*Original Article*

# The influence of hemorrhagic shock on ocular microcirculation by obtained by laser speckle flowgraphy in a white rabbit model

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## ABSTRACT

**Purpose:** To clarify the continuous changes of the retinal vessels' and choroid's microcirculation during hemorrhagic shock and resuscitation in a rabbit model.

**Methods:** Hemorrhagic shock by the removal of blood (30 ml) and resuscitation by a blood-return technique was induced in anesthetized male New Zealand White rabbits (n=10). We evaluated the retinal vessel blood flow (relative flow volume: RFV) and choroidal blood flow (mean blur rate in the choroid area: MBR-CH) by laser speckle flowgraphy (LSFG), with simultaneous measurements of systemic hemodynamics and laboratory parameters.

**Results:** RFV and MBR-CH showed significant decreases immediately after the initiation of blood removal and recovered by blood return. The lactate concentration tended to increase from baseline by the blood-removal operation, and it was significantly higher at the end of observation period. The %RFV and %MBR-CH each showed a significant positive correlation with mean arterial blood pressure, cardiac output, carotid blood flow, and central venous pressure. %RFV showed a significant positive correlation with %central venous oxygen saturation and negatively correlated with %Lactate. The %hemoglobin did not show a significant correlation with %RFV or %MBR-CH.

**Conclusion:** This rabbit hemorrhagic shock model confirmed that ocular microcirculation measurements by LSFG feasibly reflect variations of systemic hemodynamics during hemorrhagic shock and recovery.

**Keywords:** hemorrhagic shock, white rabbit, ocular circulation, laser speckle flowgraphy

**Abbreviations**

bpm: beats per minute

CF: carotid artery blood flow

CO: cardiac output

CV: coefficient of variation

CVP: central venous pressure

Hb: hemoglobin

HR: heart rate

ICC: intraclass correlation coefficient

IOP: intraocular pressure

Lac: lactic acid

LSFG: laser speckle flowgraphy

MAP: mean arterial blood pressure

MBR: mean blur rate

RFV: relative flow volume

$ScvO_2$: central venous oxygen saturation

## INTRODUCTION

Shock is a status in which the blood flow to important organs cannot be maintained, resulting in cytological tissue metabolism disorder and organ damage.[1,2] Shock-induced organ damage occurs in hemorrhagic shock and cardiogenic shock, and it can also be caused by a defect in mitochondrial oxygen utilization, as occurs in septic shock. Hemorrhagic shock presents as a decrease in the intravascular blood volume, which makes it difficult for the blood flow to adequately perfuse organs due to severe blood loss. Methods for directly evaluating the organ damage that is due to a critical microcirculation status have not been established, and thus systemic clinical signs (e.g., the cardiac output, blood pressure, and heart rate) and laboratory values (e.g., the lactate concentration) are used as surrogates for the monitoring of tissue perfusion.[3,4]

The development of novel microcirculation evaluation methods, especially for the ocular microcirculation, may be very useful for gaining a better understanding of organ tissue perfusion during shock. An optical coherence tomography angiography study of a hemorrhagic shock sheep model (the only relevant published study) clarified that the retinal vessel density was significantly decreased in shock and recovered after fluid resuscitation therapy.[5]

Laser speckle flowgraphy (LSFG), a noninvasive quantitative method for determining the ocular blood flow,[6,7] is based on the changes in the speckle pattern of laser light reflected from the fundus of the eye.[8] LSFG is dependent on the movement of erythrocytes in the retina, the choroid, and the optic nerve head, and it can be used to determine the mean blur rate (MBR), which is an indicator of the ocular blood flow.[9,10] It was reported that the MBR correlates

linearly with the capillary blood flow volume.[9,11] The relative flow volume (RFV) derived from LSFG reflects the blood flow velocity and volume in the retinal vessels.[12,13] LSFG has also been used to investigate the ocular microcirculation in a rabbit model.[9,11,14,15]

In the present study, we used LSFG to clarify continuous variations of the microcirculation in the retinal vessels and choroid during hemorrhagic shock that occurred with continuous blood removal and blood return in an experimental rabbit model, with the simultaneous measurement of systemic hemodynamics.

## MATERIALS AND METHODS

Ten male New Zealand White rabbits (16–18 weeks old, weight 2.84–3.44 kg, median 3.16 kg) housed in the same environment were evaluated. All animal experiments were approved by Toho University Laboratory Animal Research (#19-53-358) and performed in accordance with the Guiding Principles for the Care and Use of Laboratory Animals approved by The Japanese Pharmacological Society. The experiments were performed under general anesthesia: induction of anesthesia with ketamine (35 mg/kg, i.m., Ketalar®, Daiichi Sankyo Propharma, Tokyo) and xylazine (5 mg/kg, i.m., Selactar 2%®, Bayer Japan, Osaka, Japan) and maintenance with isoflurane (end-tidal concentration of 1.5%, Isoflurane®, Pfizer Japan, Tokyo). After a tracheostomy, the rabbit was mechanically ventilated ($FiO_2$=1.0, tidal volume=6 ml/kg, 40 strokes/min; SN-480-5, Shinano, Tokyo). The rabbit's body temperature was maintained at 37°C with a heating pad. Saline (15 ml/h) and rocuronium (0.6 mg

/kg/h; Rocuronium bromide®, Fuji Pharma, Toyama, Japan) were continuously administered via a left ear vein.

***Experimental protocol***

After the confirmation that the rabbit's systemic hemodynamics and ocular circulation state were stable for ≥30 min, the experiment was started. First, for the induction of hemorrhagic shock, blood was continuously removed from the central venous catheter (4-Fr. ga.) at the rate of 1 ml/min for 30 min. At the end of the 30-min blood removal period, an observation period was conducted for 20 min, and then heparinized blood (2 ml/min) was returned via the left auricular vein for 15 min. After the completion of the return of all 30 ml of blood, another observation period was conducted for 15 min. The scheme of the experimental protocol is shown in Figure 1.

***Systemic hemodynamics evaluation***

Figure 2 illustrates the apparatus used to measure the rabbit's systemic hemodynamics and ocular microcirculation. A heparinized catheter was inserted at the right brachial artery for the continuous measurement of the mean arterial blood pressure (MAP, mmHg) using transducers. For the blood removal route and central venous pressure measurement, a catheter was inserted into the femoral vein, and the tip of the catheter was placed in the inferior vena cava. The MAP, heart rate (HR, beats per minute: bpm), and central venous pressure (CVP, mmHg) were recorded using a PowerLab system® (AD Instruments, Bella Vista, New South Wales, Australia).

For the measurements of cardiac output (CO, ml/min), the origin of the aorta was exposed through a median sternotomy incision. After a blood flow meter was attached to the origin of the aorta, the inside of the thoracic cavity was simply made into a closed space using plastic paraffin film. For the measurements of carotid artery blood flow (CF, ml/min), the left common carotid artery was exposed. The CO and CF were measured with an ultrasonic blood flowmeter (TS420®, Transonic Systems, Ithaca, NY). Systemic hemodynamic parameters were measured simultaneously every 2 min during the observation periods and every 1 min during the blood removal and return periods.

***Laboratory examinations***

Hemoglobin (Hb, mg/dl), lactic acid (Lac, mmol/l), and the central venous oxygen saturation ($ScvO_2$, %) were measured by a blood analyzer (i-STAT 1 Analyzer®, Abbott, Chicago, IL). Blood samples (0.5 ml/time) of Hb, Lac, and $ScvO_2$ were collected as shown in Figure 1, at six time points.

***LSFG evaluation***

The MBR images were obtained with an LSFG-MRC™ device (Softcare Ltd., Iizuka, Japan), and the RFV in the retinal vessels and the mean MBR in the choroid area were calculated by LSFG Analyzer software (Softcare). The LSFG-MRC consists of a fundus camera equipped with a diode laser (wavelength 830 nm) and a CCD image sensor (750 × 360 pixels). The principle and application of this method have been described.[6,9,16] Rectangular bands were placed at a retinal vessel and at the choroid area avoiding retinal vessels (Fig. 3A). Within a

5-sec period tuned to the cardiac cycle, MBR images were recorded from the rectangular area, and the average MBR was displayed on a computer screen (Fig. 3B). The MBR in the choroid (MBR-CH) is also displayed on the screen.

The scheme for the measurement of the RFV in the retinal vessel is shown in Figure 4. The MBR-threshold is the threshold between the MBR values in the retinal vessels and the background choroid; f(x) is the distribution function of the MBR in a cross-sectional area of the blood vessel, and the width of the function at the MBR-threshold is represented by m and n. The RFV in the retinal vessel was calculated by subtracting the choroidal MBR value from the overall MBR value.[10,12,13]

The RFV and the MBR-CH were measured simultaneously every 2 min during the observation periods and every 1 min during the blood removal and return periods; the values were recorded three times at each time point, and the mean value was then calculated. In each experiment, the rectangular bands were stored in the software program, and the same rectangular bands were used for all analyses. The LSFG-MRC has undergone appropriate calibrations, and it has been confirmed that the MBR increases linearly with the blood flow velocity. Mydriasis was induced by topical tropicamide (Mydrin M® ophthalmic solution 0.4%, Santen Pharmaceutical Co., Osaka, Japan). Only the left eye was used in all of the experiments.

***Intraocular pressure (IOP) control***

To keep the intraocular pressure (IOP) stable during each experiment, a 25-ga. infusion cannula was inserted into the vitreous cavity of the rabbit through the

pars plana. This infusion cannula was connected to a bottle of intraocular irrigating solution (BSS Plus®; Alcon Japan, Tokyo) for the stabilization of the IOP during the experiment. The IOP was set at 10 mmHg by changing the height of the bottle. The IOP was confirmed by an ICARE Tonolab Tonometer® (Revenio Group, Helsinki, Finland) at the start and end of the experiment.

***Statistical analyses***

The data of the continuous variables are presented as the mean ± standard error. The measurement repeatability of the RFV and MBR-CH was determined based on the coefficient of variation (CV, in %) defined as the ratio of the mean standard deviation of repeated measurements to the overall mean. The intraclass correlation coefficient (ICC) for the five consecutive measurements of RFV and MBR-CH were calculated using a using a two-way analysis of variance for repeated measurements (ANOVA). The RFV and MBR-CH were also evaluated by the rate of change from the baseline value before the blood removal (% MBR, %RFV). The time courses of the changes in BP, HR, CO, CF, Hb, Lac, $ScvO_2$, %MBR-CH, and %RFV were analyzed using a repeated measurements ANOVA and the Dunnett test as a post hoc test. During the experiment, correlation coefficients between the change rate (%) of systemic hemodynamics, the % laboratory parameters, and the ocular microcirculation parameters (%RFV and %MBR-CH) were analyzed by a univariate regression. P-values $<0.05$ were accepted as significant. The JMP-10.0 program (SAS, Cary, NC) was used for the statistical analyses.

## RESULTS

The calculated repeatability indices, i.e., the CV and ICC, for the RFV and MBR-CH obtained by LSFG revealed the following. RFV: 4.21% CV and 0.97 ICC. MBR-CH: 2.04% CV and 0.97 ICC.

The time courses of changes in the HR, BP, CO, CF, CVP, LSFG parameters (%RFV and %MBR-CH), Hb, $ScvO_2$, and Lac during the experiment are shown in Figure 5. No significant change was detected in the HR, whereas the MAP, CO, CF, CVP, %RFV and %MBR-CH showed significant decreases with the blood removal. These significant differences did not disappear during the observation period, and these values were recovered to near the basic value with the blood return.

The variations from the start to the end of the blood removal phase were as follows: MAP (37.2 ± 2.0 mmHg vs. 24.1 ± 1.2 mmHg, the MAP value pre-sternotomy was 40.1±7.0 mmHg), CO (168.2 ± 15.7 ml/min vs. 105.1 ± 11.5 ml/min), CF (10.4 ± 1.5 ml/min vs. 5.8 ±0.7 ml/min) and CVP (3.7 ± 0.2 mmHg vs. 2.9 ± 0.3 mmHg). The decline rate (%) of the RFV and MBR-CH from the start to the end of the blood removal phase reached 52.6 ± 5.8% and 35.8 ± 6.8%, respectively. The Hb was significantly decreased at the end of the blood removal phase (from 9.8 ± 0.3 g/dl to 7.4 ± 0.3 g/dl), and the significant decrease continued until the end of the experiment. The $ScvO_2$ was significantly decreased by the blood removal operation (from 54 ± 4% to 39 ± 3%) and had recovered at the end of observation period. The Lac value tended to increase from 3.3 ± 0.3 mmol/l (baseline) by the blood removal and was significantly increased by the end of the observation period. The increase in Lac continued

until the end of the blood return phase (7.6 ± 0.8 mmol/l), and the increase was significant by the end of the experiment. HR was not significantly increased by the blood removal operation, but was on an upward trend (from 229 ± 18 bpm to 238 ± 18 bpm).

Figure 6 shows a representative case of the RV and MBR-CH at the baseline, the end of the blood removal, and the blood return phase. The correlation coefficients between %RFV, %MBR-CH, and the %systemic hemodynamics and laboratory parameters are shown in Table 1. The %RFV and %MBR-CH each showed a significant positive correlation with all of the %systemic hemodynamics parameters.

Among the parameters, %MAP had the strongest correlation with %RFV and %MBR-CH (r=0.77). Regarding the relationships with the %laboratory parameters, the %RFV showed a significant positive correlation with $\%ScvO_2$ and a significant negative correlation with %Lac (r= 0.32 and −0.27, respectively). The %Hb did not show a significant correlation with RFV or %MBR-CH.

## DISCUSSION

Hemorrhagic shock can involve excessive blood loss leading to decreased cardiac output, which results in decreased tissue perfusion with tissue dysoxia, organ damage, and finally death. In rare cases of hemorrhagic shock, shock-induced anterior ischemic optic neuropathy due to acute severe blood loss has been described.[17–20] It was reported that 88% of shock-induced anterior ischemic optic neuropathy occurred in both eyes, and in most cases the visual

deficit did not resolve.[17] In any case, the development of novel microcirculation evaluation methods, especially for assessing ocular microcirculation, may be useful for understanding the organ tissue perfusion during shock.

There is an only a single prior report about the relationships between ocular microvascular perfusion and critically ill statuses of hemorrhagic shock,[5] and the variations of ocular microcirculation in hemorrhagic shock and the relationships among ocular microcirculation and systemic hemodynamics in hemorrhagic shock are not fully understood. LSFG is a noninvasive quantitative method for determining the ocular microcirculation, and it can detect the blood flow in the retinal vessels, choroid, and optic nerve head continuously.[6,7,9,10] Our goal in the present study was thus to clarify the continuous changes of microcirculation in the retinal vessels and choroid during hemorrhagic shock and blood return in an experimental rabbit model examined with LSFG, while monitoring systemic hemodynamics and laboratory parameters.

The CVs and ICCs of the RFV and MBR-CH were favorable. Our present results suggest that the reproducibility of these parameters is sufficient for measuring the microcirculation in the retinal vessels and choroid of New Zealand White rabbits.

It was reported that a nearly 20% blood loss was necessary to establish a rabbit model of hemorrhagic shock.[21–23] In the present study, a total blood loss of 30 ml was needed to achieve a 20% total hemorrhage calculated from the weight of the rabbits. The blood was removed continuously at the rate 1 ml/min from the central venous catheter for 30 min. As a result, the MAP, Hb, and CO were significantly decreased (by 35%, 25%, and 37%, respectively). The mean

MAP and Lac values at the start of the blood removal phase were <40 mmHg and >2 mmol/l, respectively. All 10 of the rabbits received a median sternotomy incision for the direct evaluation of the CO before the experiment. Although the effects of a sternotomy cannot be completely ruled out, the mean MAP before the sternotomy was 40.1 mmHg, which was not significantly different between before and after the sternotomy. The rabbits' MAP levels before the experiment were the almost same as in our previous investigation using the same method without a sternotomy incision.[24] In addition, the ScvO2 was significantly decreased and Lac was significantly increased as a result of the blood removal. Earlier studies established that the ScvO2 and Lac are informative parameters even in rabbit hemorrhagic shock.[25–27] Since the standard value of HR was high at the start of the experiment, no significant increase was observed by the blood removal , but it showed an increasing tendency. We thus consider our present effort to create a hemorrhagic shock state successful.

Under the conditions used herein, we observed that the retinal vessel circulation represented by the RFV and MBR-CH were significantly decreased immediately after the start of the blood removal and were recovered by the return of the blood. These variations of ocular microcirculation are similar to the variations observed in the MAP, CO, CF and CVP of rabbits. It has been clarified that the human retinal blood flow has myogenic-dominant autoregulation to maintain the tissue blood flow against an acute rise in blood pressure,[28,29] and the human retina is capable of efficiently autoregulating its blood flow in the setting of acute systemic hypertension until there is an increase in the mean systemic blood pressure of approx. 40% above resting

levels.[28] Autoregulation has also been confirmed in the human choroidal blood flow, although it is weaker than that of the retinal blood flow.[30]

There is evidence of myogenic autoregulatory properties in the rabbit ophthalmic artery and/or choroid.[31–33] In studies of the rabbit choroidal blood flow using laser Doppler flowmetry, autoregulation was activated against an increase in the MAP, whereas a decrease in the MAP led to a decrease in the choroidal blood flow in a linear manner.[32,33] Considering these prior findings and our present results, it appears that the autoregulation of the retinal vessels and choroid under acute hemorrhagic shock is poor, and that the blood flow of both is decreasing rapidly. However, in the rabbit model used herein, the blood return operation recovered the retinal-vessel and choroidal blood flow in parallel with the recovery of the systemic hemodynamics state (Figs. 5, 6). These findings may provide important clues to the pathophysiology and prevention of shock-related ocular dysfunction such as shock-induced anterior ischemic optic neuropathy.

We next determined the correlation coefficients between the change rate (%) of systemic hemodynamics, the %laboratory parameters, and the ocular microcirculation parameters %RFV and %MBR-CH over the time course of the experiment. We observed that all of the %systemic hemodynamic parameters were significantly correlated with the %RFV and %MBR-CH, whereas the %Hb did not show a significant correlation. The %MAP had the strongest correlations with %RFV and %MBR-CH (r=0.77). These results suggest that the variations of microcirculation in the rabbit retinal vessels and choroid under critical conditions are more strongly influenced by the blood pressure than the red

blood cell count. The %$ScvO_2$ and %Lac were significantly correlated with %RFV but not %MBR-CH. This result may support the possibility that compared to the choroid vessels the autoregulation of retinal vessels is triggered more strongly in rabbits, as it is in humans.

In any case, our findings also indicate that the measurement of ocular microcirculation, especially the RFV, by LSFG is highly reproducible and well reflects the variations of systemic hemodynamics, Lac, and $ScvO_2$ during hemorrhagic shock and the recovery status. In light of our present results, it is apparent that the measurement of ocular microcirculation by LSFG could be a novel informative method for evaluations of the physiological changes during hemorrhagic shock.

There are some study limitations to address. First, the experiments were conducted with the rabbits in the supine position, and because this position is not a normal posture for rabbits, postural effects might have had an impact on the systemic and ocular hemodynamic measurements. Second, the study lacks the histological data after the animals were sacrificed. Novel and informative ocular histological data may have been missed. Third, the microcirculation in critically ill patients has been evaluated in the splanchnic region, skin, muscles, and the sublingual area.[34] We did not compare the LSFG measurements with data obtained by these methods. Finally, we used heparinized blood for the blood return phase, whereas some experimental animal models used a balanced hydroxyethyl starch solution,[5] Ringer's lactate solution,[35] or normal saline[36] for the resuscitation state. In the present study, we did not compare the effects on ocular and systemic hemodynamics based on the resuscitation fluid.

In conclusion, our experimental rabbit hemorrhagic shock model confirmed that the measurement of ocular microcirculation by LSFG is highly reproducible and well reflects variations of systemic hemodynamics during hemorrhagic shock and recovery.

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## References


1. Ohashi H, Kawasaki N, Fujitani S, Kobayashi K, Ohashi M, Hosoyama A, Wada T, Taira Y. Utility of microdialysis to detect the lactate/pyruvate ratio in subcutaneous tissue for the reliable monitoring of hemorrhagic shock. *J Smooth Muscle Res.* 2009;45:269-278.
2. Kruse JA. Searching for the perfect indicator of dysoxia. *Crit Care Med.* 1999;27:469-471.
3. Ince C. The rationale for microcirculatory guided fluid therapy. *Curr Opin Crit. Care* 2014;20:301-308.
4. Nguyen HB, Rivers EP, Knoblich BP, Jacobsen G, Muzzin A, Ressler JA, Tomlanovich MC. Early lactate clearance is associated with improved outcome in severe sepsis and septic shock. *Crit Care Med.* 2004;32:1637-1642.
5. Alnawaiseh M, Ertmer C, Seidel L, Arnemann PH, Lahme L, Kampmeier TG, Rehberg SW, Heiduschka P, Eter N, Hessler M. Feasibility of optical coherence tomography angiography to assess changes in retinal microcirculation in ovine haemorrhagic shock. *Crit Care.* 2018;*22*:138.
6. Tamaki Y, Araie M, Kawamoto E, Eguchi S, Fujii H. Non-contact, two-dimensional measurement of tissue circulation in choroid and optic nerve head using laser speckle phenomenon. *Exp Eye Res.* 1995;60:373–383.
7. Isono H, Kishi S, Kimura Y, Hagiwara N, Konishi N, Fujii H. Observation of choroidal circulation using index of erythrocytic velocity. *Arch Ophthalmol.* 2003;121:225–231.

8. Fujii, H. Visualisation of retinal blood flow by laser speckle flow-graphy. *Med Biol Eng Comput.* 1994;32:302–304.
9. Takahashi H, Sugiyama T, Tokushige H, Maeno T, Nakazawa T, Ikeda T, Araie M. Comparison of CCD-equipped laser speckle flowgraphy with hydrogen gas clearance method in the measurement of optic nerve head microcirculation in rabbits. *Exp Eye Res.* 2013;108:10-15.
10. Sugiyama T. Basic technology and clinical applications of the updated model of laser speckle flowgraphy to ocular diseases. *Photonics.* 2014;1:220-234,.
11. Aizawa N, Nitta F, Kunikata H, Sugiyama T, Ikeda T, Araie M, Nakazawa T. Laser speckle and hydrogen gas clearance measurements of optic nerve circulation in albino and pigmented rabbits with or without optic disc atrophy. *Invest Ophthalmol Vis Sci.* 2014;55:7991-7996.
12. Shiga Y, Asano T, Kunikata H, Nitta F, Sato H, Nakazawa T, Shimura M. Relative flow volume, a novel blood flow index in the human retina derived from laser speckle flowgraphy. *Invest Ophthalmol Vis Sci.* 2014;55:3899-3904.
13. Luft N, Wozniak PA, Aschinger GC, Fondi K, Bata AM, Werkmeister RM, Schmidl D, Witkowska KJ, Bolz M, Garhöfer G, Schmetterer L. Measurements of retinal perfusion using laser speckle flowgraphy and Doppler optical coherence tomography. *Invest Ophthalmol Vis Sci.* 2016;57:5417-5425.
14. Shibata M, Oku H, Sugiyama T, Kobayashi T, Tsujimoto M, Okuno T, Ikeda T. Disruption of gap junctions may be involved in impairment of

autoregulation in optic nerve head blood flow of diabetic rabbits. *Invest Ophthalmol Vis Sci.* 2011;52:2153-2159.

15. Shibata M, Sugiyama T, Kurimoto T, Oku H, Okuno T, Kobayashi T, Ikeda T. Involvement of glial cells in the autoregulation of optic nerve head blood flow in rabbits. *Invest Ophthalmol Vis Sci.* 2012;53:3726-3732.
16. Sugiyama T, Araie M, Riva CE, Schmetterer L, Orgul S. Use of laser speckle flowgraphy in ocular blood flow research. *Acta Ophthalmol.* 2010;88:96-102.
17. Hollenhorst RW, Wagener HP. Loss of vision after distant hemorrhage. *Am J Med Sci.* 1950;219:209-214.
18. Chisholm IA. Optic neuropathy of recurrent blood loss. *Br J Ophthalmol.* 1969;53:289-295.
19. Drance SM, Morgan RW, Sweeney VP. Shock-induced optic neuropathy. A case of nonprogressive glaucoma. *N Engl J Med.* 1973;288:392-395.
20. Jaben SL, Glaser JS, Daily M. Ischemic optic neuropathy following general surgical procedures. *J Clin Neuroophthalmol.* 1983;3:239-244.
21. Lee J, Kim JG, Mahon S, Tromberg BJ, Mukai D, Kreuter K, Saltzman D, Patino R, Goldberg R, Brenner M. Broadband diffuse optical spectroscopy assessment of hemorrhage- and hemoglobin-based blood substitute resuscitation. *J Biomed Opt.* 2009;14:044027.
22. Lee J, Cerussi AE, Saltzman D, Waddington T, Tromberg BJ, Brenner M. Hemoglobin measurement patterns during noninvasive diffuse optical spectroscopy monitoring of hypovolemic shock and fluid replacement *J Biomed Opt.* 2007;12:024001.

23. Lee J, Saltzman DJ, Cerussi AE, Gelfand DV, Milliken J, Waddington T, Tromberg BJ, Brenner M. Broadband diffuse optical spectroscopy measurement of hemoglobin concentration during hypovolemia in rabbits. *Physiol Meas.* 2006;27:757-767.
24. Sakuma K, Shimoda A, Shiratori H, Komatsu T, Watanabe K, Chiba T, Aimoto M, Nagasawa Y, Hori Y, Shirai K, Takahara A. Angiotensin II acutely increases arterial stiffness as monitored by cardio-ankle vascular index (CAVI) in anesthetized rabbits. *J Pharmacol Sci.* 2019;140:205-209.
25. Chen S, Zhu X, Wang Q, Li W, Cheng D, Lei C, Xiong L. The early effect of Voluven, a novel hydroxyethyl starch (130/0.4), on cerebral oxygen supply and consumption in resuscitation of rabbit with acute hemorrhagic shock. *J Trauma.* 2009;66:676-682.
26. Zhao L, Luo L, Jia W, Xiao J, Huang G, Tian G, Li J, Xiao Y. Serum diamine oxidase as a hemorrhagic shock biomarker in a rabbit model. *PLOS ONE.* 2014;9:e102285.
27. Jacobson YG, Keller M, Mundth ED, Defalco AJ, McClenethan JE. Hemorrhagic shock. Influence of hyperbaric oxygen on metabolic parameters. *Calif Med.* 1966;105:93-96.
28. Robinson F, Riva CE, Grunwald JE, Petrig BL, Sinclair SH. Retinal blood flow autoregulation in response to an acute increase in blood pressure. *Invest Ophthalmol Vis Sci.* 1986;27:722-726.
29. Nagaoka T, Mori F, Yoshida A. Retinal artery response to acute systemic blood pressure increase during cold pressor test in humans. *Invest Ophthalmol Vis Sci.* 2002;43:1941-1945.

30. Polska E, Simader C, Weigert G, Doelemeyer A, Kolodjaschna J, Scharmann O, Schmetterer L. Regulation of choroidal blood flow during combined changes in intraocular pressure and arterial blood pressure. *Invest Ophthalmol Vis Sci.* 2007;48:3768-3774.
31. Delgado E, Marques-Neves C, Rocha I, Sales-Luís J, Silva-Carvalho L. Intrinsic vasomotricity and adrenergic effects in a model of isolated rabbit eye. *Acta Ophthalmol.* 2009;87:443-449.
32. Kiel JW, van Heuven WA. Ocular perfusion pressure and choroidal blood flow in the rabbit. *Invest Ophthalmol Vis Sci.* 1995;36:579-585.
33. Kiel JW, Shepherd AP. Autoregulation of choroidal blood flow in the rabbit. Autoregulation of choroidal blood flow in the rabbit. *Invest Ophthalmol Vis Sci.* 1992;33:2399-2410.
34. den Uil CA, Klijn E, Lagrand WK, Brugts JJ, Ince C, Spronk PE, Simoons ML. The microcirculation in health and critical disease. *Prog Cardiovasc Dis.* 2008;51:161-170.
35. Gao C, Xu DQ, Gao CJ, Ding Q, Yao LN, Li ZC, Chai W. An exogenous hydrogen sulphide donor, NaHS, inhibits the nuclear factor κB inhibitor kinase/nuclear factor κb inhibitor/nuclear factor-κB signaling pathway and exerts cardioprotective effects in a rat hemorrhagic shock model. *Biol Pharm Bull.* 2012'35:1029-1034.
36. Abdel-Zaher AO, Abdel-Aal RA, Aly SA, Khalifa MM. Adenosine for reversal of hemorrhagic shock in rabbits. *Jpn J Pharmacol.* 1996;72:247-254.

## Figure legends

**Fig. 1.** The experimental protocol.

**Fig. 2.** The continuous monitoring by LSFG and the heart rate (HR), mean arterial pressure (MAP), cardiac output (CO), carotid blood flow (CF), and central venous pressure (CVP) in an anesthetized rabbit. bpm: beats per minute, Hb: hemoglobin, Lac: lactate, $ScvO_2$: central venous oxygen saturation.

**Fig. 3.** The method for analyzing the mean blur rate in the choroid area (MBR-CH) and relative flow volume (RFV) using the LSFG-MRC device. **A:** The color-scale map of the total measurement area. The rectangular measurement areas were placed at the retinal vessel (1) and choroid area (2). **B:** The pulse waves show changes in the MBR (which is tuned to the cardiac cycle for 5 sec), and the average MBR-CH is determined from this panel.

**Fig. 4.** The relative flow volume (RFV) in a rabbit retinal vessel. The mean blur rate (MBR)-threshold is the threshold between the MBR values in the retinal vessels and the background choroid; f(x) is the distribution function of the MBR in a cross-sectional area of the blood vessel; the width of the function at the MBR-threshold is represented by m and n. The RFV is calculated by subtracting the choroidal MBR from the overall MBR.

**Fig. 5.** Time courses of the effects of blood removal and blood return on the heart rate (HR), mean arterial pressure (MAP), cardiac output (CO), carotid blood flow (CF), central venous pressure (CVP), %RFV (relative flow volume), %MBR-CH (mean blur rate in the choroid area), hemoglobin (Hb), central venous oxygen saturation ($ScvO_2$), and lactate (Lac) in the anesthetized rabbits (n=10). *Closed symbols:* A significant differences from the corresponding control value of each parameter by $p<0.05$.

**Fig. 6.** A representative case showing the RFV and MBR-CH at the baseline (**A:** RFV = 218.4, MBR-CH = 4.5), at the end of blood removal (**B:** RFV = 81.2, MBR-CH = 2.8), and at the blood return phase (**C:** RFV = 182.6, MBR-CH = 4.9).

**Table 1.** Correlation coefficients between the %RFV, %MBR-CH, and %systemic hemodynamics and laboratory parameters

| **Objective variable:** | **RFV** | **%MBR-CH** |
|---|---|---|
| **Explanatory variable:** | **r** | **r** |
| %HR | 0.14* | 0.18* |
| %MAP | 0.77* | 0.77* |
| %CO | 0.53* | 0.57* |
| %CF | 0.61* | 0.57* |
| %CVP | 0.35* | 0.51* |
| %Hb | 0.19 | 0.04 |
| %$ScvO_2$ | 0.32* | 0.27 |
| %Lac | −0.27* | 0.01 |

*p<0.05. bpm: beats per minute, CF: carotid blood flow, CO: cardiac output, CVP: central venous pressure, Hb: hemoglobin, HR: heart rate, Lac: lactate, MAP: mean arterial pressure, MBR-CH: mean blur rate in the choroid area, RFV: relative flow volume, $ScvO_2$: central venous oxygen saturation.

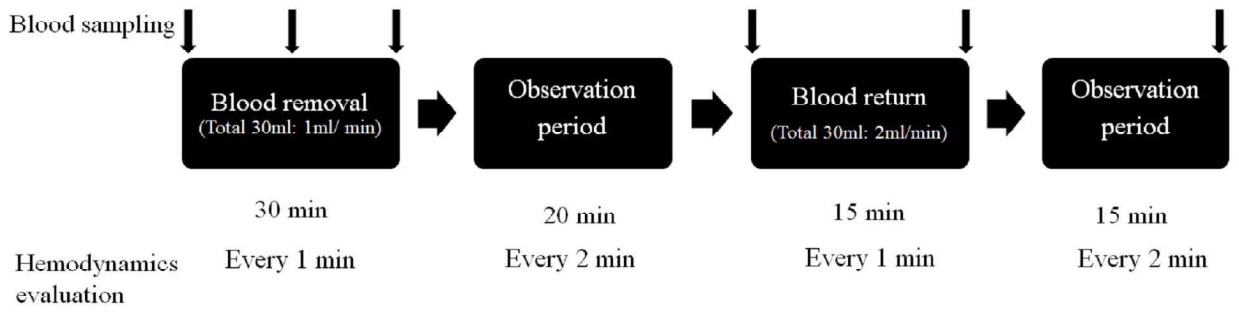



Fig.1

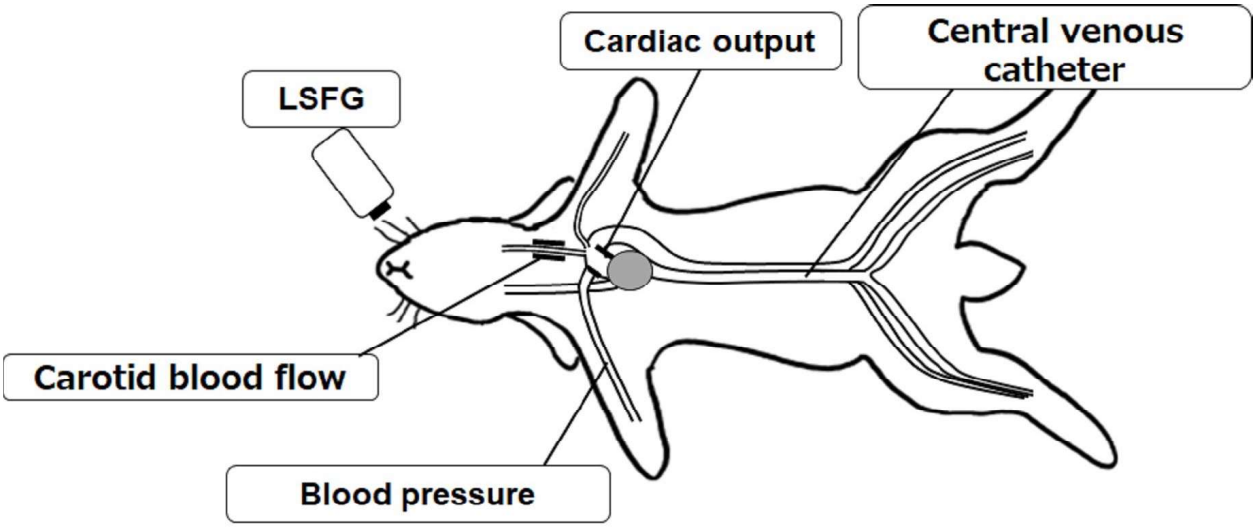



Fig.2

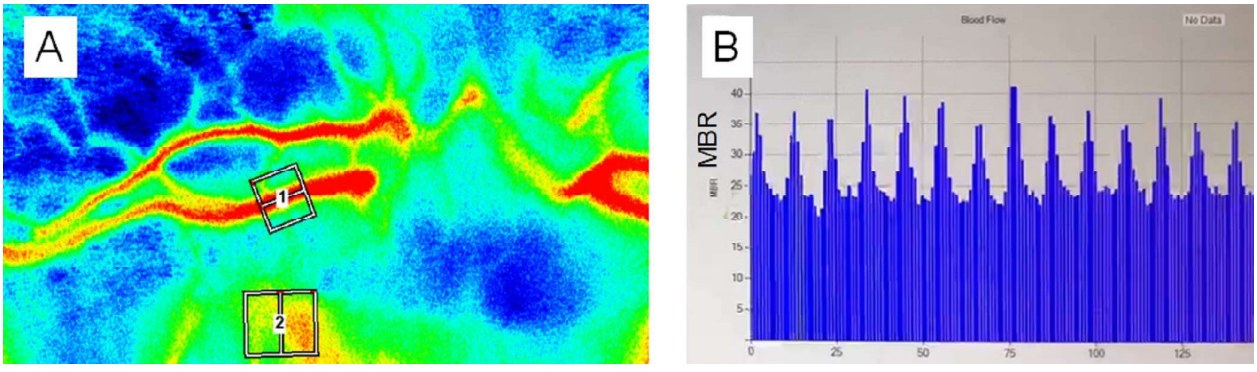
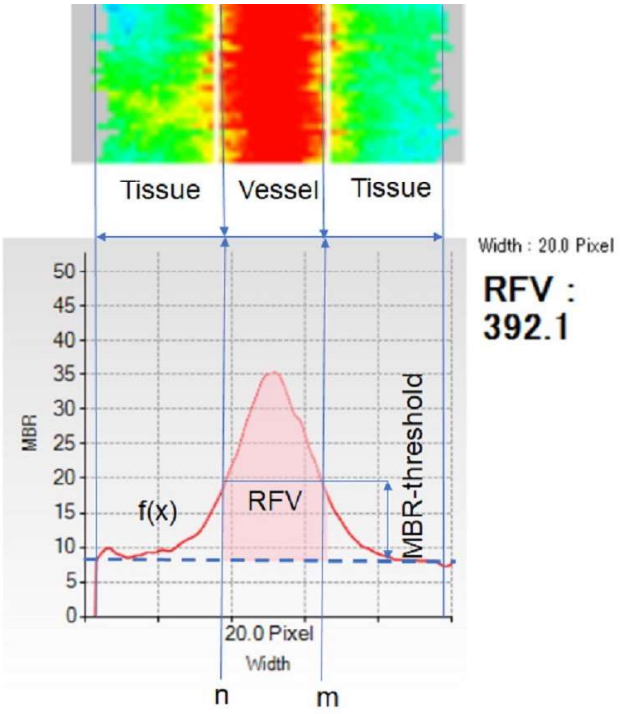



Fig.3


Tissue
Vessel
Tissue
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RFV :
392.1
MBR
50
45
40
35
30
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20
15
10
5
0
f(x)
RFV
MBR-threshold
20.0 Pixel
Width
n
m

Fig.4

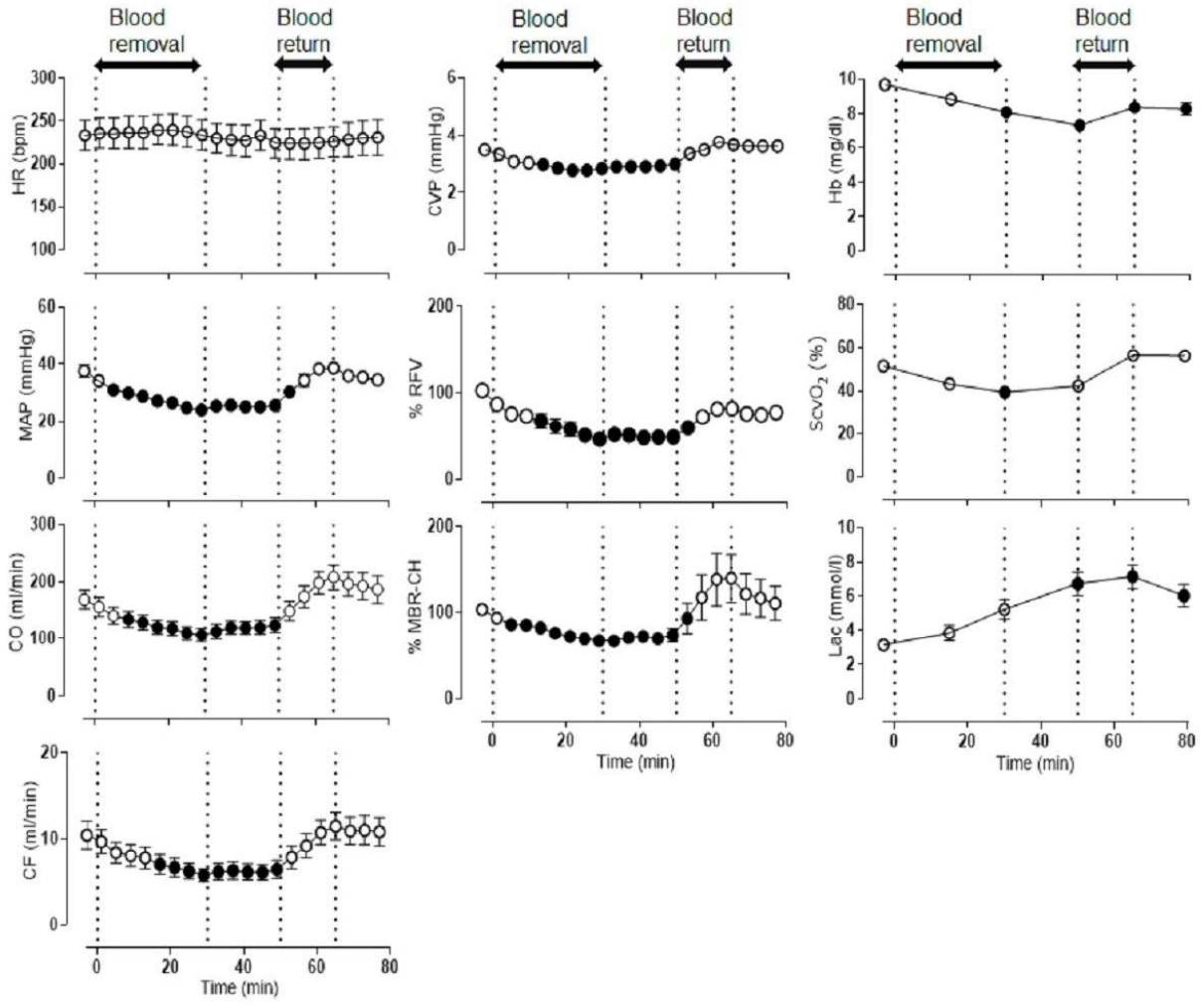
Blood removal
Blood return
HR (bpm)
MAP (mmHg)
CO (ml/min)
CF (ml/min)
CVP (mmHg)
% RFV
% MBR-CH
Hb (mg/dl)
ScvO2 (%)
Lac (mmol/l)
Time (min)

Fig.5

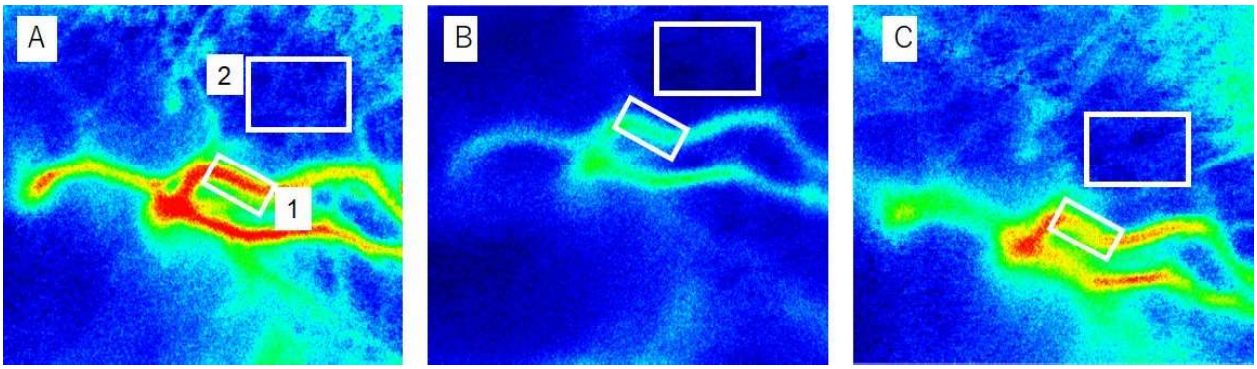


Fig.6