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ANALYSIS OF CONJUNCTIVAL VASCULAR DENSITY IN SCLERAL CONTACT LENS WEARERS USING OPTICAL COHERENCE TOMOGRAPHY ANGIOGRAPHY

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A R T I C L E I N F O

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A B S T R A C T

Purpose: To investigate conjunctival vascular density (VD) using Anterior Segment Optical Coherence Tomography Angiography (OCTA) in Scleral Contact Lens (ScCL) wearers.
Methods: In this cross-sectional study, the conjunctival blood VD was assessed using OCTA with an anterior segment lens adapter. The nasal surface of each eye (6 ×6 mm) was scanned to a depth of 800 µm with ScCL and fifteen minutes after removing the lens. Conjunctival VD was defined as the percentage of the scanned volume occupied by vessels in which blood flow was measured. Measures of limbal indentation were subjectively determined by two independent observers using Anterior Segment Optical Coherence Tomography (AS-OCT). Comparisons between VD measurements before and after ScCL removal and correlations between conjunctival VD, time of use, vault and indentation values were also investigated.
Results: A total of 23 patients (3 females, 20 males) with keratoconus, with a mean age (years±SD) of 38.74±10.38 were included in the study. VD was significantly higher without ScCL (71.75%±2.97) than VD measured with ScCL (69.81%±2.63), p=0.02. A moderately negative correlation was found between indentation and vault (r=-0.44, p<0.05) and a positive tendency regarding the time of wearing ScCL and indentation (r=0.11 and r=0.068, respectively).
Conclusions: Using OCTA with an anterior segment lens adapter, the ocular surface blood VD was imaged and assessed with good repeatability and reliability. This study presents a new possible application of OCTA to investigate and monitor conjunctival vasculature in ScCL wearers. This results cautiously suggest that the repeated use of ScCL can cause vascular alterations in conjunctiva of the eyes of ScCL wearers, possibly due to a hidden hypoxia caused by prolonged limbal indentation.

1. Introduction

Over the last decade, Scleral Contact Lenses (ScCL) have improved dramatically with the addition of sophisticated lens designs. In consequence, they have increasingly been prescribed globally and gained renewed interest mainly due to advances in manufacturing and the benefits of using them. Optimal fitting has become increasingly challenging, because of complex lens geometries and great regional differences worldwide. Despite the new developments in this field, reducing mechanical stress on the cornea is a challenge with every keratoconus lens fit, even for the experienced contact lens practitioner. For true corneal clearance without any mechanical involvement, it is recommended to avoid any contact between the lens and the cornea by bridging or vaulting over it [1]. This vaulting over the cornea and limbus produces a reservoir of fluid that neutralizes irregular anterior corneal astigmatism, allowing the correction of higher-order aberration, improving visual quality and providing corneal protection from mechanical factors and dryness, creating a therapeutic environment in patients with severe ocular surface disease [2,3]. Despite the improvements in fitting ScCL, the primary concern continues to be the adverse effects associated with use, namely infections, inflammatory-related responses and hypoxia-related complications [4]. Regarding the last-named, although complications associated with the lack of oxygen in the underlying ocular surface have been reduced, compared to the use

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of rigid contact lenses, they have not been eliminated, and some of these may be a challenge in managing each patient [4,5]. The normal conjunctiva is composed of an epithelium, which contains goblet cells involved in mucus secretion, and a stroma or chorion, which combines connective tissue and the normal conjunctival vascular pattern with capillary, arterioles, and venules in several dispositions [6]. Specifically, in the superficial layer, the vessels extended radially and smaller vessels existed among them from the limbus to the periphery, being the density and diameter of the superficial vessels greatest in the nasal quadrant [6]. However, during and following scleral lens wear, some morphological alterations could be observed, directly causing tissue compression, reduction in central clearance, variation of tear film stability and post-lens suction forces (i.e. negative pressure) [4,7]. Firstly, the thickness profile of the tissue placed under or adjacent to the haptic zone is distorted, which can lead to macroscopic alterations such as conjunctival prolapse or a compression ring or points of conjunctival indentation, which can be observed after lens removal. Secondly, some vascular changes could be detected, namely the dilatation or compression of bulbar conjunctival vessels and episcleral [7].

The introduction of new diagnostic tools, such as Anterior Segment Optical Coherence Tomography (AS-OCT), allowed study and better understanding of the ocular surface, leading to the formulation of new scleral lens designs and techniques, improving management features related to the fitting relationship with the sclera [8,9].

Despite the optical considerations and advantages of AS-OCT, it does not provide information related to the scleral vasculature or hypoxia.

With the development of Optical Coherence Tomography Angiography (OCTA), it could be possible to study vascular abnormalities and quantify Vascular Density (VD) by visualization of blood flow in vessels through motion contrast imaging of erythrocyte movement across sequential B-scans [10]. Firstly developed to quantify VD in the posterior pole, and commonly used for imaging the retinal vasculature, it has recently emerged as an essential tool used in imaging the anterior segment (AS) of the eye [11,12].

Currently, there is no research on the vascular alterations caused by repetitive use of ScCL in the conjunctiva of ScCL wearers.

Using OCTA, this study aimed to evaluate the VD of the conjunctiva of patients with keratoconus (KC) who use ScCL, before and after removal of the lens, in addition to investigating the association between VD measurements and indentation values of the lens.

2. Materials and Methods

This is a prospective cross-sectional study conducted for analysis of VD measurements in ScCL lens wearers. Volunteer patients were recruited at the Department of Ophthalmology, at Centro Hospitalar Entre Douro e Vouga, Santa Maria da Feira, Portugal. The study was performed in accordance with the Declaration of Helsinki and its later amendments, and ethics approval was obtained from the internal Ethics Committee. All patients completed and signed informed consent forms. The study included 23 eyes from 23 patients with keratoconus diagnosed by a Corneal Expert in our Center. All use ScCL. Eleven patients used Irregular Corneal Design (ICD®) lens, 4 patients Comfort 15® lens and 8 patients ZenLens® lens.

Each patient underwent an evaluation of visual acuity (VA) without correction, with glasses and with ScCL. Biomicroscopy of the anterior and posterior segment of the eye, and AS photography using a slit-lamp mounted digital camera system (SL-D Digital Slit-Lamp; Topcon, Tokyo, Japan) were also performed. After that, each patient completed AS-OCT to quantify conjunctival and scleral compression during scleral lens wear. Subsequently, OCTA is performed with ScCL and fifteen minutes after removing ScCL. The inclusion criteria included naïve users of ScCL for a period from 1 to 3 years for the correction of keratoconus. On the day of the exam, the study participant must not have used the lens for more than five consecutive hours. Exclusion criteria included not neophyte users of scleral lenses, users of ScCL over a period more than 3 years, the presence of congenital eye disorders, the presence of significant corneal opacity or evidence of corneal neovascularization and low-quality images obtained with OCTA. OCTA images of the nasal conjunctiva were used for quantitative assessment of the VD with and without ScCL in the nasal quadrant by binary vessel maps generated from suitable slabs analyzed using ImageJ software.

Relationships between VD, indentation and time of use were also investigated.

2.1. AS-OCT

The shape of the limbal junction, where the cornea meets the sclera, informs contact lens design and influences the lens fit, and potentially patient comfort during ScCL wear [7]. The angle of the corneoscleral junction varies with the ocular meridian, being sharpest (a less obtuse angle) nasally and flattest (closest to a tangent) at the top [7,13]. For the indentation measurements, AS-OCT (SPECTRALIS®, Heidelberg Engineering, Heidelberg, Germany) was used with a line scan set of 6 mm. The scan is centered in the nasal zone so that the landing is in the middle of the AS-OCT image, to see the sclera curvature and the limbal clearance simultaneously. The measures of limbal indentation were subjectively determined by two independent masked observers. Two parallel lines from the limbus to the lens edge with the sclera thickness were drawn and the indentation value corresponded to the distance between the parallel lines where the depression was greater (Fig. 1).

2.2. OCT Angiography

OCT angiograms of the AS were taken by spectral domain OCTA system (Avanti XR AngioVue, Optovue, Inc., Fremont, CA, USA) using the Angio Retina mode (split-spectrum amplitude-decorrelation angiography), adapted for AS imaging by coupling the long cornea adaptive module lens (CAM-L; Optovue, Inc). Blood flow is detected through the decorrelation signal generated from the red blood cell motion through consecutive A-scans of the same location, generating a volumetric blood flow analysis. All OCTA scans were conducted by one expert professional operator. To fully expose the nasal conjunctiva, the subjects gazed upon a fixation point located on the temporal side of the eye. Although the software could automatically segment the retina into four layers, due to the lack of a standard protocol for imaging of the AS with OCTA, a manual adjustment of focus was necessary to obtain a correctly segmented angiogram in the conjunctiva layer. OCTA 6×6mm scans (250×250 pixels) were performed to a depth of 800 μm in nasal quadrants along the limbus. A square of 2.5×2.5mm (150×150 pixels) tangent to the corneoscleral margin, which comprises the greatest part of limbal vascular networks, at the 3 o'clock position in the RE and 9 o'clock position in the LE, was defined as the representative ROI (Fig. 2).

2.3. Image Processing and Vessel Density measurements

VD was defined as the percentage area occupied by the large vessels and microvasculature in the analyzed region. The OCTA images were exported from the equipment in Portable Network Graphics (PNG) image file for further analysis, into Fiji Software, a distribution of the open-source Java-written software *ImageJ*, (V.1.49p, National Institutes of Health, Bethesda, Maryland, USA) [14] focusing on biological-image analysis. To determine the VD, the OCTA images were binarized and converted to an 8-bit image for processing (Fig. 3). The program adjusts threshold with the *Shanbhag* method applied to highlight blood vessels and reduce the surrounding noise, and the contrast of the images was adjusted using the Equalize Histogram of this algorithm. VD was expressed as a percentage by taking the ratio of the total vessel area to the total area of the analyzed region (area with the highest number of whites - vessel pixels = 1; background = 0). Only clear images were studied, and poor quality images caused by motion artifacts such as poor fixation and blinking artifacts were excluded.

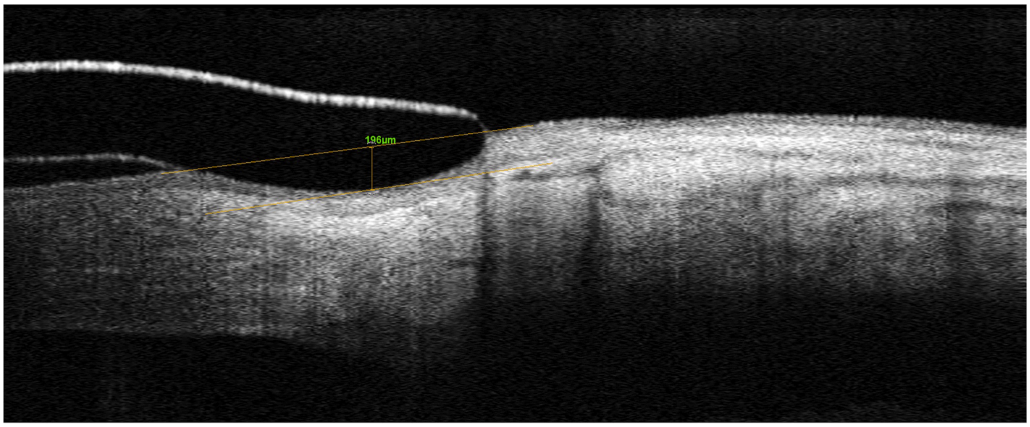

Fig. 1. Assessment of indentation values using anterior AS-OCT. The value corresponded to the distance between the two orange parallel lines manually assessed where the depression is greater.

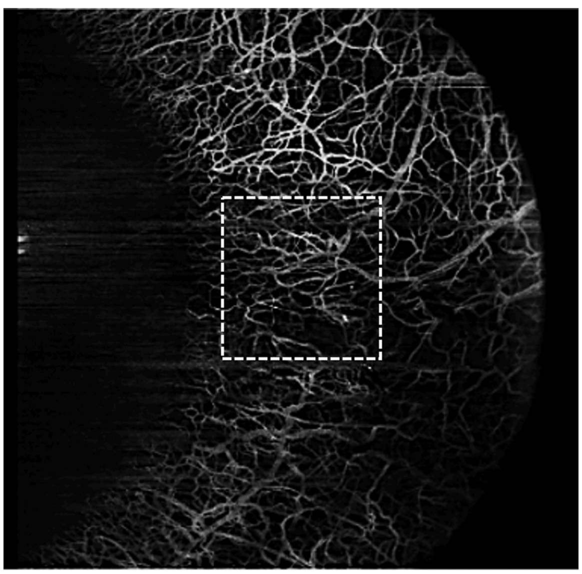

Fig. 2. Representative region of interest in the OCTA slab. The dotted-line square shows the 2.5×2.5mm (150×150 pixels) ROI used to assess the vascular density of the conjunctiva.

2.4. Statistical Analysis

Statistical analysis was performed with the Statistical Package for Social Sciences (SPSS for Mac, version 25; IBM/SPSS, Chicago, IL). The normality of the sample distribution was confirmed using the Shapiro-Wilk test ($p>0.05$). The main outcome parameters included the percentage of conjunctival VD detected by OCTA with and without ScCL, expressed as mean±SD, and the correlations between the indentation, VD and time of use. Comparisons between VD measurements before and after ScCL removal were calculated using Student's paired t-test and the correlation analysis was investigated by *Pearson's* correlation test. A *p value* ≤ 0.05 was considered of statistical significance.

3. Results

The demographic and clinical characteristics of the participants are summarized in Table 1. A total of 23 patients (3 females, 20 males), with a mean age (years±SD) of 38.74±10.38 were included in the study. The sample showed a mean indentation value of 190.26±47.5 (µm±SD). Corneal asphericity (Q value) was -1.14±0.81 (Q±SD) and the mean value of the keratometry was 52.01±7.38 (D±SD). The mean usage time of ScCL is 23.48±12.11 (months±SD). The mean VA was significantly better with pos fitting ScCL (0.81±0.21 vs. 0.12±0.15 without correction, $p<0.01$, and vs. 0.29±0.48 with glasses, $p<0.01$). The image quality score of the scan with and after removal of the ScCL is not different in the evaluation (5.48±2.06 vs. 5.48±1.68, $p>0.05$).

The statistical analysis is presented in Table 2 and Table 3. VD was significantly higher after removing the ScCL (71.75%±2.97), compared to VD measured with ScCL (69.81%±2.63), $p=0.02$ (Table 2). A

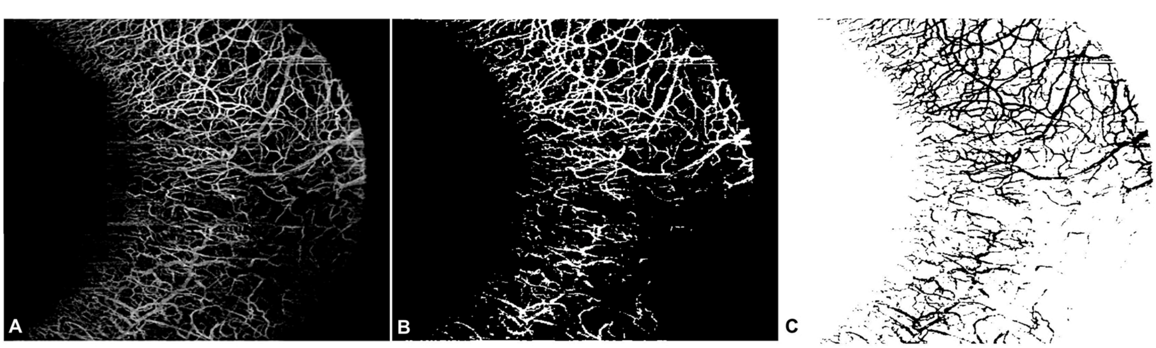

Fig. 3. Illustration of optical coherence tomography angiography (OCTA) image analysis. (A) The OCTA scans were adjusted to highlight the vessels in the region of interest and reduce the surrounding noise. (B) The images were then exported from the system into the Image J software (V.1.49p, National Institutes of Health, Bethesda, Maryland, USA) and the contrast of the images was adjusted using the Equalize Histogram of this algorithm. (C) The Vessel Analysis Complete framework of Image J was used to export the resulting vascular tree as a binary image for analysis.

Table 1
Demographic and clinical Parameters of the study subjects

	Age (years)	Indentation (μm)	Q-value	Vault (μm)	Usage time of ScCL (months)	VA 1 (decimal)	VA2 (decimal)	VA3* (decimal)	KM (D)
Valid	23	23	23	23	23	23	23	23	23
Missing	0	0	0	0	0	0	0	0	0
Mean	38.739	190.261	−1.142	250.783	23.478	0.123	0.293	0.809	52.009
Std. Deviation	10.737	47.523	0.811	63.114	12.109	0.152	0.248	0.207	7.380
Minimum	22.000	102.000	−2.680	146.000	13.000	0.010	0.010	0.400	39.400
Maximum	70.000	290.000	0.400	351.000	34.000	0.500	0.700	1.000	66.700

VA 1 = Visual acuity without correction; VA 2 = Visual acuity with glasses; VA 3 = Visual acuity with pos fitting ScCL.
* Statistical difference between VA1 and VA3 and VA2 and VA3 (both $p<0.01$).

Table 2
Descriptive statistics of Vessel Density measurements between groups.

Group Descriptives					
	Group	N	Mean	SD	SE
VD*(%)	With ScCL	23	69.805	2.625	0.547
	Without ScCL	23	71.754	2.969	0.619

VD=Vessel Density; SD=Standard Deviation; SE=Standard Error.
* $p=0.01$ *paired t-test*.

moderately negative correlation was found between indentation and vault ($r=-0.44$, $p<0.05$) (Table 3). Regarding the time of wearing ScCL and indentation, a weak positive tendency ($r=0.11$ and $r=0.068$, respectively) was found between the variables and the VD measurements (all $p>0.05$).

4. Discussion

ScCL have been successfully fitted over the years with a range of therapeutic and optical applications. Before the OCTA era, AS-OCT imaging allowed the quantification of contact lens fit assessment, by improving initial diagnostic lens selection and decreasing fitting-related ocular complications [15]. While some technical manufacturing challenges have been virtually eliminated for the practitioner due to technological advances in lens selection and materials, a number of other challenging questions emerged and remained to be answered, such as the effects and consequences of long-term ScCL use, including perilimbal conjunctival compression in the eye. OCTA, which was initially used for structural and flow estimation of the retinal vascular system, is still being tested for other clinical applications, and is a promising tool for evaluating conjunctival and intrascleral vasculatures [6,16,17]. In line with other studies, the OCTA system was used with an anterior segment lens adapter, to obtain measurements of the limbal conjunctival blood vessel density in ScCL wearers, before and after lens application. To our knowledge, this is a pilot clinical study in using OCTA to evaluate the affected limbal vascularization in the eyes of patients with ScCL, suggesting that OCTA may be a promising non-invasive imaging tool for studying and monitoring conjunctival vasculature. The major result of the study is that the VD was significant higher in eyes after removal of the ScCL. This result raises the hypothesis of a possible hidden hypoxia, caused by compression of the lens in the conjunctiva. In some areas of greater compression, the vascular density was impossible to measure, and was represented by black points in binary OCTA images, when comparing with the same patient, in the same area, without ScCL. This theory may raise questions regarding the prolonged use of contact lenses, due to possible alterations in the vascular conjunctival layer with still unknown consequences. The data in the literature showed that insufficient limbal clearance or mechanical compression may result in superficial corneal staining, corneal swelling, epithelial breakdown, peripheral neovascularization, or scarring [4,18]. Regarding the indentation values, no significant results were found and further studies are needed to clarify this result and the tendency towards greater indentation with longer usage time. Although OCTA technology has advantages and technological improvements over other imaging modalities, some limitations must be noted. Firstly, in the study design, the first priority was to understand if there were some alterations in the vascular pattern soon after removing the lens. Here, as the duration of the changes induced by scleral contact lens remains unclear, and previous studies report that some alterations could reverse immediately after removal, we consider a period of 15 minutes for the study, and subsequent measurements (after 1 hour, 4 hours, 6 hours, e.g.), or evaluation on subsequent visits, were not included [5,7,19]. To ensure that the full conjunctiva was included, the scan depth was extended to the outer surface of the sclera. However, the ability to segment the images to separate the conjunctival vessels from the superficial scleral vessels was not optimal. Thus, improvements in the segmentation software and in motion correction are needed to enhance the reliability of the data. Secondly, the indentation value was measured manually because there is still no specific measure for that purpose. Although this was a subjective evaluation, the values were measured by two independent observers in a masked way, with inter operator reproducibility. The values obtained may suggest a possible way for comparative evaluations between patients.

This study demonstrates that OCTA measurement of vessel density is feasible and reliable. This approach will provide better means of

Table 3
Correlation analysis between the variables.

Pearson Correlations		VD	Q-value	Usage time	Indentation	Vault
VD	Pearson's r					
	p-value					
Q-value	Pearson's r	−0.253				
	p-value	0.244				
Usage Time	Pearson's r	0.114	−0.084			
	p-value	0.603	0.703			
Indentation	Pearson's r	0.068	0.148	−0.110		
	p-value	0.756	0.500	0.618		
Vault	Pearson's r	−0.253	0.103	−0.155	−0.438*	
	p-value	0.244	0.638	0.481	0.037	

* $p < .05$.

examination of ScCL users and could be useful in evaluating the long-term consequences of repetitive use of ScCL. This study considers that vessel density monitoring will be helpful for the exercise of adapting contact lenses, and for the user, making it possible to differentiate normal and abnormal conjunctival blood vessels. Besides, the OCTA could be used for the follow-up and early detection of hypoxia or ischemia and posterior conjunctival neovascularization due to the prolonged use of ScCL.

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References

[1] Van der Worp E. A Guide to Scleral Lens Fitting. College of Optometry. Pacific University; 2010.
[2] Van der Worp E, Bornman D, Ferreira DL, Faria-Ribeiro M, Garcia-Porta N, González-Meijome JM. Modern scleral contact lenses: A review. Cont Lens Anterior Eye 2014;37(4):240–50. https://doi.org/10.1016/j.clae.2014.02.002.
[3] Bennett E. Contemporary Scleral Lenses: Theory and Application. Optom Vis Sci 2018. https://doi.org/10.1097/OPX.0000000000001259.
[4] Walker MK, Bergmanson JP, Miller WL, Marsack JD, Johnson LA. Complications and fitting challenges associated with scleral contact lenses: A review. Contact Lens Anterior Eye 2016. https://doi.org/10.1016/j.clae.2015.08.003.
[5] Fadel D. Scleral Lens Issues and Complications Related to a Non-optimal Fitting Relationship between the Lens and Ocular Surface. Eye Contact Lens 2019. https://doi.org/10.1097/ICL.0000000000000523.
[6] Akagi T, Uji A, Huang AS, et al. Conjunctival and Intrascleral Vasculatures Assessed Using Anterior Segment Optical Coherence Tomography Angiography in Normal Eyes. Am J Ophthalmol 2018. https://doi.org/10.1016/j.ajo.2018.08.009.
[7] Walker MK, Schornack MM, Vincent SJ. Anatomical and physiological considerations in scleral lens wear: Conjunctiva and sclera. Contact Lens Anterior Eye 2020. https://doi.org/10.1016/j.clae.2020.06.005.
[8] Di Lee W, Devarajan K, Chua J, Schmetterer L, Mehta JS, Ang M. Optical coherence tomography angiography for the anterior segment. Eye Vis 2019;6(1):1–9. https://doi.org/10.1186/s40662-019-0129-2.
[9] Tom LM, Jacobs DS. Advances in Anterior Segment OCT For the Design and Fit of Scleral Lenses. Int Ophthalmol Clin 2019. https://doi.org/10.1097/IIO.0000000000000284.
[10] Spaide RF, Klancnik JM, Cooney MJ. Retinal vascular layers in macular telangiectasia type 2 imaged by optical coherence tomographic angiography. JAMA Ophthalmol 2015. https://doi.org/10.1001/jamaophthalmol.2014.3950.
[11] Tan ACS, Tan GS, Denniston AK, et al. An overview of the clinical applications of optical coherence tomography angiography. Eye 2018;32(2):262–86. https://doi.org/10.1038/eye.2017.181.
[12] Ang M, Sim DA, Keane PA, et al. Optical Coherence Tomography Angiography for Anterior Segment Vasculature Imaging. Ophthalmology 2015;122(9):1740–7. https://doi.org/10.1016/j.ophtha.2015.05.017.
[13] Hall LA, Hunt C, Young G, Wolffsohn J. Factors affecting corneoscleral topography. Invest Ophthalmol Vis Sci 2013. https://doi.org/10.1167/iovs.13-11657.
[14] Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. Nat Methods 2012;9(7):671–5. https://doi.org/10.1038/nmeth.2089.
[15] Vincent SJ, Fadel D. Optical considerations for scleral contact lenses: A review. Contact Lens Anterior Eye 2019. https://doi.org/10.1016/j.clae.2019.04.012.
[16] Zhao F, Cai S, Huang Z, Ding P, Du C. Optical Coherence Tomography Angiography in Pinguecula and Pterygium. Cornea 2020. https://doi.org/10.1097/ICO.0000000000002114.
[17] Patel CN, Antony AK, Kommula H, Shah S, Singh V, Basu S. Optical coherence tomography angiography of perilimbal vasculature: Validation of a standardised imaging algorithm. Br J Ophthalmol 2020. https://doi.org/10.1136/bjophthalmol-2019-314030.
[18] Vincent SJ, Alonso-Caneiro D, Collins MJ. Optical coherence tomography and scleral contact lenses: clinical and research applications. Clin Exp Optom 2019. https://doi.org/10.1111/cxo.12814.
[19] Soeters N, Visser ES, Imhof SM, Tahzib NG. Scleral lens influence on corneal curvature and pachymetry in keratoconus patients. Contact Lens Anterior Eye 2015. https://doi.org/10.1016/j.clae.2015.03.006.