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Ali Hariri,a Junxin Wang,a Yeji Kim,b Anamik Jhunjhunwala,c Daniel L. Chao,d and Jesse V. Jokersta,e,f,*

Abstract. Chorioretinal imaging has a crucial role for the patients with chorioretinal vascular diseases, such as neovascular age-related macular degeneration. Imaging oxygen gradients in the eye could better diagnose and treat ocular diseases. Here, we describe the use of photoacoustic ocular imaging (PAOI) in measuring chorioretinal oxygen saturation (CR – sO\(_2\)) gradients in New Zealand white rabbits (n = 5) with ocular ischemia. We observed good correlation (\(R^2 = 0.98\)) between pulse oximetry and PAOI as a function of different oxygen percentages in inhaled air. We then used an established ocular ischemia model in which intraocular pressure is elevated to constrict ocular blood flow, and notice a positive correlation (\(R^2 = 0.92\)) between the injected volume of phosphate buffered saline (PBS) and intraocular pressure (IOP) as well as a negative correlation (\(R^2 = 0.98\)) between CR – sO\(_2\) and injected volume of PBS. The CR – sO\(_2\) was measured before (baseline), during (ischemia), and after the infusion (600-\(\mu\)L PBS). The ischemia-reperfusion model did not affect the measurement of the sO\(_2\) using a pulse oximeter on the animal’s paw, but the chorioretinal PAOI signal showed a nearly sixfold decrease in CR – sO\(_2\) (n = 5, \(p = 0.00001\)). We also observe a sixfold decrease in CR – sO\(_2\) after significant elevation of IOP during ischemia, with an increase close to baseline during reperfusion. These data suggest that PAOI can detect changes in chorioretinal oxygenation and may be useful for application to imaging oxygen gradients in ocular disease.

Keywords: photoacoustic ocular imaging; chorioretina oxygen saturation; oxygen tension; ischemia reperfusion.

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1 Introduction

The eye is a critical light-sensitive organ\(^1\) divided into anterior and posterior segments.\(^2\) The anterior segment includes the cornea, iris, pupil, and ciliary body. The posterior segment includes the vitreous body, retina, choroid, and optic nerve.\(^3\) The retinal pigment epithelium mostly contains melanin\(^4\) and plays a major role in energy and oxygen consumption.\(^5\) Retinal and choroidal circulation delivers oxygen to the inner retinal tissue and photoreceptors, respectively.\(^6\) Focal retinal hypoxia is thought to underlie the pathophysiology of many ocular diseases, such as diabetic retinopathy,\(^7,8\) glaucoma,\(^9,10\) and retinal venous occlusion.\(^11\) Thus, the ability to image retinal oxygen gradients could be very valuable in treating and diagnosing retinal disease. Also, chorioretinal vasculature imaging has a significant role for diseases, such as neovascular age-related macular degeneration (AMD).\(^12\)

There are four requirements for ocular imaging. First, motion artifacts and image distortion need to be compensated either through high-speed imaging or eye tracking software. Second, imaging should be noninvasive to decrease the risk of side effects. Third, light illumination should be below safety limits to prevent damaging the retina.\(^13\) Fourth, resolution is an important element of all imaging techniques. However, there are some applications where low-resolution imaging techniques are useful, such as magnetic resonance imaging\(^14\) and ultrasound.\(^15\)

Current clinical ophthalmology imaging modalities such as optical coherence tomography (OCT),\(^16,17\) confocal scanning laser ophthalmoscopy,\(^18,19\) and fundus photography\(^20\) provide anatomic data on the retina but do not measure oxygen gradients. Hyperspectral imaging\(^21,22\) has shown potential for imaging oxygen gradients in the eye but still has not been systematically validated, and an artifact of spectral noise from pigmentation, ocular media, etc. can affect hemoglobin oxygen gradients.

More recently, photoacoustic imaging (PAI) has been described as a noninvasive and nonionizing imaging technique that combines optical absorption and ultrasound detection.\(^23–25\) Photacoustic signal is generated due to absorption and thermal expansion of endogenous or exogenous molecules using a nanosecond laser pulse followed by detection with a wideband ultrasound transducer.\(^26–31\) This modality offers high penetration depth due to its weak ultrasound scattering in biological tissue. Oxyhemoglobin and deoxyhemoglobin (HbR and HbO\(_2\)) as well as melanin are the main optical absorbers in tissue. PAI can measure oxygen saturation (sO\(_2\)) variations based on differences in HbR and HbO\(_2\) absorption.\(^32–35\) In eye diseases, oxygenation is dysregulated due to increased intraocular pressure (IOP)—the choroidal blood flow decreases as the IOP increases.\(^36\) In turn, oxygen availability decreases with decreasing CR – sO\(_2\).\(^36\) Although PAI is ideally suited to measure oxygen saturation, somewhat surprisingly, most studies in the field have focused only on anatomic imaging.

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Examples of anatomic imaging include de La Zerda et al.\(^3\) who utilized PAI in swine and rabbit eyes. Their system could image both the anterior and posterior segments of the ocular system. However, 90 min was required to image a 12-mm × 8-mm area. Hu et al.\(^3\) utilized optical resolution photoacoustic microscopy (OR-PAM) to do in vivo imaging of the anterior segment of a mouse eye; axial and lateral resolution of 15 and 5 \(\mu\)m were reported, but 120 min was required to cover only a 2-mm × 2-mm area. Liu et al.\(^3\)\(^9\) and Wu et al.\(^3\)\(^0\) improved their OR-PAM system’s acquisition time to 20 min for a 3-mm × 3-mm image and 6.5 min for a 2-mm × 2-mm image, respectively. Jiao et al.\(^4\)\(^1\) Song et al.\(^4\)\(^2\),\(^4\)\(^3\) and Liu et al.\(^4\)\(^2\) proposed multimodal ocular imaging using PAI with an optical scanning method and decreased the acquisition time to 2.7 s for a 2-mm × 2-mm image. Recently, Tian et al.\(^4\)\(^5\) designed chorioretinal imaging of integrated PAM and OCT for rabbits. The acquisition time for a 3-mm × 3-mm area was 65 s with PAM lateral and axial resolution values of 4.1 and 37 \(\mu\)m, respectively.

Other groups have used microscopy techniques to image oxygen saturation in rodent eyes. Although these techniques offered very high spatial resolution, the penetration depth was limited to a few mm, and the technique required many minutes to map only a few mm\(^2\) of tissue. An example of this includes Song et al.\(^4\)\(^3\) who quantified retinal oxygen saturation in rats. They combined OCT and PAM to measure the retinal oxygenation.

However, to the best of our knowledge, only one study has yet evaluated oxygen saturation in a large animal eye using PAI. In that study, Hennen et al.\(^4\)\(^6\) used acoustic resolution photoacoustic tomography (AR-PAT) to image the conjunctiva and other areas on the “anterior” of rabbit eyes with 2-Hz temporal resolution. However, a remaining fundamental limitation is oxygen saturation imaging of the “posterior” (chorioretina) of a large animal eye. This has not yet been reported. Indeed, as Hennen and coworkers\(^4\)\(^6\) stated, such a technique would be useful for evaluating the role of oxidative damage, hypoxia, and ischemia in pathogenesis of various ocular diseases, such as glaucoma, diabetic retinopathy, AMD, and cataract.

Here, we describe high-speed photoacoustic ocular imaging (PAOI) to detect chorioretinal oxygen gradient in an in vivo model of hypoxia as well as ischemia reperfusion model on albino live rabbits. This PAOI approach is faster and has a simpler optical design than acoustic resolution photoacoustic tomography and PAM techniques. PAOI could monitor CR − sO\(_2\) changes in real time as demonstrated with oxygen tension experiments and ischemia-reperfusion models.

2 Methods and Materials

2.1 Animal Procedures

New Zealand white rabbits (2 to 3 kg) were used as animal models. All animal experiments were performed in compliance with the Institutional Animal Care and Use Committee established by University of California San Diego. Ketamine (35 mg/kg) and xylazine (5 mg/kg) were given via intramuscular injection for anesthesia. The pupils were dilated and anesthetized using 2.5% phenylephrine hydrochloride, 0.5% proparacaine hydrochloride, and 1% tropicamide. The heart rate, peripheral capillary oxygen saturation, respiration rate, and temperature were monitored during the experiment. Animals were placed on a recirculating water blanket kept at 39°C. An ocular speculum was used to keep the rabbit’s eye open and ultrasound gel coupled the eye to the transducer.

2.2 Photoacoustic Ocular Imaging

All in vivo imaging was performed using a laser integrated high-frequency ultrasound system (Vevo LAZR, VisualSonics Inc.). A linear array transducer was used with optical fiber bundles integrated to each side (LZ-201, fc = 15 MHz, axial resolution was 290 \(\mu\)m, lateral resolution = 580 \(\mu\)m, B-mode photoacoustic frame rate = 6 Hz, B-mode CR − sO\(_2\) map rate = 0.8 Hz). These fiber bundles delivered light from a tunable laser (680 to 970 nm). A Q-switched Nd:YAG laser (4- to 6-ns pulse width) with optical parametric oscillator and a repetition rate of 20 Hz was used to deliver laser energy to the surface of the eye at 8 ± 0.5 mJ. The transducer can be scanned in one direction to acquire three-dimensional (3-D) data.

2.3 Oxygen Saturation Measurement Using Photoacoustic Imaging

The photoacoustic intensity is proportional to the local absorption coefficient (\(\mu_a\)), and thus the relative concentration of oxy- and deoxyhemoglobin (HbO\(_2\) and HbR) can be determined by solving the following equation:

\[
\mu(\lambda_i) = \epsilon_{HbR}(\lambda_i)[HbR] + \epsilon_{HbO2}(\lambda_i)[HbO2],
\]

where \(\epsilon_{HbR}(\lambda_i)\) and \(\epsilon_{HbO2}(\lambda_i)\) are the known molar extinction coefficients (cm\(^{-1}\) M\(^{-1}\)) of HbR and HbO\(_2\) at wavelength of \(\lambda_i\), respectively. To calculate HbO\(_2\) and HbR at least two different wavelengths are needed, and sO\(_2\) can be obtained as following:

\[
sO_2(\%) = \frac{[HbO2]}{[HbO2] + [HbR]} \times 100.
\]

In our PAOI, 750 and 850 nm were used to differentiate the oxy- and deoxyhemoglobin. We used 750 and 850 nm to differentiate the oxy- and deoxyhemoglobin. These wavelengths have lower light attenuation in tissue and are in the so-called “optical window.” This makes them suitable for imaging hemoglobin and deoxyhemoglobin. The 750-nm peak corresponds to deoxyhemoglobin, and the oxyhemoglobin has a peak at 850 nm. Both are in this optical window (600 to 1100 nm). Known values for molar extinction coefficients were embedded in the VisualSonics software.\(^4\)\(^8\)

2.4 Oxygen Saturation Measurement Using Pulse Oximeter

Pulse oximetry is a noninvasive method to measure the oxygen saturation (sO\(_2\)) of the blood.\(^3\)\(^9\) A commercial peripheral pulse oximeter (CMS60D-VET, Contec Co., UK) was utilized on the rabbit’s back paw to validate our oxygen saturation measurement using PAOI.

2.5 Oxygen Tension Experiment

To show PAOI’s capability to monitor CR − sO\(_2\) and evaluate the response of photoacoustic signal to oxygen tensions, five anesthetized rabbits were administered different percentages of oxygen [5%, 10%, 15%, 21% (air), 30%, and 100%] with the balance as nitrogen. Pulse oximeter measured sO\(_2\) from...
the paw at the same time. We also demonstrated the capability of real-time measurements of CR− sO2 using PAOI with alternating changes in the level of oxygen from normal air to 100% oxygen (hyperoxic condition). The results were evaluated with a pulse oximeter. Hyperoxia and normal air were applied to the animal. A maximum intensity projection (MIP) method was utilized to generate 3-D images.

2.6 Ischemia Reperfusion Model

A well-established ischemia-reperfusion model was used to generate chorioretinal intravascular gradients in rabbits (n = 5). In this model, a 25-gauge needle was placed in the anterior chamber while the animal was sedated and breathing normal air. A 10-mL syringe containing phosphate buffered saline (PBS) was connected to a syringe pump. PBS then was infused to increase the IOP and induce ischemia. A Tonopen (Reichert Inc.) was used to measure the IOP in this study. To investigate the relationship between IOP, injected PBS volume, and CR− sO2, we injected 100, 200, 300, and 400 μL PBS into the eye. Before and after each injection, the IOP and CR− sO2 were measured using a Tonopen and PAOI, respectively. The flow rate for this experiment was fixed at 10 mL/h. Each IOP measurement in this article using Tonopen is an average of three readings.

Next, we confirmed the ability to do real-time imaging using PAOI. Five rabbits were imaged and scanned in this experiment. The flow rate and injection volume were set to 20 mL/h and 600 μL, respectively. A pulse oximeter measured the sO2 from the animal’s paw at the same time. The IOP was measured using a Tonopen before ischemia (baseline), during ischemia, and after reperfusion.

3 Results

Figure 1 shows the experimental setups in this study. Figure 1(a) shows the oxygen tension experiment, and Fig. 1(b) shows the ischemia-reperfusion setup.

3.1 Oxygen Tension Experiment

We first validated whether PAOI could measure sO2 in the eye by comparing PAOI − CR− sO2 measurements and pulse oximetry measurements in animals breathing different percentages of oxygen. Figure 2(a) shows the B-mode photoacoustic/ultrasound image of a rabbit eye at 750 nm. The PAOI field of view is limited—only part of the chorioretina (40% of whole chorioretina) can be imaged using PAOI. This is a result of the light propagation [Fig. 1(b)] and can be improved by tilting the transducer. The acquisition time for one B-mode CR− sO2 map is 0.8 frame per second. We see a very strong correlation (R² = 98, n = 5) between PAOI and pulse oximetry as a reference for sO2 measurement. This result validates the PAOI and oximetry methods.

Also, see in Fig. 3 when one animal transitioning from room air to 100% N2 and the accompanying change in CR− sO2.

Next, we further validated the kinetics of measurement. Figure 4(a) shows synchronization between pulse oximetry and PAOI. The temporal resolution of PAOI was 0.8 Hz, and the refresh rate on the pulse oximeter was 1 Hz. We also validated the reproducibility across 100 B-scans and created 20-mm MIP maps of CR− sO2 during hyperoxia and normal conditions [Figs. 4(b) and 4(c)]. The coefficient of variation across 100 B-scans was 1.45% and 2.15% for normal and hyperoxia conditions, respectively. The CR− sO2 values measured with PAOI are significantly lower than the sO2 measurements with a pulse oximeter.

3.2 Ischemia Reperfusion Model

We next evaluated the ability of PAOI to detect CR− sO2 gradients in an ischemia reperfusion model [Fig. 5(a)]. Figures 5(b)–5(e) show the B-mode CR− sO2 map after injection of 100, 200, 300, and 400 μL PBS, respectively. We first validated the model by injecting increasing volumes of PBS and noted a proportional increase in IOP as validated with a Tonopen [Fig. 5(f)]. As IOP increases, we see a corresponding decrease in CR− sO2 levels [Fig. 5(g)]. The injection of 400-μL PBS
resulted in a 4.8-fold increase in IOP and a 1.6-fold decrease in R − sO2 as measured by Tonopen and PAOI, respectively.

We evaluated whether PAOI could measure changes in chorioretinal oxygenation in an ischemia reperfusion model. IOP was elevated to occlude ocular blood flow using injection of PBS into the anterior chamber. After elevated IOP, we see a steady decrease in CR − sO2 over 50 s that reached steady state [Fig. 6(e)]. After decreasing the IOP with subsequent reperfusion, the CR − sO2 increased—close but not statistically identical to—the baseline values (n = 5, p = 0.03) [Figs. 6(f) and 6(g)].

Figure 6(a) is a B-mode CR − sO2 map that was imaged before the start of the ischemia experiment. Figures 6(b) and 6(c) show CR − sO2 maps 5 and 15 s after the start of the injection of 600-μL PBS in the anterior segment, respectively.

Figure 6(c) shows an obvious decrease in blood oxygenation during reduced blood flow due to elevated pressure. This pressure was measured with a Tonopen both at baseline and after the injection of 600-μL (ischemia). We then removed the needle from the eye and measured the IOP and CR − sO2 15 min later (reperfusion). Figure 6(d) shows the B-mode CR − sO2 map in the reperfusion condition. The reperfusion map is not in the exact same plane as the previous condition, because

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**Fig. 2** Evaluation of the PAOI response to oxygen tensions. (a) B-mode photoacoustic/ultrasound image at baseline condition (breathing normal air) including the cornea, conjunctiva, lens, and chorioretina. The image depth is 20 mm. (b) The B-mode CR − sO2 map when the rabbit is given 5% O2 and 95% N2, (c) 10% O2 and 90% N2, (d) 15% O2 and 85% N2, (e) normal air, (f) 30% O2 and 70% N2, and (g) 100% O2. For PAOI, the CR − sO2 measurement frame rate is 0.8 Hz. (h) PAOI—CR − sO2 measurements show linear dependence (R² = 0.98, n = 5) with pulse oximeter measurements at different oxygen tensions. The error bars show the standard deviation of different five different eyes. All scale bars represent 5 mm.

**Fig. 3** Still frame from a video showing oxygen tension experiment for one animal transitioning from room air to 100% N2 and the accompanying change in CR − sO2 (Video 1, mp4, 785 KB [URL: https://doi.org/10.1117/1.JBO.23.3.036005.1]).

**Fig. 4** Validation of real time measurements of CR − sO2 using PAOI. (a) Real-time monitoring of sO2 using a pulse oximeter and PAOI in the normal and hyperoxic conditions. PAOI has a frame rate of 0.8 Hz. (b) MIP CR − sO2 map hyperoxia and (c) MIP CR − sO2 map during normal breathing condition. The scan size was 20 mm, and the scale bars represent 5 mm.
we moved the PA/US transducer to measure the IOP and then repositioned it back onto the eye after the IOP measurement. The PAOI showed a nearly sixfold decrease in CR – sO2 post-injection of 600 μL (n = 5, p = 0.00001). The sO2 measurement for control areas (conjunctive) confirms that the ischemia only affects the sO2 in the chorioretina and not other parts of the eye. The IOP did not fully return to baseline after reperfusion. It remained elevated relative to baseline [n = 5, p = 0.03; Figs. 6(f) and 6(g)]. The CR – sO2 and IOP are negatively correlated [Figs. 6(f) and 6(g)]. The CR – sO2 decreased with increased IOP (n = 5, p = 0.00002). Figure 6(e) shows real-time monitoring of both the pulse oximeter and the PAOI during the ischemia-reperfusion experiment. As expected, the ischemia-reperfusion model did not affect sO2 measurements via the pulse oximeter on the animal’s paw.

Figure 7 presents the sequence of increase in IOP and subsequent decrease in CR – sO2.

4 Discussion

We demonstrate high speed, simple, and noninvasive PAI to provide spectral, spatial, and temporal data with sufficient resolution to visualize chorioretinal oxygen gradients. This is the first measurement of chorioretinal oxygen gradients using an ischemia-reperfusion model and PAI. Rabbits with pigmented eyes (Dutch belted rabbit) were also evaluated using our approach. This strain has pigmented eyes, and we attempted to measure the chorioretinal oxygen gradients using PAOI. The retinal pigmented epithelium is a pigmented layer that is firmly attached to the underlying chorioretinal vessels. This layer contains melanin and absorbs light to generate strong photoacoustic signal. Therefore, we cannot measure the photoacoustic signals from the chorioretina in the pigmented rabbit eye. We used a commercial PAI system (Vevo LAZR, VisualSonics Inc.) and showed that this system is reliable and reproducible for measurement of CR – sO2 with the frame rate of 0.8 Hz, which is significantly higher than those in the literature. Using this tool, we observed CR – sO2 gradients both by changing oxygen tension as well as through an ischemia-reperfusion model. Our paper is the first to demonstrate the ability of PAOI to monitor oxygen level on the posterior segment of the eye over such a wide range of oxygen levels. Ketamine-xylocaine can reduce oxygen saturation on room air. In addition, we were using the pulse oximeter on the rabbit’s paw, which had much more hair than a human finger. This could result in an artificially low oxygen saturation reading. However, during our initial calibration using oxygen/nitrogen mixtures (Fig. 2), the oxygen saturation reported using PAOI was consistently lower than that reported by the pulse. This difference has been reported in hyperspectral computed tomography system and AR-PAM as well. One possible explanation for the difference is that while the peripheral pulse oximeter measures arterial sO2, the PAOI quantifies the CR – sO2 using the average of both arterial and venous vessels. Also, the large number of photons are absorbed and attenuated in the anterior segment of eye, such as iris and lens and only limited number of photons is delivered through the eye. Oxygen saturation measurement using PAOI depends on light fluence. The reason of having different numbers from pulse oximeter can be the change of fluence spectrum in depth. Nevertheless, the PAOI CR – sO2 measurements strongly correlated with systemic measurements of oxygen saturation (pulse oximeter).

One limitation of this approach is balancing spatial resolution and penetration depth. Rabbits’ retinal vessels are 20-mm deep. Therefore, we used a 15-MHz transducer with 580 and 290 μm as lateral and axial resolution, respectively. Although this could not discriminate individual vessels as in photoacoustics microscopy, we could achieve much deeper penetration. An ideal technique would have good depth penetration and
5 second after ischemia

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Fig. 6 Real-time evaluation of ischemia-reperfusion model using PAOI. (a) B-mode CR − sO₂ map at baseline condition (no PBS injection-no IOP increase); a 25-gauge needle is inside the anterior segment of eye. Red and yellow dotted rectangles show the retina and control areas, respectively. The CR − sO₂ is 68.76 ± 2.34 at baseline (no IOP increase; 10.5 ± 0.33 mm Hg). (b) The CR − sO₂ map 5 s after injection of PBS in the eye. Here, the change in the retina map to blue indicates a decrease in CR − sO₂ after 5 s of IOP. (c) CR − sO₂ gradients after injection of PBS (15 s after start of injection). The IOP increased to 46.5 ± 2.54 mm Hg, and the CR − sO₂ decreased to 12.3 ± 3.54. (d) CR − sO₂ map in reperfusion condition. The imaging plane is different from other three because we had to remove the transducer and measure the IOP with a Tonopen. CR − sO₂ recovered to 58.87 ± 3.23 after the IOP returned to 16.55 ± 1.67 mm Hg. (e) Real-time monitoring of CR − sO₂ and sO₂ using PAOI and pulse oximetry. The Δ shows sO₂ difference between baseline and ischemia. (f) Statistical analysis of CR − sO₂ for five eyes under different conditions including baseline, ischemia, and reperfusion using PAOI. The PAOI shows sixfold decrease in CR − sO₂ during ischemia (n = 5). There was a very significant change between baseline and ischemia and a significant different in CR − sO₂ from baseline to reperfusion. (g) Statistical analysis of IOP for five eyes at baseline, ischemia, and reperfusion using Tonopen. There is negative correlation between IOP and CR − sO₂. Error bars shows the standard deviation of five different eyes. Here, * indicates p < 0.05 and ** indicates p < 0.0001.

50-micron resolution to determine if specific sections of the retina are hypoxic. This current PAOI approach cannot discriminate between the retina and the choroid, and the area of study includes both features.

Previous work22 applied ischemia-reperfusion model on a rabbit’s eye and measured the CR − sO₂ using hyperspectral computed tomography system. This work used an elevated reservoir of balanced salt solution (BSS) to increase the IOP, but there was no precise control on the amount of injected BSS and IOP in the eye. Here, we used a syringe pump to more precisely control the injected volume (Fig. 5). With this model, the CR − sO₂ decreased with increasing volume of injected PBS due to interrupted blood flow. In CR − sO₂ map, we found that 10 s after starting the PBS injection, blood flow to the chorioretinal rapidly dropped (Fig. 6). The CR − sO₂ during reperfusion was lower than baseline (n = 5, p = 0.03) likely because of compensatory mechanisms of repair in the eye.
Another limitation is the use of a Tonopen designed for human on rabbit subjects. Mermoud et al. demonstrated that the transducer pressure and Tonopen as well. This means that the IOP numbers reported with the Tonopen on rabbits might be lower than the true IOP. The IOP numbers are likely 10 to 25 mmHg, because the Tonopen was used after the needle was removed from the eye after injection. Nevertheless, the good correlation seen here between injected volume of saline, Tonopen, and PAOI suggests that this model is indeed creating ischemia. Figure 6 shows changes in IOP (from baseline to 46.5 ± 2.54 mmHg) with just one step injection of 600 µL PBS in the eye.

The maximum permissible exposure (MPE) from the American National Standard Institute for 700- to 1050-nm laser light with 10-s illumination is 5.0CA,Cx × 10−7 J/cm². Here, there is a 20-Hz repetition rate and a 5-nm pulse width. This results in ~1 µs of actual laser pulse illumination time. With 700- to 1050-nm laser light and based on illumination specifications, CA,Cx = 102.0±0.786 and Cx = α/(τmax−τmin). Here, λ and α are the radiation wavelength and the full angle of actual laser pulse illumination time. We also already characterized this the limited PAI system in terms of penetration depth. Temporal resolution up to 30 Hz and signal-to-noise ratio of higher than 3 can be achieved for embedded pencil lead inside the chicken breast. We also evaluated application of LED system for monitoring chorioternea vessels on ex vivo rabbit eyes with frame rate of 15 Hz.

5 Conclusion

In this paper, we describe a high-speed PAOI technique to monitor chorioternea oxygen gradients in vivo. This the first report of PAI applied to CR − S0 measurements of ischemia-reperfusion in a large animal eye. To the best of our knowledge, a frame rate of 0.8 Hz for measuring CR − S0 is the highest frame rate yet reported, allowing for dynamic measurements of chorioternea oxygenation in real time. We demonstrate a very strong correlation between PAOI CR − S0 and pulse oximetry with varying levels of inspired oxygen, and we demonstrate a dramatic decrease in oxygen gradients during ocular ischemia. These experiments demonstrate that PAOI can serve as a dynamic measurement oxygen gradients in the eye and may be useful in the future for applications in ocular disease.

Disclosures

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