Assessing hemorrhagic shock: Feasibility of using an ultracompact photoacoustic microscope

Qian Chen¹,² | Heng Guo¹,² | Weizhi Qi¹,² | Qi Gan³ | Lei Yang⁴ | Bowen Ke⁴ |
Xingxing Chen² | Tian Jin¹,² | Lei Xi¹,²*

¹Department of Biomedical Engineering, Southern University of Science and Technology, Shenzhen, China
²School of Physics, University of Electronic Science and Technology of China, Chengdu, China
³Department of Neurosurgery, West China Hospital Sichuan University, Chengdu, China
⁴Department of Anesthesiology and Critical Care Medicine, West China Hospital Sichuan University, Chengdu, China

*Correspondence
Lei Xi, Department of Biomedical Engineering, Southern University of Science and Technology, Shenzhen, Guangdong, 518055, China.
Email: xilei@sustc.edu.cn

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Hemorrhagic shock, as an important clinical issue, is regarded as a critical disease with a high mortality rate. Unfortunately, existing clinical technologies are inaccessible to assess the hemorrhagic shock via hemodynamics in microcirculation. Here, we propose an ultracompact photoacoustic microscope to assess hemorrhagic shock using a rat model and demonstrate its clinical feasibility by visualizing buccal microcirculation of healthy volunteers. Both functional and morphological features of the microvascular network including concentration of total hemoglobin (CHbT), number of blood vessels (VN), small vascular density (SVD) and vascular diameter (VD) were derived to assess the microvascular hemodynamics of different organs. Animal studies show the feasibility of the proposed tool to assess and stage the hemorrhagic shock via microcirculation. In vivo oral imaging of healthy volunteers indicates the translational possibility of this technique for clinical evaluation of hemorrhagic shock.

1 | INTRODUCTION

Hemorrhagic shock remains an important clinical issue, which may lead to hemodynamic instability, decreases in oxygen delivery, insufficient tissue perfusion, cellular hypoxia, organ damage and even death [1–4]. All the clinical imaging techniques including X-ray computed tomography (CT), ultrasonography and magnetic resonance imaging (MRI) are limited to assess the hemorrhagic shock via hemodynamics in microcirculation [5–7]. However, microcirculation, which is inaccessible to clinical imaging modalities, plays a more important role to assess and stage the hemorrhagic shock and fluid resuscitation [8]. With the rapid development of laser and light detection techniques, various optical imaging modalities including near-infrared spectroscopy (NIRS), pure optical microscopy, orthogonal polarization spectral imaging, optical intrinsic signal imaging and laser speckle contrast imaging have been widely applied to study microcirculation of hemorrhagic shock [9–11]. Among all these optical imaging modalities, NIRS suffers from low spatial resolution, fluorescence microscopy introduces the external contrast agents, both optical intrinsic and laser speckle imaging lack of depth resolving capability. Apart from pure optical imaging modalities, optical resolution photoacoustic microscopy (ORPAM), featuring rich optical contrast, deep penetration depth, multiscale resolving capability and ultrahigh sensitivity to hemoglobin, shows significant advantages on visualizing microcirculation of rodents [12–14]. Unfortunately, clinical translation of ORPAM is still challenging due to the bulky size and inconvenience of the equipment. Recent clinical investigations show that the microcirculatory indices in sublingual or buccal mucosa reflect the systemic microcirculation which is tightly associated with hemorrhagic shock [8]. Our group proposed a rotatory-scanning-based portable ORPAM and reported the
first study of human buccal microcirculation [15]. Although it is portable relative to tabletop ORPAMs and achieves high-resolution imaging of buccal microcirculation in human revealing the potential for clinical use, it is still heavy and bulky for handheld devices and difficult to hold stably for a long time. To further reduce the portable probe and make it suitable for clinical use, we utilized a two-dimensional (2D) electrothermal-bimorph-actuation based micro-electro-mechanical system (MEMS) scanner to develop a miniaturized ORPAM system, a handheld microscope and an ultracompact ORPAM (U-ORPAM) probe [16–19]. This probe weights 20 g and has an outer size of $22 \times 30 \times 13 \text{ mm}^3$, a high lateral resolution of 3.8 $\mu\text{m}$, an effective imaging domain of $2 \times 2 \text{ mm}^2$ and a maximal full-view frame rate of 2 Hz.

In this study, our purpose is to investigate the microvascular hemodynamics of different organs during the entire course of hemorrhagic shock and resuscitation with the U-ORPAM, which has been successfully applied to monitor hemodynamics in human buccal vasculatures.

2  | MATERIALS AND METHODS

2.1  | Animal preparation

The experimental protocol was approved by the Animal Ethics Committee at the Southern University of Science and Technology (SUSTech). Female Sprague-Dawley rats, weighting 250 to 300 g ($n = 60$), were equally divided into four groups. The rats were anesthetized by intraperitoneal injection of chloral hydrate (50 mg/kg). Anesthesia was maintained by additional supplements of chloral hydrate at intervals of approximately 30 to 45 minutes. We carried out experiments of four organs including brain, intestine, ovary and bladder. The rats in each group were evenly divided into three sub-groups: (a) shock group: the rats received surgeries, induction of hemorrhagic shock and blood resuscitation; (b) control group: the rats undertook surgeries without inducing shock; (c) death group: the rats received surgeries as well as the induction of shock without resuscitation.

2.2  | The shock models

We established two rat shock models including the standard one and the rapid one. We evaluated the cerebral hemodynamics with standard shock model. However, considering that the internal organs cannot be exposed to the air for a long time, we only carried out experiments for internal organs based on the rapid shock model. For both models, Two PE-50 cannulas were inserted into the femoral arteries on both sides. One cannula was connected to a pressure transducer to monitor the mean arterial pressure using a multi-parametric physiological recording device (RM6240, Chengdu Instrument Inc., Chengdu, China). We extracted blood from the other cannula to induce hemorrhagic shock. One more cannula was inserted into the femoral vein on the right side to infuse blood mixed with heparin sodium solution. A feedback-controlled heating pad was used to maintain the body temperature at $37.0 \pm 0.5^\circ\text{C}$ over the entire course of the experiment. For brain imaging, we did craniotomies to remove both the scalp and skull. The dura was kept intact and continually bathed with artificial cerebrospinal fluid. For experiments of abdominal organs, we expose the organ with minimal invasive incision to avoid unnecessary exposure of other organs.

For the experiments with the standard shock model, we carried out full-view imaging of the brain with an interval of 2 minutes within 10 minutes prior to the extraction of blood. Then, the blood was extracted with a constant speed from the femoral vein using a syringe pump to target a mean arterial pressure of 40 mm Hg within 15 minutes. We captured 10 more images during bleeding. Hypovolemia was maintained for additional 1 hour and we performed 18 experiments to monitor the microvascular hemodynamics. In the stage of resuscitation, the blood was reinfused via femoral vein and additional six images were captured within 20 minutes. We spent another 80 minutes to monitor the rat with 24 experiments to monitor the microvascular hemodynamics post resuscitation. For the rapid model, we firstly carried out five full-view imaging experiments as the resting state within 5 minutes, then extracted the blood with a constant velocity and continued to image the microvascular hemodynamics every minute until the arterial pressure dropped to 40 mm Hg within another 5 minutes. We imaged the same region (15 images) when the arterial pressure was maintained around 40 mm Hg for additional 15 minutes. After that, the blood resuscitation (5 images) was carried out within 5 minutes. Post the resuscitation, we carried out 15 experiments to monitor the microvascular hemodynamics until the arterial pressure return to a stable level.

2.3  | The imaging system

Supplementary Figure S1, Supporting Information presents the configuration of the system, which is detailed described in Supplementary Section I. Figure 1A shows the photograph of the system integrating all the optical and electrical components inside a movable cart. The probe highlighted in Figure 1B has an outer size of $22 \times 30 \times 13 \text{ mm}^3$, a weight of 20 g, a lateral resolution of 3.8 $\mu\text{m}$ (Supplementary Figure S1B), an axial resolution of 104 $\mu\text{m}$ (Supplementary Figure 1C) and a frame rate of up to 2 Hz with a frame size of 500 $\times$ 500 pixels in a $2 \times 2 \text{ mm}^2$ imaging domain.

2.4  | Image reconstruction

The raw photoacoustic signals were filtered using a bandpass filter (5-20 MHz) to remove both low-frequency and high-frequency electromagnetic noise. All depth-resolved A-lines were processed using a Hilbert transform and directly back-projected to a rectangular coordinate. We showed all
volumetric data through projecting the maximal amplitude of each photoacoustic signal (MAP). The movie was performed using Matlab (MathWorks Inc., Natick, Massachusetts) by stacking MAP images captured at different time points. We derived the concentration of total hemoglobin (C_HbT), number of blood vessels (VN), vascular density of small blood vessels (SVD) and vascular diameter (VD) to assess the hemorrhagic shock and associated blood resuscitation using a home-developed algorithm described in Supplementary Section II.

2.5 | Statistical analysis

We carried out the graphic display of data and statistical analysis using Graphpad Prism 6 (San Diego, California), and reported the values as mean ± standard deviation (SD). Kolmogorov-Smirnov test was used to confirm normal distribution of experimental data. The parametric test (unpaired t test) and nonparametric test (Mann-Whitney U test) were carried out to do comparison between time-based experimental data within each group. Two-way ANOVA (Tukey’s multiple comparisons test) was performed between shock-normal, shock-death groups for each organ to compare relative microvascular hemodynamics. We consider $P < 0.05$ as statistically significant.

2.6 | The human experiments

We imaged the tongues and lips of three male volunteers. All volunteers wore the protection glass to avoid potential laser exposure to the eyes. The lips and tongues were attached to the imaging interface via drinkable water. After the experiments, dentists continued to examine the imaged area for 7 days and no abnormal symptom was observed. We have obtained the written consent forms from all the volunteers participating in the experiments.

3 | RESULTS

Figure 1C shows a photograph of a volunteer participating in the human experiment, in which an operator holds the
probe and images the region of interest in the oral cavity. Figure 1D,E present the MAP images of microvasculature located on the superficial surfaces of the upper lip and hypogloeeis. A large number of blood vessels forming an ultra-dense microvascular network are clearly visualized. On the top surface of the buccal mucosa, there exists many capillary loops which change at the beginning of hemorrhagic shock.

Figure 2 shows the MAP images of a typical rat brain obtained at different stages of standard hemorrhagic shock. Figure 2A shows the photoacoustic image of cerebral cortex prior to bleeding, referred to as the baseline. Figure 2B,C present the cerebral hemodynamics of the rat with the loss of 20% and 35% of the total blood volume, respectively. Due to insufficient supply of blood, $C_{\text{HbT}}$ declines to 55% and 35% of the baseline (Figure 3), respectively. Post bleeding, as shown in Figures 2D,E and 3, $C_{\text{HbT}}$ slightly recovers to 60% of the baseline due to the compensated perfusion by autoregulation mechanism of the body and then declines to 35% of the normal status because of the failure in compensatory mechanism. The close-up views of sub-region indicated by white dashed boxes in Figure 2C-E are presented in the bottom of Figure 2, marked by I, II and III. The quantitated photoacoustic intensities of a selected blood vessel reveal the compensatory mechanism. Figure 2F-H shows the recovery of the microcirculation during and post the procedure of blood resuscitation. Owning to the blood infusion, $C_{\text{HbT}}$ gradually return to the baseline. Figure 2I shows the change of the same blood vessel, marked by blue, green and red dashes, respectively. We can see that there is limited change of vessel diameter, but significant change of photoacoustic amplitude after bleeding.

We notice that VN, SVD and VD changed mildly while the $C_{\text{HbT}}$ has a more intense change over the entire course of hemorrhagic shock and blood resuscitation (Figure 3). VN, SVD and $C_{\text{HbT}}$ show a consistent change tendency within the entire course of hemorrhagic shock, while the recovery of vessel diameter is not obviously. It is possible that blood redistribution and vasodilatation, which change the diameter of blood vessels, is much more complicated than VN, SVD and $C_{\text{HbT}}$. Visualization 1 depicts the microvascular kinetics over the entire period of the hemorrhagic shock and resuscitation.

Figure 4 presents the hemodynamics in the microcirculation of intestine, ovary and bladder. Visualization 2 shows the longitudinal monitoring of the microvascular hemodynamics of a selected intestine, ovary and bladder over the entire course of experiments. We selected five images at the stages of baseline, bleeding, shock, blood infusion and postinfusion. We observe more intense changes of microvascular morphology compared with that of cerebral cortex. For example, with the loss of blood, a large amount of small blood vessels disappear. In
FIGURE 3  Quantitative analysis of functional and morphological features of the cerebral cortex over the entire course of hemorrhagic shock. Relative changes of vascular number (A), small vascular density (B), total hemoglobin concentration (C) and vessel diameter (D). We normalized all the data for each parameter and show the data at stages of baseline (0-10 minutes), rapid bleeding (10-25 minutes), compensatory (25-35 minutes), decompensatory (35-85 minutes), rapid blood infusion (85-105 minutes) and postresuscitation (105-185 minutes). \( P < 0.05: *; \ P < 0.01: **; \ P < 0.001: ***; \ P < 0.0001: ****\)

FIGURE 4  Microvascular hemodynamics of internal organs during hemorrhagic shock and blood resuscitation. A, Five selected MAP images of an intestine at stages of baseline, bleeding, shock, infusion and post infusion. B, Five selected MAP images of an ovary at stages of baseline, bleeding, shock, infusion and post infusion. C, Five selected MAP images of a bladder at stages of baseline, bleeding, shock, infusion and post infusion. Yellow rows indicate typical vessels in different organs, which shrink with the loss of blood and recover post the resuscitation. Scale bar: 400 \( \mu m \)
addition, the vasoconstriction of large vessels indicated by the yellow arrows is significant. Furthermore, we find that the loss of blood will result in the shrink of the organs and further lead to the slight motion of the imaging domain at different stages. It is worth to notice that, organs could not fully recover after the blood perfusion within a short time. Apart from the microvascular hemodynamics in the abdominal organs, there is no loss of the small vessels and limited morphological change of large and medium vessels in the cerebral cortex during the entire course of bleeding and infusion.

Figure 5 shows the microvascular hemodynamics of control and death groups. In the control group, there is no obvious change of microcirculation. Visualization 3 shows the microvascular hemodynamics of a representative intestine, ovary and bladder during the experiments in the control groups, respectively. In contrast, there is no recovery of vascular hemodynamics in the death group. Visualization 4 presents the longitudinal data of a representative intestine, ovary and bladder in the death groups, respectively.

Figure 6 shows the quantitative analysis of \( C_{HBT} \), VN, SVD and VD of the abdominal organs in different groups. In
control groups, all the derived parameters slightly decline due to the long-time exposure of organs to the air, and do not return to the base line after the infusion of blood. The parameters of both shock and death groups have the similar variation tendency at the stages of baseline, bleeding and shock. As we expected, the functions of vasculature partially return to normal status after the blood resuscitation in shock groups, while, the organs of death groups fail to recover due to the severe loss of blood. Supplementary Tables S1 and S2 summarize the statistical analysis of the data in shock-control groups and shock-death groups.

4 | DISCUSSION

Generally, a typical hemorrhagic shock includes three major stages: compensatory-, decompensatory- and refractory-stage [8, 11, 20]. In compensatory-stage, abnormal bleeding triggers the auto-regulatory mechanism of the body, which redistributes the blood in microvasculature and peripheral organs to maintain the minimum perfusion of vital organs. In decompensatory-stage, the microvascular congestion of organs leads to the failure of auto-regulatory mechanism and potential damage to vital organs. In the last stage featuring refractory hypotension and failure of microcirculation, the body will suffer from irreversible cell damage, organ disability and even death. The cerebral data clearly presents the suddenly rise in $C_{HbT}$ after the termination of bleeding, which represents the occurrence of compensating mechanism of the body. In addition, we observe slight vasoconstriction of vessels, which possibly aims to moderate the systemic arterial pressure by increasing systemic vascular resistance [21]. However, we note that the vessel number and small vascular density do not variate dramatically during the entire course of hemorrhagic shock and blood resuscitation. Evidence indicating that the cerebral flow is redirected from peripheral organs to the brain has been documented [22]. Furthermore, the unique response of cerebral microcirculation to hemorrhagic shock, in which microcirculatory flow is sustained at essentially normal levels, has been reported [8, 11, 23]. Benefiting from the principle of photoacoustic effect which is ultrasensitive and positively proportional to the concentration of total hemoglobin at 532 nm, we clearly observe significantly decline of $C_{HbT}$ at the stages of bleeding and shock. Even the auto-regulatory mechanism partially compensates the decline of $C_{HbT}$ and protects the
cerebral tissue from ischemic injury, \( C_{\text{HBT}} \) will eventually decline due to the insufficient supply of blood.

Apart from cerebral microcirculations, abdominal organs including intestine, ovary and bladder, which are less essential than brain, have much more significant microvascular hemodynamics during the entire course of hemorrhagic shock and resuscitation. Similar to cerebral microcirculation, \( C_{\text{HBT}} \) declines with bleeding and recovers with infusion. However, we observe the disappearance of small blood vessels, especially capillaries, within the stages of bleeding and shock, which is totally different from the cerebral hemodynamics. In addition, the vasoconstriction of the vessels in abdominal organs is more obvious than that of cerebral cortex. Interestingly, we notice that intestine and ovary are capable of fully recovering, while bladder cannot return to the normal status even at several hours post the blood infusion. The major reasons may include: (a) the importance of abdominal organs is different resulting in the diverse response to resuscitation; (b) the response speed of each organ is variated.

In human experiments, we observe ultra-dense vascular networks formed by large, medium and small vessels as well as capillary loops which connects the venule and arteriole, will change at the early stage of bleeding and is tightly associated with the severity of hemorrhagic shock. Successful visualization of human buccal microcirculation in three-dimensional with capillary-level resolving capability reveals the great clinical translational potential of this technique.

Although our study is encouraging, we still recognize some important limitations in the experimental method and the interpretation of our findings. Firstly, the use of single wavelength illumination only enables the derivation of limited parameters which are insufficient to evaluate hemorrhagic shock in clinic. However, this is not the fundamental issue of photoacoustic. Through the utilization of multispectral strategy and acoustic Doppler effect, it is feasible to estimate the concentrations of oxygen- and dioxygen- hemoglobin, oxygen saturation and blood flow, making the assessment more accurate [24, 25]. Secondly, the imaging depth is still limited by both the transparency of tissue and excitation wavelength. With tissue clearing techniques [26] and near-infrared illumination [27], we will observe more blood vessels in deep locations. Thirdly, it is hard to explain how the responses of peripheral organs to hemorrhagic shock or ischemia are different based on current data. In further studies, other optical imaging techniques such as optical coherence tomography (OCT), laser speckle imaging et al. should be combined with the proposed techniques to derive more functional, morphological and molecular parameters for further assessment of organ response to hemorrhagic shock and fluid resuscitation. Last, it is feasible to increase the imaging speed by using a faster scanner, making it possible to capture fast events of microcirculations during hemorrhagic shock.

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**Author contributions**

L.X. conceived the concept, proposed the experimental plans, supported and supervised the project. L.X., Q.C., H.G., X.C. and T.J. built the system, acquired and processed the data. W.Q. and H.G. provided the assistance in operation, assembly of the MEMS scanner. Q.G., L.Y. and B.K. assisted the establishment of the disease model. L.X., Q.C. and W.Q. prepared the manuscript.

**ORCID**

Qian Chen https://orcid.org/0000-0002-0815-5336

Lei Xi https://orcid.org/0000-0002-2598-6801

**REFERENCES**


SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1 The schematic of the system configurations and the ultracompact imaging probe (A). Estimation of the lateral (B) and axial (C) resolutions of the system. PC, personal computer; OL, objective; DAQ, data acquisition card; Amp, amplifier; PH, pinhole; L, convex lens; SMF, single mode fiber; CL, collimation lens; DL, doublet lens; P, right-angle prism; MEMS, micro-electro-mechanical system; GC, glass cover; FG, function generator (motor control); T, transducer.

Figure S2 The flowchart of imaging postprocessing.

Table S1 Statistical analysis of derived parameters between shock and control groups.

Table S2 Statistical analysis of derived parameters between shock and death groups.

Video S1 The longitudinal monitoring of rat cerebral cortex and recording of mean arterial pressure during the entire course of hemorrhagic shock and blood resuscitation within 3 hours.

Video S2 The longitudinal monitoring of three representative internal organs and recording of mean arterial pressure during the entire course of hemorrhagic shock and blood resuscitation within 45 minutes.

Video S3 The longitudinal monitoring of three representative internal organs and recording of mean arterial pressure in the control group within 45 minutes.

Video S4 The longitudinal monitoring of three representative internal organs and recording of mean arterial pressure in the death group within 45 minutes.

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