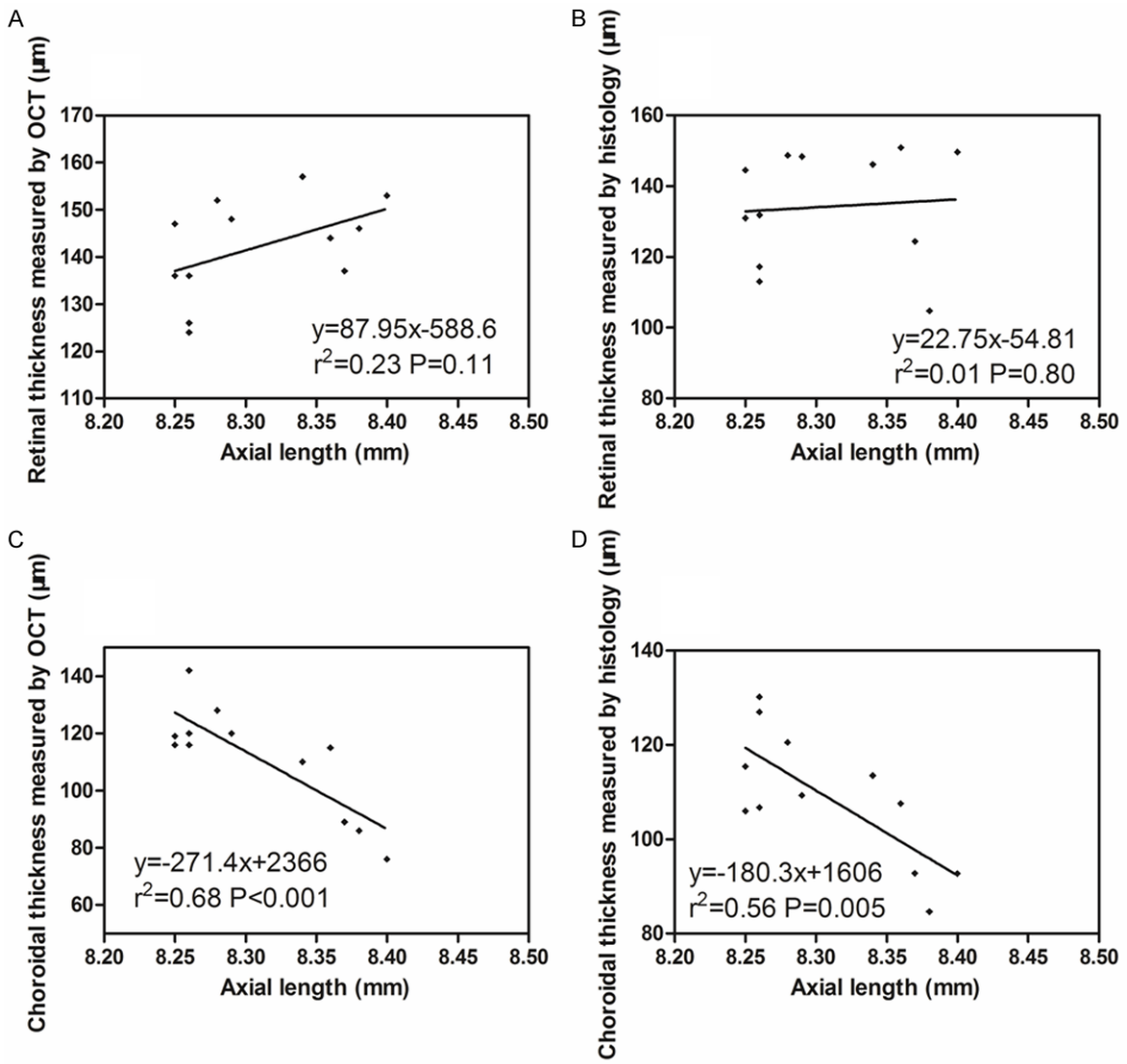

Figure 8. Axial length and retinal thickness measured by OCT (A) and histology (B); axial length and choroidal thickness measured by OCT (C) and histology (D). Significant correlations are seen between spherical equivalent and choroidal thickness measured by OCT (r^2 = 0.68, P < 0.001) and histology (r^2 = 0.56, P = 0.005). In contrast, there are no significant correlations between spherical equivalent and retinal thickness measured by OCT (r^2 = 0.23, P = 0.11) and histology (r^2 = 0.01, P = 0.80).

not use Bland-Altman analysis. In this study, we calculated the retinal and choroidal thicknesses in a larger sample (n = 12). The Bland-Altman analysis showed that the retinal and choroidal thicknesses between the OCT and histology were in reasonable agreement. Furthermore, our results demonstrated that the thicknesses of retina and choroid directly measured by histology without calibration were significantly correlated to OCT.

In the present study, the choroidal thickness was slightly thinner than the retinal thickness, which is similar to that measured by ultrasound and OCT [23, 24]. Lu et al found [24] the estimated choroidal thickness by OCT in the defocused eyes of guinea pigs reduced during optical defocus, but retinal thickness did not significantly change. They thought that the changes in choroidal thickness were due to regulation of the choroidal perfusion rather than morphological changes in the choroids [24]. The results were similar to our findings from this study. But Lu and her co-workers used only 3 guinea pig cadaver eyes to verify the tissue interfaces defined by the in vivo OCT measurement, and

they did not compared the OCT and histology values. Furthermore, our findings about the relationships between retinal and choroidal thicknesses with SE and axial length were also similar to some studies reported in human [25-27], suggesting similarity between guinea pig and human eyes.

One of the limitations of the current study is that only one location in the retina and choroid was measured in this study. Regional thickness differences have been reported in the retina of guinea pigs [19]. Further study will be warranted to measure the retinal and choroidal thicknesses at multiple locations. Furthermore, additional noise components, including inherent irregularity of histologic boundaries, subjective judgment required in positioning histologic boundaries, would act to reduce the correlations between OCT and histology.

In conclusion, retinal and choroidal thicknesses could be well measured from OCT images and histological sections. Bland-Altman plot suggests they are in reasonable agreement. Furthermore, the degree of myopia and elongation of the globe are associated with thinning of choroidal thickness, while not with retinal thickness.

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Disclosure of conflict of interest

None.

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