A flexible optoacoustic blood stethoscope for non-invasive multiparametric cardiovascular monitoring

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Article

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Abstract

Quantitative and multiparametric blood analysis is of great clinical importance in cardiovascular disease diagnosis. Current blood analysis methods are typically restricted to invasiveness, bulky instruments and complicated clinical procedures. Wearable devices can realize on-skin assessment of several vital signals but generally exhibit very limited blood analyzing functions. Here, we report a flexible optoacoustic blood stethoscope (OBS) for multiparametric cardiovascular monitoring, bypassing the need for invasive procedures. The device features the light delivery elements for blood illumination and the piezoelectric acoustic elements capable of capturing the light-induced acoustic waves. This flexible device can adhere to the skin for continuous and non-invasive in-situ monitoring of multiple cardiovascular biomarkers, including hypoxia, drug concentration decay, and hemodynamics, which can be further visualized with the tailored 3D algorithm. Demonstrations on both *in-vivo* animal trials and human subjects highlight the OBS's unique potential to provide a new avenue for cardiovascular disease diagnosis and prediction.

Introduction

Multifunctional personal healthcare at the wearable level is the key to advancing the next generation of precision medicine. Currently, by collecting a broad spectrum of physical or chemical information from superficial skin, an emerging class of flexible wearable devices showcase their ability for in-situ analysis of body temperature¹, surface electrophysiological signals², sweat^{3,4}, saliva⁴, etc. Wearable monitoring of clinically significant physiological signatures from deeply embedded blood vessels, however, remains a challenge. Blood carries abundant clinical information but is challenging to be accessed non-invasively in the presence of a skin barrier. It is of great significance to directly measure multiple physical and chemical blood dynamics properties for early detection or treatment of cardiovascular diseases^{5,6}. For example, continuous blood oxygen saturation assessment, especially on patients, elders, and infants, can help to reflect the patient's oxygen supply capacity⁷; the tracking of chemical compositions, e.g. drug concentration in blood, offers meaningful pharmacokinetic information⁸; furthermore, the hemodynamics analysis can be harnessed for cardiovascular disease diagnosis⁹.

With the advances of optoelectronics, the optical sensors can be crafted into flexible and thin films, thereby allowing conformal adhesion to the skin for wearable purpose^{10,11}. Therefore, many optical-based non-invasive blood measurements devices are utilized for characterizing cardiovascular-related parameters, including oxygen saturation¹², glucose level¹³, blood pressure¹⁴ and blood flow¹⁵. However, due to the rapid photon diffusion and scattering inside human tissues¹⁶, optical solutions can only statistically read out the light radiation region's gross physiological information but fail to resolve any specific blood vessel in deep tissue. Similarly, the detection of the blood flow, based on thermal transportation properties^{17,18}, for skin physiology investigation only enables access to the superficial vasculature. Besides the above optical solutions, flexible vibration sensors also can evaluate cardiovascular-related hemodynamics with the recorded pulse transmitted from the blood vessels. These kinds of vibration receivers have been well miniaturized and made skin-conformable^{19,20}; and one

particular design can realize deep vascular measurement by adopting active ultrasound testing ²¹. Benefited from the versatile directivity and penetration ability of ultrasound waves, such an active sensor can focus its region of interest (ROI) for locating a single blood vessel in deep tissue. Nevertheless, vibration is only sensitive to mechanical properties and unable to perceive the variation in blood physiological biomarkers. Other techniques like electrochemical method ²² perform non-invasive epidermal sensing of external bodily fluids like sweat, tears, saliva to infer the blood parameters indirectly. Still, the composition of external fluids is only partially dependent on the blood ²³. In short, current blood sensors are unable to locate the blood vessels underneath the skin to provide direct information of distributed blood properties. Beyond that, they can only offer single physiological vital measurement, capping their capability towards comprehensive, multi-dimensional assessment of cardiovascular health status.

Here, we present a flexible optoacoustic blood stethoscope (OBS), non-invasive and skin-conformal (Fig. 1a), for 3D multiparametric cardiovascular monitoring. The OBS can detect multiple physiological vital signs from the 3D blood vessel model reconstructed with our tailored algorithm to provide remarkable insight into cardiovascular functions from various aspects. The device is rooted from the optoacoustic (OA) effect ²⁴: it delivers light into blood, triggering mechanical vibration of blood chromophore, and receives the blood signal in the form of acoustic waves. The OBS comprises a micro-lens array with pre-drilled holes for light delivery and an alternant piezoelectric sensor array as the acoustic receiver. The name of the device, blood stethoscope, is derived from its rationale, where the acoustic waves are 'heard' for characterizing the blood properties. The reconstruction algorithm was also developed for imaging blood vessels buried in heterogeneous layered human skin. Most of all, the designed OBS is made skin-conformal, as a fundamental advancement over any other existing OA implementations. *In-vivo* animal experiments and on-body human trials demonstrate that the OBS could non-invasively locate the blood vessels and monitor rich blood signatures directly correlated to multiple cardiovascular conditions, such as hemodynamics, hypoxia and drug metabolism. The OBS provides an unprecedented solution to measure venous distensibility and arterial endothelial function for cardiovascular study. Our proposed conformal scheme avoids complex fixture setup in the traditional methods and is more versatile and accurate for cardiovascular diseases prediction and diagnosis.

Results

The OBS device is made lightweight and skin-conformal. It integrates multi-layered optical delivery and acoustic receiving elements with dense interconnections and array electrodes (see Methods and Supplementary Fig. 1,2 for detailed fabrication processes). Our OBS comprises 25 optical and 36 acoustic nodes for precisely positioning a single blood vessel, with its stack structure exploded in Fig. 1b. Polydimethylsiloxane (PDMS)-fabricated convex micro-lens array works as the optical focusing component to deliver light, and a piezoelectric polyvinylidene fluoride (PVDF) film is implemented as the acoustic receiver. All the layers are soft and bendable under external strain (Fig. 1c), and the OBS can conformally contact on hand, wrist, or any other part of the body (Fig. 1d). In the optical and acoustic

node design, each optical node is surrounded by four acoustic nodes, and each acoustic node has four light focal spots around its corners. This cross-distributed design of the nodes enables efficiently light-acoustic coupling (Fig. 1e). The light illuminating the OBS will be focused into small spots with the optical node (Fig. 1f) and pass it through the pre-drilled holes in the laminated films, then it penetrates human skin and stimulates the ultrasonic emission from the blood, and finally, the light-induced ultrasound waves are captured by the acoustic receiving nodes (Fig. 1g). The OBS is versatile to monitor and image various blood characteristics by targeting different blood vessels or selecting different light wavelengths.

PVDF is selected as the piezoelectric layer in the acoustic sensor for two reasons. Firstly, the material is soft by itself, while other bulk piezoelectric borrowing flexibility from the soft substrates, named island-bridge structure, is complex to fabricate²¹. Most island-bridge structures require wide inter-node spacing for the bending-supported soft substrates. Therefore the receiving sensors are dispersed distributed, leading to low efficiency in the area utilization both acoustic-wisely and optical-wisely. Secondly, PVDF is excellent in ultrasound receiving due to its high piezo-stress constant and low acoustic impedance. In traditional ultrasound applications, PVDF is relatively weak in ultrasound emission compared with other bulk piezoelectric materials. Still, for our applications, unlike the ultrasound actuator, the ultrasound waves are excited by laser illumination, and PVDF is only responsible for acoustic receiving. Hence, PVDF is highly suited for the OBS's design therein. The metal electrodes patterned on the PVDF are made from silver, serves as a main conductive layer that provides excellent electrical characteristics while reflecting light out of the aperture. Thus, it could reduce the undesired thermal damage from redundant illumination on electrodes and other polymer layers. PDMS is regarded as the most appropriate material for the micro-lens array due to its easy-to-mould capability and high transparency in a wide range of the spectrum (400–1100 nm, transmittance > 90%)²⁵.

Optical and acoustic elements design and characterization. The OBS requires an optical element for light delivery and an acoustic element for detecting stimulated ultrasound waves from blood. The optimal functioning of the OBS requires a dedicated design of the optical and acoustic elements. Unfortunately, the tradeoff in the area between these two elements should be carefully balanced for the best detecting efficiency: Increasing the acoustic receiving areas may block the light and reduce the amount to be delivered into tissue, while overgrowing the coverage of optical elements inevitably degrades the acoustic sensitivity and may lead to reconstruction artifacts. Traditionally, optoelectronic devices adopt transparent substrates and electrodes (ITO, indium tin oxide) for optimal light delivery. However, such a solution may severely deteriorate the bendability of OBS and the acoustic receiving performances due to its high resistance. Therefore, we propose an extraordinary micro-lens array with nested pre-drilled holes for light delivery in our OBS. The micro-lens array, featuring 5 x 5 hemispherical lenses made from PDMS, can focus incoming light and deliver it through pre-drilled holes into tissue. The thickness of the micro-lens array layer should be carefully determined, which affects the light delivery efficiency (Supplementary Note 1) and the acoustic characteristics and device flexibility. A thin layer cannot guarantee sufficient light delivery, while an excessively thick one will destroy the flexibility of the OBS. Benefiting from the high

transparency of PDMS, the total optical transmission coefficient of the OBS could reach up to more than 65% with a PDMS thickness of 500 μm , which is comparable to other piezoelectric devices with transparent electrodes (Supplementary Note 2). Our optical element design can well maintain excellent light transmission without deteriorating the piezoelectric sensor's acoustic sensitivity. For other transparent piezoelectric devices, increasing the piezoelectric layer thickness can enhance the acoustic sensitivity, but at the cost of reducing light transmission. Our design allows light delivery through a thicker piezoelectric layer via pre-drilled holes for an improved acoustic sensitivity without affecting light transmission. Similarly, the transparency of ITO electrodes is also determined by the electrode thicknesses. For 400 nm ITO, its transparency is close to our light delivery solution, while the electric resistance is up to several orders of magnitude higher than Ag. In our solutions, the Ag electrodes are 7 μm in thickness, and the interconnection is made of 10 μm silver paste to keep a low resistance in long-distance transmission. According to references^{26,27}, a considerable resistance not only induces a signal loss in circuit transmission but also constructs an output RC low-pass filter, as a side effect that causes the acoustic resolution deterioration.

For OBS, achieving uniformity of the delivered light beam in a very short distance is critical to avoid any additional pre-diffusion layer and thus maintain minimum thinness of the entire device. To show the superiority of our optical element design in the aspect of light delivery, Monte Carlo simulation was conducted to compare light fluence distributions of our micro-lens array method (Fig. 2a) with other mostly adopted schemes, including the side illumination method (Fig. 2b)^{28,29} and the center window method (Fig. 2c)³⁰. The light transmission medium is assumed to be dermis tissue with optical scattering and absorption coefficients of 357 cm^{-1} and 0.46 cm^{-1} , at 532 nm wavelength, respectively³¹. See Supplementary Note 3 for the detailed Monte Carlo simulation setup³². Due to the light diffusion and scattering, the light fluence uniformity quickly developed with the penetration depth. Nevertheless, neither the side illumination method nor the center window method could achieve a distribution uniformity with shallow penetration. To facilitate quantitative analysis, the region with a light intensity drop over 20 dB below the maximum is defined as the blind region at a specific depth. Here, at a depth of 1 mm (the typical depth for some superficial veins), both side illumination and center window methods have a large blind region under 532 nm light illumination (Fig. 2e,f); hence a light pre-diffusion layer is on-demand in these methods, which inevitably increases the device thickness³⁰. In contrast, our micro-lens array with multi-focusing capability brings about a relatively high light distribution uniformity, with the blind region only laying at the four corners, as shown in Fig. 2d. A similar comparison of light illumination pattern at 1000 nm wavelength can be found in Supplementary Fig. 3, and the percentage of the effective illumination areas at different depths for 532 nm and 1000 nm lights are illustrated in Supplementary Fig. 4. Our micro-lens array shows the highest effective light illumination area at various depths among the designs. For the broad spectrum from 532–1064 nm at 1 mm depth (Fig. 2g), the effective illumination area exceeds 90%, proving that our micro-lens array can realize more uniform and larger area light delivery than classical methods. Therefore, no additional pre-diffusion layer is required, and the device can be fabricated into a thin film. To experimentally demonstrate the excellent light uniformity of the micro-lens array on our OBS, a beam of light (633 nm) irradiated through the OBS (Fig. 2m), with one

layer (0.2 mm) (Fig. 2n) and two layers of papers (0.4 mm), as the optical diffusion medium, overlaid from the undersurface (Fig. 2o), respectively. The transmitted light pattern shows a uniform distribution behind a thin diffusion layer (Supplementary Note 4). Apart from this, a phantom absorption experiment verified that the OBS's capability for multi-spectral imaging (Supplementary Note 5).

The design principle of acoustic elements, which resembles the general rule of the ultrasound sensor array, includes evaluating the electromechanical characteristics (resonance and antiresonance frequencies), acoustic axial resolution, and acoustic field of view (AFOV). The mean resonance frequency and antiresonance frequency (Fig. 2k) are 7.64 MHz and 8.01 MHz with s.d. (standard deviation) of 38.4 kHz and 39.2 kHz, respectively. The centre frequencies of the acoustic sensors average 7.8 MHz (s.d. = 37.7 kHz) according to the phase angle spectra (Fig. 2l). The stable electromechanical performances indicate the good uniformity among the acoustic elements produced from a reliable fabrication process. The thickness of the PVDF used is 110 μm with a bare resonance frequency of 10 MHz, which is decreased with the coating layer of silver electrodes. A thinner silver electrode layer favours higher resonance frequency but may result in excess electrical signals loss during signal transmission³³. A multi-layered Manson's model (Supplementary Note 6) allows for pre-evaluation of the electrical impedance and phase angle, enabling the prefabricated optimization with the fine-tuned thickness of the electrode as well as other matching and backing layers. After integrating the acoustic element into the OBS system, the acoustic element's impulse responses could be characterized using a point-like black tape target. As shown in Fig. 2m, most of the centre frequencies peak around 7 ~ 8 MHz, and about 78% of sensors have a bandwidth of over 100%. The corresponding attainable axis resolution can be worked out to be 0.2 mm in tissues with a sound speed of 1500 m/s (Supplementary Note 7).

Similar to the criteria for optical illumination, the AFOV uniformity within the whole ROI should also be characterized (Supplementary Note 7). The receiving beam pattern of a single sensor at a depth of 1 mm is illustrated in Fig. 2n. The effective spatial response region (-6 dB) swiftly exceeds the sensor area of 2.28 mm² and reaches 5.5 mm². Profited from the small gaps (0.15 mm) between acoustic sensors, the superimposed AFOV of the whole OBS at depth 1 mm reveals a superb uniformity (Fig. 2o); the mean versus maximum acoustic amplitudes from the effective AFOV is about -6.5 dB with an s.d. of 2.3 dB which can be further reduced to 2 dB at a depth of 2 mm. Meanwhile, the small gap size also avoids the grating lobe induced artifacts. In addition to simulation, a bending test was conducted by sticking the sensors firmly onto different rigid curved surfaces (with a radius of 40 mm, 30 mm, and 20 mm, respectively) to compare the spectra of the received signal (Supplementary Fig. 5). The spectral results (Fig. 2p) suggest that the OBS can substantially maintain its receiving performance under these bending conditions.

3D imaging algorithm. The received raw data from OBS should be processed with the ultrasound image reconstruction methods before the blood physiological information extraction. The classical reconstruction method, based on the delay-and-sum (DAS) principle and implemented in time-space domain³⁴, is not suitable for skin conformal sensing and imaging in terms of both accuracy and efficiency. The fundamental misconception of the DAS-based method is that the skin should be

considered as a heterogeneous layered medium rather than a simple homogenous component. Each layer possesses different acoustic properties that cause a serpentine receiving path (Fig. 3a), making it challenging to accurately evaluate DAS's delay values. Moreover, as the acoustic element is bent and tightly attached to the non-planar skin surface, the path calculation will become even more complicated due to inter-sensor displacement. Also, to enhance the resolution of the blood vessels 3D structures, numbers of interpolations are required before rendering at a substantial computational cost in DAS-based reconstructions. Here, we propose a phase shift non-uniform Fast Fourier transform (PS-NUFFT) algorithm to reconstruct the 3D image in a general heterogeneous medium setup. This method decomposes the wave components and utilizes wavefield extrapolation to separately examine the propagation of each wave component (Fig. 3b), which avoids the calculation of complicated receiving path. Moreover, pre-migration technique³⁵ could be integrated into the PS-NUFFT algorithm to adapt to the curved acoustic elements distribution problem. The detailed derivation of the reconstruction method can be found in Supplementary Note 8. Thanks to its highly low-cost computational memory and complexity, the PS-NUFFT is eminently suited for our lightweight OBS device. In summary, the whole reconstruction process involves four main steps. First, the received raw data is converted into wavenumber domain with a 3D Fast Fourier transform as

$$P(k_x, k_y, z=0, \omega) = \frac{1}{8\pi^3} \iiint p(x, y, z=0, t) e^{-ik_x x} e^{-ik_y y} e^{i\omega t} dx dy dt, \quad (1)$$

where $p(x, y, z=0, t)$ is the pressures received by the acoustic element. Subsequently, the phase shift is used to extrapolate the wavefield onto the boundary of each layer

$$P(k_x, k_y, z=z_L, \omega) = P(k_x, k_y, z=z_L, \omega) \prod_{l=1}^{L-1} e^{ik_z(d_l)}, \quad z_L = \sum_{l=1}^{L-1} d_l, \quad l=1, 2, 3 \dots L-1, \quad (2)$$

where d_l represents the thickness of layer l . Then an integral transformation is implemented on the extrapolated wavefields with a non-uniform interpolation expressed as

$$P_l(k_x, k_y, k_z) dk_x dk_y dk_z = P(k_x, k_y, z=z_L, \omega(k_x, k_y, k_z)) c k_z / \sqrt{k_x^2 + k_y^2 + k_z^2} \Phi D_p dk_x dk_y d\omega, \quad (3)$$

where Φ is a sinc interpolation basis, D_p is a down-sampling function, and c is the sound speed of tissue. Finally, the 3D image is obtained by a 3D inverse Fast Fourier transform on $P_l(k_x, k_y, k_z)$ as

$$S(x, y, z) = \iiint P_l(k_x, k_y, k_z) e^{ik_x x} e^{ik_y y} e^{ik_z z} dk_x dk_y dk_z, \quad z \in [z_l, z_{l+1}], \quad (4)$$

where $S(x, y, z)$ represent the acoustic sources excited by the laser in the l -th layer. The completed acoustic sources are obtained from combining all the $S(x, y, z)$ in different layers. The total computational complexity for 3D imaging is $O(Ln^3\log_2(n^3))$, where L represents the total layer number. Since the number of the skin layers usually does not exceed 5, the overall computational complexity reduces below that of the DAS-based solution ($O(n^5)$) while achieving a much higher reconstruction resolution and accuracy in a compatible heterogeneous setup. Generally, the interpolation is performed to smooth out the sawtooth effect in low-resolution images. In PS-NUFFT, the final image can be conveniently interpolated by zero-padding the spectrum of k_x and k_y or control the down-sampling function D_p prior to the 3D inverse Fast Fourier transform; nevertheless, such operation barely imposes an additional computational burden.

A numerical simulation was run to verify the performance of the PS-NUFFT. The detailed simulation setup is described in Supplementary Note 9, and the acoustic source pattern was depicted in Fig. 3c. The images reconstructed by the DAS-based and our PS-NUFFT methods are shown in Fig. 3d and Fig. 3e. Clearly, PS-NUFFT reconstructed image shows a much higher resolution close to the sources. A quantitative comparison on the image resolution could be evaluated by slicing the data amplitude profile along Line-A for both images (Fig. 3f). Using full width half maximum (-6 dB) as the resolution metric, the resolution of a point source at $x = 3.5$ mm is 0.4 mm for the PS-NUFFT method and 1.5 mm for the DAS method, indicating our method outperforms the DAS method by 308%. Additionally, the PS-NUFFT outperforms the DAS method with a superior mean-square error (RMSE), 0.54 vs 0.93. Therefore, the accuracy of our method is about 1.6 times better than the DAS method (Fig. 3g). In completing such a 3D imaging task with a large data size of $256 \times 256 \times 300$ ($x \times y \times z$) pixels, our PS-NUFFT only consumes about 2 seconds on the image reconstruction with a lightweight laptop (ASUS 430UN, CPU8550U @ 1.8 GHz). In comparison, DAS spends 2 seconds only to obtain one piece of depth information and would cost nearly 600 seconds on the whole data set.

Hypoxia and drug concentration decay tests on animal model. The OBS with optimized optical and acoustic coupling can characterize blood physiological information from measured OA signals, which was first verified with in-situ hypoxia monitoring in mice. The principal function of blood is to deliver oxygen from alveoli to tissues. Consequently, the pathological hypoxia, likely due to COVID-19, will cause physical dysfunction and even directly endanger human life and personal safety³⁶. Therefore, monitoring hypoxia status should aid in the early detection and prevention of related physiological abnormalities. The hypoxia test was conducted with the OBS attaching on mice abdomen skin (Fig. 4a). The mouse was anesthetized first and then supplied with two types of oxygen-isoflurane mixture: A mixture dosage (oxygen 3 L/min, isoflurane 1%) can provide a normal oxygen condition, and another mixture dosage (oxygen 1 L/min, isoflurane 3%) can imitate an oxygen-poor condition (hypoxia). With the two types of the mixture supplied alternatively, the OBS continuously monitored the blood status of the mice under different conditions. Under the oxygen-poor condition, the hypoxia leads to lower oxygen saturation. Each hypoxia cycle takes about 10 mins, and the oxygen saturation continuously decreased within these periods. The OBS recorded such a pathological hypoxia change as the OA signal amplitude decreased by

24.4% $\pm$ 4.7%, as shown in Fig. 4b. Meanwhile, the OBS can provide 3D imaging of the blood hypoxia status (Supplementary Video 1) and give a quantitative mapping of the hypoxia region (Fig. 4c).

Besides hypoxia status, the drug concentration decay in blood vessels can also be monitored with the OBS. Tracking the drug concentration in the bloodstream demonstrates its importance in pharmacokinetics and pharmacodynamic analysis, contributing to optimizing the prescription of medicine dosage, dosing interval, and the suppression of the toxic side effects³⁷. Traditional drug concentration monitoring is based on the intermittent invasive blood test, while the OBS provides the feasibility of the approach for non-invasive and continuous monitoring of the decay. The mouse was anesthetized in the drug concentration tests, intravenously injected with 200 μ L Rhodamine B (2 mg/mL) from the tail as a label drug. After the drug is stably metabolized (about 1 hour after injection), the OBS was attached for continuously monitoring the mouse abdomen region, providing continuous 3D imaging of mouse blood vessels (Supplementary Video 2). The trend line of OA signals (Fig. 5d) captured a decay of Rhodamine B level in the blood, where the value was reduced by 28% at 2000 s. Meanwhile, during the metabolism, the drug was released from the blood into tissues. Such diffusion phenomenon could be visually discriminated from the photographs of mouse abdomen region, taken before and after drug injection (Supplementary Fig. 6); the mean intensity of the red colour (RGB mode) was elevated from 143 to 156 (colour scale 0-255) after injection. Generally, the decay rate of Rhodamine B in the tissues was much slower than that in the blood, and as a result, the trend line of tissue was reduced only 10% at 2000 s. Considering the tissue signal as the baseline, the decay of Rhodamine B in the blood conformed to the exponential decay curve with a decay coefficient of about -3.6×10^{-4} per second (Fig. 4e). For comparison, blood sampling was taken every 5 mins concurrently for the in vitro measurement of the Rhodamine B concentration with fluorescence tests (See Supplementary Note 10). The decay coefficient of Rhodamine B obtained from in vitro test was -3.2×10^{-4} per second, which was comparable to the OBS result (Fig. 4f). It is worth noting that the OBS measurement provides better R-square and root mean square (RMS). Moreover, the decay of Rhodamine B was discernible from the snapshot images at sequential time points (Fig. 4g).

In-Vivo human trial for cardiovascular care

a. Compliance for Veins

A venous system nearly houses 70% blood volume that significantly influences cardiac output by regulating blood flow back to the heart, which intensely relies on the mechanical properties of the venous wall. The compliance of the venous wall is a crucial indicator in the evaluation of cardiovascular status and predicting venous diseases³⁸. Some studies have shown that venous thrombus can increase the expression of MMP-9, a matrix remodelling protein known for its ability to lyse both elastin and collagen. However, MMP-9 is also responsible for degrading the extracellular matrix and basement membrane of the venous wall leading to a decrease in wall compliance of veins³⁹. In this work, before evaluating the properties of the venous wall, a set of blood perfusion test was employed to explore the venous reactions during vascular occlusion and release. The venous perfusion experiment was targeted at the human

dorsal venous network (Fig. 5a). After attaching the OBS to the dorsum of the hand (Fig. 5b), the vascular occlusion was initiated, and the complete experimental process was recorded in Supplementary Video 3. The amplitudes of the trend line measured from the venous OA signals were increased by 23% during vascular occlusions. The main reason is the inflation of the cuff worn on the arm blocked the return of blood, resulting in blood pooling in veins. After releasing the pressure, the trend line rapidly dropped. A sequence of snapshot 3D images (Fig. 5d) visually displays the amplitude variation of blood signals for the venous volume change's subsequential characterization. Such a phenomenon is cross-validated with ultrasound imaging (detail setup see Method) with a similar trend line denoting the evolution of the cross-sectional area of the vein (Supplementary Fig. 7 and Supplementary Video 4).

A further quantitative experiment was conducted to explore the venous volume change under different occlusion pressures. For inducing adequate vasodilation, the cuff was worn at the upper arm upright of the subjects ($n = 3$) as described in Method. The occlusion pressure was adjusted from 110 mmHg to 0 mmHg with several step release (30 mmHg decrement in the first three releases and 20 mmHg for the last decrement) (Fig. 5e). According to reference ⁴⁰, the typical venous pressure of the dorsal hand vein was about 30 mmHg for the upright sitting posture (Supplementary Fig. 14). To prevent complete flattening of the blood vessels during measurement, only amplitude ratios recorded at occlusion pressure of > 40 mmHg were considered valid; the amplitude ratios 102%, 108% and 114% were obtained from the conditions where the occlusion pressures are 50 mmHg, 80 mmHg, 110 mmHg, respectively. Such a decreasing trend was consistent with the venous volume measured with ultrasound imaging but reduced errors (Fig. 5f). Since the amplitudes of recorded acoustic signals were proportional to the cross-sectional area of the vein as mentioned in the perfusion test above, it could be used to estimate the vena cava volume versus transmural pressure ^{38,41}. The compliances of veins ($\Delta R/\Delta P$, R: amplitude ratio, P: pressure) were about $0.196\% \text{ mmHg}^{-1}$ and $0.185\% \text{ mmHg}^{-1}$ at ranges of 50–80 mmHg and 80–110 mmHg, respectively in our experiment. Measured with venous-occlusion plethysmography (VOP) ⁴² setup, venous compliance is prone to be overestimated; this is because the approach focuses not solely on venous but is also susceptible to the volume change in the other tissues and blood vessels including the arterioles and capillaries. Ultrasound imaging is another alternative solution to directly observe the venous volume changes, but the complex proximal supporting skeleton and bulky setup are incompatible for long term monitoring. Our OBS demonstrated its promise of becoming a paradigm-shift technology for venous diseases care due to its positioning capability for specific veins.

b. Flow-mediated Dilation for Artery

Human arterial hemodynamic studies have enormous implications for cardiovascular diseases prediction and diagnose. Diseases like atherosclerosis, stroke, and thrombosis could be directly or indirectly reflected from the blood pressures, blood flow, shear stress in hemodynamic analysis ^{43–45}. Several cardiovascular-related parameters of the artery, including the flow-mediated dilation (FMD), were measured with OBS in this study. Firstly, an arterial perfusion test set was employed to study the arterial reactions on the radial artery (Fig. 6a, b). Contrary to the veins experiment, the amplitudes of arterial signals dropped 37% during the vascular occlusion since the blood flows into the wrist was blocked

(Supplementary Video 5). Correspondingly, upon releasing the pressure, the blood flushed into the radial artery. The sudden influx of blood immediately recovered the signal amplitudes and even above their baselines for a short period (Fig. 6c,d). The trend line was also further compared with medical ultrasound results, coinciding with a similar curve pattern (Supplementary Fig. 8 and Supplementary Video 6).

Many studies have revealed the endothelial dysfunction is deleterious and implicated as a key factor in the initiation and progression of the atherogenic process that underlies coronary artery disease, peripheral ischemia, and some forms of stroke ⁴⁶. The FMD has been widely used as a medical standard to examine endothelial dysfunction for predicting cardiovascular risks ⁴⁷. Classical FMD tests utilize ultrasound imaging to continuously track the maximum diameter variations within one minute after the occlusion release. The low contrast of ultrasound and the high sensitivity to the measurement position heavily relies on bulky auxiliary fixtures of the ultrasound system to eliminate the motion-induced measurement error ⁴⁸ (Supplementary Fig. 9). Taking the advantages of OA effect, our OBS simplifies the measurement system while keeping pertinent specificity. Figure 6e - g shows the typical evolution of OA signals during the FMD tests ($n = 3$), where Peak A denotes the maximum vascular volume within one minute after the release of the cuff. The relative signal changes p_A were + 16.3%, + 15.5% and + 9.3% at Peak A, respectively. Since the signal amplitudes are approximately proportional to the vascular volume changing in a small range, the OBS estimated vascular diameter changes

$$(\sqrt{1 + p_A}) -$$

were 7.8%, 7.2% and 4.6%, which were also consistent with the ultrasound imaging result of 8%, 7.1% and 5.6% in Fig. 6h (Supplementary Fig. 10 and Supplementary Video 7). Note that, a typical FMD value is around 6.7% for healthy people in the clinic, and every 1% of the decrease in FMD implicates a 12% increase of hazard ratio for cardiovascular system ⁴⁹.

Discussion And Conclusion

We have reported a flexible optoacoustic blood stethoscope for multiparametric cardiovascular care and verified its applications in monitoring hypoxia, drug concentration decay, and measuring cardiovascular-related critical hemodynamical parameters. Taking advantage of optical spectral specificities and acoustic high resolution, the OBS allows imaging to analyze multiple physiological vitals of blood quantitatively. Besides, the flexibility and bendability enable the device to attach conformally to human skin for physiological monitoring. 3D imaging provides a new perspective for the quantitative and specific studies of the prediction of cardiovascular disease.

The applications of the OBS are not only confined to the aspects discussed in this paper. By selecting a specific or a combination of laser wavelength, the probe can also be potentially extended to measure pH values ⁵⁰, blood glucose ⁵¹, blood temperature ⁵², and even monitoring the treatment of tumours ⁵³. Moreover, multi-wavelength measurement provides rich characteristic parameters to decouple the multi-variable estimation. In other words, the OBS has the potential to monitor and analyze the multiple blood

parameters simultaneously. Since the receiving elements are made of piezoelectrical materials, the OBS could be considered a 2D array transducer for 3D ultrasonic imaging. Compared with traditional ultrasonic imaging systems, the OBS can passively excite ultrasound waves by collecting the light diffused and reflected from the skin structure, without any ultrasound transmitting systems. Such a phenomenon, referred to as passive ultrasound^{54, 55}, is observed in our experiments (Supplementary Fig. 11). Future studies will focus on further improvement of device performance by integrating denser acoustic and optical elements, incorporating LED and laser diode-based light sources, compacting acquisition circuits and wireless transmissions system based on system-on-a-programmable-chip, for distributed, mobile, and real-time monitoring of animal and human from various aspects. Also, further clinical validation may broaden the applications of the OBS to accelerate the development of mobile medical, as well as to advance current state-of-the-art clinical practices for prevention and investigation of cardiovascular-related diseases.

Methods

Fabrication of the flexible optoacoustic blood stethoscope

The piezoelectric acoustic element was fabricated from a 110 μm -thick polyvinylidene difluoride (PVDF) film (Measurements Specialties Inc, TE Connectivity Ltd). After cutting the PVDF film into the desired size, the thin films were rinsed in ethanol in the ultrasonic bath for 20 minutes and subsequently rinsed in DI water for cleaning. After the PVDF film was wholly dried in the air, the first layer of top electrodes was screen printed with a 7 μm silver paste layer (Qingdao Poten Technology Co., Ltd) patterned by a shadow mask (designed by AutoCAD software). Then a UV curable dielectric ink (Jelcon IN-15M JUJO Chemical Co, LTD) was screen-printed on the top layer as insulation and cured with 120W/cm UV lamps for 10 mins. The second layer of the top electrodes, which serves as the output wiring for later measurements, was screen-printed on top of the insulation layer with 10 μm silver paste. Following the top layer fabrication, a bottom electrode layer of 7 μm silver was deposited with screen printing. The pre-drilled holes on the acoustic sensor were pattern-cut with an electronic cutting machine (Silhouette Cameo).

The micro-lens array was manufactured by casting a mixture of PDMS elastomer and crosslinker (Sylgard 184) in 10:1(w/w) ratio in the micromachining-fabricated mould (Computer numerical control) and then spin-coated at 250 rpm/min (EZ4 Spin Coater, Lebo Science). After that, the mixture was degassed in a vacuum oven and cured at 60 °C for 6 hours. Next, the micro-lens array was peeled off from the mould. After that, the micro-lens array was mounted on top of the as-fabricated acoustic sensor array and secured with deposition of PDMS precursor over its edge. Two vertical interconnect access (VIA) holes filled with conductive silver paste on each side of the device are utilized to route the bottom electrodes to the top plane before the final PDMS encapsulation.

Light source system

The schematic of the light source system setup is illustrated in Supplementary Fig. 12. A tunable laser (Radiant 532LD, Opotek Ltd, UK) was used to generate light pulses at point **a** (5-7 ns pulse width, 1-20 mJ energy, and tunable 680-2600 nm wavelength). Meanwhile, the laser pulses with a base wavelength at 532nm were excited from point **b**. The optical paths of tunable and base light sources were merged by a dichroic mirror (HGMP-650-D25, Hengyang Optics Ltd, China) placed at position **c**. The output of **c** is free space light that could be directly used for imaging, while for wearable applications, a fibre-coupled light source is preferred. A short-focus convex lens (LB1761, Thorlabs, US) was placed at position **d** to focus the free space light into the multi-mode fibre bundle (BF20LSMA02, Thorlabs, US). Finally, the fibre-coupled light was guided and collimated through a customized parabolic mirror, providing broad and well-patterned illumination at the output port of the adaptive mirror holder, which can be conveniently attached to the OBS optical element.

Data Acquisition System

The data acquisition system to read out electrical signals of the OBS is shown in Supplementary Fig. 13. The system consists of customized 36-channel two-stage pre-amplifiers (MAX4805A, maxim integrated, and TLV3544, Texas Instruments), analogue multiplexers (TMUX1108, Texas Instruments), a low-noise amplifier (5072PR, Olympus), an oscilloscope (Lecroy), a multi-channel data acquisition box (SonixDAQ) and an FPGA board (Minized, AVENT). The FPGA board functioned as a lower computer responsible for coordinating the laser, data acquisition box, oscilloscope, and multiplexers to realize channel switching. After the laser illuminated the biological tissue, the laser-induced ultrasound signals would be received by OBS and then amplified by the two-stage pre-amplifiers. The two-stage amplifiers were fabricated on a flexible substrate for directly bonding to the OBS without introducing any interconnection cable to minimize the possibly additional imposed interference and signal loss. A total gain of about 30 dB is provided to ensure a sufficient signal intensity to confront the loss and noise in long-distance signal transmission. The multiplexers selected one specific channel, supplied with an additional gain of 30-50 dB (5072PR amplifier), for convenient waveform display and monitoring. At the same time, the multi-channel data acquisition box recorded all the data channels for imaging. The oscilloscope converted the single-channel analogue signal into a digital form with a 100 MHz sampling rate and sent it to the laptop for real-time display. Meanwhile, the data acquired by the data acquisition box were also amplified with a gain of 35-51 dB and sampled with a 40 MHz sampling rate. After that, these data were sent to the laptop via USB, processed with the PS-NUFFT reconstruction method, and was finally post-rendered for the final 3D display.

Animal Procedures

4 Nude mice (CrTac: NCr-Foxn1nu, Male, 6-8 weeks, Invivos) were involved for oxygen saturation and drug concentration decay tests. The mouse was fixed in an animal holder with a gas-supply apparatus mounted on the head. The apparatus was supplied by the anaesthesia machine (R540IE, BIOSEB) with a mixture of oxygen and isoflurane. The concentration of oxygen inhaled was controlled by adjusting the flow rate ratio of oxygen and isoflurane. A heating pad was placed under the mouse to maintain its body

temperature, and the ultrasound gel was applied to ensure efficient acoustic coupling between the skin and the OBS receiver. All animal procedures and cares were executed under protocols approved by the Nanyang Technological University Institutional Animal Care and Use Committee (IACUC) per federally approved guidelines.

Human Trial Protocols

Perfusion Experiment: The volunteers sat on the chair with a cuff worn on the upper arm. Before applying vascular occlusion, ultrasound imaging was acquired to locate the blood vessels. The arteries can be easily distinguished from the veins due to the periodic vasodilation-induced motion. Then, the OBS was attached to the desired region for imaging. Our perfusion protocol involves the following five steps: (a) volunteers seated and rested for 1 min, (b) the pressure cuff was inflated to 150 mm Hg and kept for 40 seconds to induce vascular occlusion, (c) the cuff was released entirely and held for 40 seconds, (d) the 150 mm pressure was again applied for another 40 seconds, (e) and at last, the pressure was again utterly released.

Venous Distensibility: During the venous distensibility test, to prevent complete flattening of the blood vessel, the initial venous pressure was maintained at about 30 mmHg. Since the venous pressure was directly determined by body posture, volunteers were required to bend their elbows in 90 degrees to achieve an approximated 40 cm difference in height between the testing area and neck (Supplementary Fig. 14). The test was started by inflating the pressure cuff to 110 mmHg to produce venous occlusion. Then, the pressure was step-released from 110 mmHg to 0 mmHg with each step of 30 mmHg. The time interval between each release was about 20 seconds.

Flow-mediated Dilation—The FMD measurement adopted the distal testing setting with a cuff worn on the upper arm. Simultaneously, the OBS was attached to the lower end wrist to characterize the FMD of the radial artery. In the beginning, 160 mmHg of total occlusion pressure was applied for 2 mins (cuff-on period: the gross occlusion pressure induced by a cuff equals the systolic blood pressure plus 50 mmHg) before the cuff was released. Then the recording of data only started after the signal amplitudes first time recovered to its baseline. A classical FMD indicator estimates the relative ratio of increment in hyperaemia status artery diameter to baseline artery diameter.

All the human trial protocols are approved under the Nanyang technological University Institutional Review Board (IRB) per federally approved guidelines.

Ultrasound imaging for Cross-validation

The medical ultrasound system (SonixRP, Ultrasonix, Canada) with a linear array transducer (L14-5/38) was used to monitor the veins and arteries dynamically. The image acquisition frame rate of the medical ultrasound machine was 55 Hz. The images were pre-processed with morphological methods to estimate the vascular cross-sectional areas (Supplementary Note. 11).

Declarations

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Author contributions

H.J., Z. Z., and Y. Z. designed the study. H. J performed theoretical analysis, device design and interpreted the data. Z. Z. performed in-vivo mice and human experiments. Z. C., Y. J. G. C, and J. W. fabricated and upgraded the device. Z. W. performed the mice experiments. C. Y. and W. S. developed the data acquisition system. H.J., Z. Z, W. L., Z. C., X.C., and Y. Z composed the paper. All authors discussed the results and commented on the manuscript. Y. Z. supervised the work.

H. J. and Z. Z. contribute equally to this work.

Competing interests

The authors declare no competing interests.

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Figures

Figure 1

Design and working principle of the OBS. a, Schematic illustration of the OBS conformally laminated on the surface of the hand. The OBS is based on the optoacoustic effect and can be employed for monitoring hypoxia, drug decay, vascular compliance, and endothelial dysfunction. Meanwhile, OBS can map the blood information to a 3D distribution model. b, Schematic layout of the OBS: a multi-layered integration of optical (micro-lens), acoustic (piezo-layer) elements with connection, insulation, electrode, and encapsulation layers. See Methods and Supplementary Fig. 1-2 for detailed OBS fabrication process. c, Photograph of the OBS under bending. d, Photograph of the OBS with flexible connecting circuits conformally pasted on the human wrist. e, The cross-distributed acoustic and optical nodes. f, An exploded view of a single optical node. g, An exploded view of a single acoustic node.

Figure 2

The design and characterization of optical and acoustic elements. a-c, Schematic diagram of Monte Carlo simulation under different optical illumination settings. Our micro-lens array method (a) is compared with other classical methods, including the center window method (b) and the side illumination method (c). d-e, The 532 nm light fluence distributions after penetrating 1 mm tissue. Our micro-lens array method (d) shows a larger effective illumination area (determined by -20 dB illumination intensity) than

the center window method (e) and side illumination method (f). g, The proportion of effective illumination area at 1 mm tissue depth with different laser wavelengths. h-j, Photographs of illumination pattern of 633 nm laser after passing through the OBS (h), the OBS with one layer of paper at bottom (i), and the OBS with two layers of paper at bottom (j). k, Impedances of 36 acoustic sensors (sorted by descending order). l, Phases of 36 acoustic sensors (sorted by descending order). m, The histogram of the bandwidths and centre frequencies of 36 acoustic sensors. n, The receiving field intensity of a single acoustic sensor at depth 1 mm. o, The AFOV of 36 sensor elements at 1 mm depth. p, The response spectra of acoustic sensors under different bending conditions.

Figure 3

3D imaging algorithm. a, Schematic illustration of ultrasound receiving paths in traditional DAS method. A correct path takes account of refraction at the layer interface, and the time flight becomes complicated to calculate. b, Our method decomposes the wave components and separately extrapolates the wavefields in different layers for image reconstruction. c, The phantom image for numerical simulation. d, Image reconstructed by traditional DAS method. e, Image reconstructed by our method. f, The amplitudes profiles, along with Line-A data of the ground truth and the images reconstructed by the DAS and our methods, respectively. With the full width half maximum (-6 dB) used as the resolution metric, our method

outperforms the DAS method by 308%. g, Quantitative comparisons between the DAS and our method in terms of accuracy and resolution.

Figure 4

Hypoxia and drug concentration decay tests on mice. a, The hypoxia experiment setup for in-vivo animal tests. b, The trend lines of peak-to-peak values of the OA signals measured by OBS in the hypoxia test. Light yellow regions represent periods with sufficient oxygen supply, while light blue regions represent

periods with low oxygen supply. M1~M3 stand for different mice samples. c, Snapshot images of the reconstructed 3D mice blood oxygen mapping at 400 s, 1000 s, 1500 s, 2000 s, and 2400 s. d, The trend lines of peak-to-peak values of OA signals measured by OBS for drug concentration monitoring. Rhodamine B metabolism in blood vessels (red line) and the tissues (blue line) show the trend of drug decay over time. e, The decay trend of Rhodamine B in blood measured from the OBS. f, The decay trend of Rhodamine B in blood measured from the in vitro fluorescence method. g, Snapshot images of 3D mice drug concentration mapping at 200 s, 700 s, 1200 s, and 1900 s in drug concentration decay test. Rhodamine B in the blood is rendered with orange, whilst Rhodamine B in tissues is rendered with purple.

Figure 5

Vascular occlusion tests on veins. a, Photograph of the outlined dorsal venous network, in which the image region is denoted with a white dotted box. b, Photograph of the OBS worn on the hand. c, The trend lines of peak-to-peak values of the OA signals from the OBS. d, Snapshots of 3D vascular images at 20 s, 70 s, 120 s, and 170 s with the dashed line showing the venous network contour. e, The comparison of peak amplitude and volume ratios under different pressures. Both the peak amplitudes and volumes decreased along with the reduction of occlusion pressure. f, the linear correlation between OBS measured

peak amplitude ratio changes and ultrasound measured venous volume ratio changes under different cuff pressures.

Figure 6

Vascular occlusion tests on arteries. a, Photograph of the outlined radial artery, in which the image region is denoted with white dotted box b, Photograph of the OBS worn on the wrist. c, The trend lines of peak-to-peak values of recorded signals. d, Snapshots of 3D blood images at 20 s, 70 s, 120 s, and 160 s with the dashed line showing the artery vessel contour. e - g, The FMD tests with OBS. All the signals are recorded within 60 seconds after occlusion was released. Peak A represents the maximum vascular volume within one minute after releasing the cuff. h, A quantitative comparison of our OBS and ultrasound imaging in measuring FMD.

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