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Research article

In vivo oximetry of human bulbar conjunctival and episcleral microvasculature using snapshot multispectral imaging

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ABSTRACT

Multispectral imaging (MSI) is a well-established technique for non-invasive oximetry of retinal blood vessels, which has contributed to the understanding of a variety of retinal conditions, including glaucoma, diabetes, vessel occlusion, and retinal auto-regulation. We report the first study to use snapshot multi-spectral imaging (SMSI) for oximetry of the bulbar conjunctival and episcleral microvasculature in the anterior segment of the eye. We report the oxygen dynamics of the bulbar conjunctival and episcleral microvasculature at normoxia and at acute mild hypoxia conditions.

A retinal-fundus camera fitted with a custom Image-Replicating Imaging Spectrometer was used to image the bulbar conjunctival and episcleral microvasculature in ten healthy human subjects at normoxia (21% Fraction of Inspired Oxygen [FiO_2]) and acute mild hypoxia (15% FiO_2) conditions. Eyelid closure was used to control oxygen diffusion between ambient air and the sclera surface. Four subjects were imaged for 30 seconds immediately following eyelid opening. Vessel diameter and Optical Density Ratio (ODR: a direct proxy for oxygen saturation) of vessels was computed automatically. Oximetry capability was validated using a simple phantom that mimicked the scleral vasculature.

Acute mild hypoxia resulted in a decrease in blood oxygen saturation (SO_2) (i.e. an increase in ODR) when compared with normoxia in both bulbar conjunctival ($p < 0.001$) and episcleral vessels ($p = 0.03$). Average episcleral diameter increased from 78.9 ± 8.7 μm (mean ± standard deviation) at normoxia to 97.6 ± 14.3 μm at hypoxia ($p = 0.02$). Diameters of bulbar conjunctival vessels showed no significant change from 80.1 ± 7.6 μm at normoxia to 80.6 ± 7.0 μm at hypoxia ($p = 0.89$). When exposed to ambient air, hypoxic bulbar conjunctival vessels rapidly reoxygenated due to oxygen diffusion from ambient air. Reoxygenation occured in an exponential manner, and SO_2 reached normoxia baseline levels. The average ½ time to full reoxygenation was 3.4 ± 1.4 s. As a consequence of oxygen diffusion, bulbar conjunctival vessels will be highly oxygenated (i.e. close to 100% SO_2) when exposed to ambient air. Episcleral vessels were not observed to undergo any significant oxygen diffusion, instead behaving similarly to pulse oximetry measurements.

This is the first study to the image oxygen dynamics of bulbar conjunctival and episcleral microvasculature, and consequently, the first study to directly observe the rapid reoxygenation of hypoxic bulbar conjunctival vessels when exposed to ambient air. Oximetry of bulbar conjunctival vessels could potentially provide insight into conditions where oxygen dynamics of the microvasculature are not fully understood, such as diabetes, sickle-cell diseases, and dry-eye syndrome. Oximetry in the bulbar conjunctival and episcleral microvasculature could be complimentary or alternative to retinal oximetry.

1. Introduction

Multispectral imaging (MSI) is a well established technique for non-contact oximetry of blood vessels (D J Mordant et al., 2011a; David J Mordant et al., 2011b) which has enhanced the

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understanding of a variety of retinal conditions, such as diabetes (Hammer et al., 2009; Hardarson and Stefánsson, 2012; Isenberg et al., 1986), glaucoma (Boeckaert et al., 2012; Mordant et al., 2014; Olafsdottir et al., 2011), and vessel occlusion (Eliasdottir et al., 2014), and additionally, has provided insight into the retinal autoregulatory response to flicker stimulation (Hammer et al., 2011) and acute mild hypoxia (Choudhary et al., 2013). However, oximetry of retinal capillaries is beyond the technical capabilities of MSI-enabled retinal fundus cameras. The anterior segment provides two alternative ocular microvascular beds that are easily accessible for multispectral imaging the bulbar conjunctival and episcleral microvascular beds. These microvascular beds could be used to probe ocular blood oxygen saturation (SO_2) and potentially provide new physiologically-relevant information. This study is the first to use MSI to non-invasively measure the oxygen saturation of bulbar conjunctival and episcleral microvasculature with high spatial and temporal resolution, revealing rapid oxygen diffusion from ambient air into bulbar conjunctival vessels.

The episcleral microvasculature is located within the scleral tissue, with few episcleral vessels visible near the scleral surface. In contrast, the bulbar conjunctival microvasculature is semi-mobile above the sclera, and presents many arterioles, venules, and capillaries for imaging (Meighan, 1956). Groups of individual red blood cells can be observed to flow in bulbar conjunctival capillaries if imaged with high magnification (Jiang et al., 2014). The bulbar conjunctiva may be unique in that it is the only microvascular bed in the human body (excluding the alveolar capillaries) which is directly exposed to ambient air. Fig. 1a shows generalised positions of bulbar conjunctival and episcleral vessels with respect to the sclera and Fig. 1b shows a representative image of bulbar conjunctival and episcleral vasculature in a single subject.

MSI oximetry is based on the SO_2-dependent optical absorption spectra of haemoglobin. Changes in SO_2 can be inferred by imaging blood vessels at two wavelengths: one wavelength where optical absorption is sensitive to variations in SO_2, and at another wavelength which is insensitive to SO_2 variations (i.e. isobestic). From images of vessels, the optical density (OD) of vessels at each wavelength can be calculated, allowing the calculation of optical density ratio (ODR), which is directly proportional to SO_2. ODR may be empirically calibrated to SO_2 by assuming local arterial SO_2 is equal to systemic arterial SO_2 as measured by pulse oximetry (Beach et al., 1999), or by using reference SO_2 values from previous studies. (Hardarson et al., 2006).

To the best of our knowledge there are no reported MSI oximetry studies of the bulbar conjunctival or episcleral microvasculature. Instead, insights into the oxygen dynamics of microvasculature have generally been indirectly inferred from vessel-diameter or blood-flow measurements (Jiang et al., 2013; Shahidi et al., 2010; Wanek et al., 2013); however these parameters may be affected by factors other than changes in SO_2, such as conjunctival or episcleral inflammation. Direct measurement of the partial pressure of oxygen (pO_2) of the palpebral conjunctival microvasculature has been achieved with Clark-type electrodes (Chapman et al., 1986; Iguchi et al., 2005; Isenberg et al., 2002; Kwan and Fatt, 1971; Mader et al., 1987), but these electrodes have insufficient spatial discrimination for localisation of oximetry to blood vessels and crucially, block oxygen diffusion between ambient air and any blood vessels under study.

In this study, we report the use of a retinal fundus camera modified for Snapshot Multispectral Imaging (SMSI) to non-invasively quantify the oxygen dynamics of both bulbar conjunctival and episcleral microvasculature in healthy human subjects. The high temporal resolution of the SMSI system (10 ms exposure, 1 Hz image acquisition rate) enables observation of fast biological processes (Fernandez Ramos et al., 2014). We observe rapid oxygen diffusion from ambient air into bulbar conjunctival vessels; such observations are not possible with time-sequential MSI or Clarke-type electrodes because these techniques lack sufficient temporal and spatial resolution respectively.

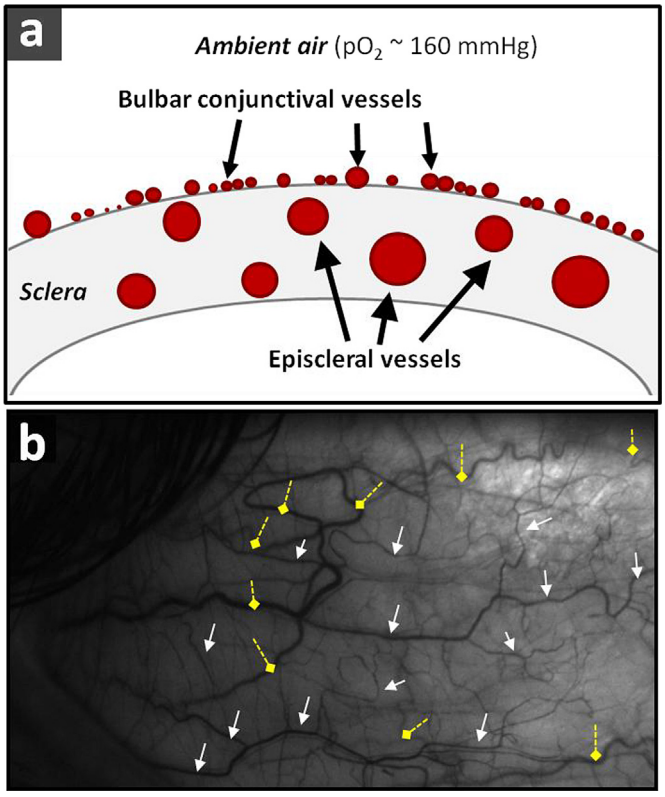

Fig. 1. **(a)** Simplified diagram showing position of bulbar conjunctival and episcleral vasculature with respect to the sclera and ambient air. **(b)** Representative image of vasculature observed when imaging the sclera. Bulbar conjunctival vessels are marked with white arrows and episcleral vessels are marked with yellow dashed diamond arrows. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2. Material and methods

2.1. Subject recruitment

This study was approved by the Ethics Committee of the University of Glasgow, College of Medical, Veterinary and Life Sciences. All volunteers provided written informed consent before participation and all procedures were performed in accordance with the tenets of the Declaration of Helsinki. Ten healthy volunteers (age 25 ± 2 years, six males and four female) were recruited. Subjects reported no history of ocular, respiratory, or vascular disease. Volunteers that regularly wore contact lenses or who were suffering from allergic conjunctivitis were excluded because this may induce fluctuating bulbar conjunctival vasodilatation (Gartner, 1944; Cheung et al., 2012; Jiang et al., 2014).

2.2. Imaging system

The imaging system consisted of a commercial retinal fundus camera (Topcon TR50-DX; Topcon, Itabashi, Tokyo, Japan), fitted with an Image Replicating Imaging Spectrometer (IRIS) and a cooled sCMOS camera (Zyla 5.5; Andor, Belfast, United Kingdom). IRIS is discussed in detail elsewhere (Harvey et al., 2005; Alabboud et al., 2007; Gorman et al., 2010; Fernandez Ramos et al., 2014); but