Non-invasive volumetric optoacoustic imaging of cardiac cycles in acute myocardial infarction model in real-time

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ABSTRACT

Extraction of murine cardiac functional parameters on a beat-by-beat basis remains challenging with the existing imaging modalities. Novel methods enabling *in vivo* characterization of functional parameters at a high temporal resolution are poised to advance cardiovascular research and provide a better understanding of the mechanisms underlying cardiac diseases. We present a new approach based on analyzing contrast-enhanced optoacoustic (OA) images acquired at high volumetric frame rate without using cardiac gating or other approaches for motion correction. Acute myocardial infarction was surgically induced in murine models, and the method was modified to optimize for acquisition of artifact-free optoacoustic data. Infarcted hearts could be differentiated from healthy controls based on a significantly higher pulmonary transit time (PTT: infarct 2.07 s vs. healthy 1.34 s), while no statistically significant difference was observed in the heart rate (318 bpm vs. 309 bpm). In combination with the proven ability of optoacoustics to track targeted probes within the injured myocardium, our method is capable of depicting cardiac anatomy, function, and molecular signatures on a beat-by-beat basis, both with high spatial and temporal resolution, thus providing new insights into the study of myocardial ischemia.

Keywords: acute myocardial infarction, real-time imaging, heart rate, optoacoustic imaging, pulmonary transit time

1. INTRODUCTION

Coronary heart diseases remain a leading cause of death in the world [1, 2], which accentuates the demand for new methods for the prevention and treatment of these conditions. Animal models represent a fundamental tool for the understanding of the pathophysiological mechanisms underlying myocardial ischemic injury and reperfusion as well as for the development and optimization of new therapeutic approaches [3]. Technical and economic constrains promote the wide use of small mammals, particularly mice, to mimic heart conditions, and the availability of a myriad of genetically manipulated mice strains further contributes to the importance of this animal model [4, 5, 6].

Imaging technologies are a fundamental tool for in vivo biological studies with mice models [7, 8]. Cardiovascular magnetic resonance imaging (MRI) is commonly the method of choice due to its high resolution structural imaging capability, which enables the characterization of organ-level functional parameters such as cardiac volume or ejection fraction [9, 10]. However, the relatively low temporal resolution of MRI requires the use of time-gating approaches for cardiac imaging, and beat-by-beat characterization of the heart remains inaccessible with this imaging technology,

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particularly in mice (400-600 beats per minute). Pulsed echo and Doppler ultrasound (US) can render real-time images of the heart [11]. However, US is prone to motion artefacts and intraobserver variability, and the fast dynamic imaging performance is generally limited to two dimensional B-scans. Confocal fluorescence microscopy and other optical imaging modalities have also been used to image heart tissue and detect targeted molecules with high resolution and sensitivity [12-14]. However, optical methods are strongly susceptible to scattering. Thereby, the penetration depth is extremely limited for imaging of whole organs.

Recently, we have demonstrated the unique capability of optoacoustic (OA) tomography for cardiac dynamic imaging with a sufficiently high spatio-temporal resolution to characterize the fast beating mouse heart in vivo [15-17]. Vital cardiac anatomy was clearly resolved, and functional cardiac parameters could be quantified. Of particular importance is the feasibility to measure the pulmonary transit time (PTT), i.e., the time required for blood to travel through the pulmonary circulation from the right to the left ventricles, by imaging the cardiac perfusion of a contrast agent at a very high frame rate in three dimensions [17]. Herein, we build upon the previous studies to explore cardiac function in disease murine models. A new transducer array system with increased number of detection elements and angular aperture has been designed for an enhanced performance. As a result, both spatial resolution and sensitivity were improved, accompanied with enriched depth information. We further established a mouse model of myocardial infarction compatible with optoacoustic tomography. Indeed, strongly absorbing black hair and sutures in standard infarct models hamper optoacoustic cardiac imaging. Herein, albino black mice were used, and glue was further employed to close the wound. In this new framework, we explore the use of an optoacoustic tomography system for real-time visualization, acquisition, and processing of for cardiac function investigations, and compare results between infarct and healthy murine models.

2. METHODS

Optoacoustic (OA) imaging set-up

Based on a previously existing spherical ultrasound array design [16], a matrix array of 512 piezoelectric elements with approximately 100% -6dB bandwidth around a central frequency of 5 MHz were densely placed on a spherical surface with radius 40 mm. The diameter of the individual elements is approximately 2.5 mm. The spherical surface covers an angle of 140º, which is much higher than the 90º coverage of the previous design. This in turn allows reducing limited-view effects and hence improving image quality for deep tissue observations.

During an optoacoustic in vivo experiment, a newly-developed spherical concave transducer array was oriented up-wards and the entire hemispherical cap was filled with agar to guarantee acoustic coupling. The imaged mouse was lying in a prone position over the active detector elements, shown in figure 1.

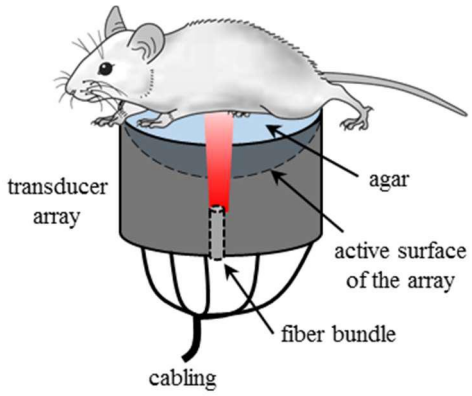

Figure 1. Optoacoustic imaging setup. The spherical transducer array was oriented up-wards, filled with agar to provide acoustic coupling. The imaged mouse was lying in a prone position over the active detector elements. The illumination was delivered through a fiber bundle.

The illumination source was a fast tuning pulsed laser based on an optical parametric oscillator (OPO), delivering <10 ns pulses with approximately 20 mJ energy per pulse (Innolas Laser GmbH, Krailling, Germany). The wavelength of the laser can be tuned between 700–900 nm on a per pulse basis and the repetition rate can be set to 10, 25, 50, and up to 100 Hz. The laser output is guided to the center of the spherical ultrasound probe by a custom made fiber bundle (Ceram Optec GmbH, Bonn, Germany), with the beam diameter reaching ~1 cm at the surface of the mouse. The optoacoustic signals were simultaneously sampled for all transducer elements at 1006 instants at a rate of 40 MSamples/s with a custom-made data acquisition system (DAQ) consisting of 512 parallel analog to digital converters. The data acquisition process was controlled by a computer software (Matlab, Massachusetts, USA), where the DAQ was triggered with the Q-switch output of the laser at a rate of 50 Hz. For each ICG injection event, 2000 frames (corresponding to 40 s) excited at 800 nm were recorded.

Infarct mouse model

Procedures involving animals and their care were conducted in conformity with institutional guidelines and with approval from the Government of Upper Bavaria (Protocol Number 55.2-1-54-2532-85-13) and conformed to Position of the American Heart Association on Research Animal Use. An infarct model suitable for optoacoustic imaging was established by modifying a conventional surgical method where sutures were used to achieve permanent ligation in the heart muscle approximately 2 mm underneath the tissue. Black suture and dark pigmented glue was used to close the ribcage and skin respectively, resulting in severe scarring of the tissue. Figure 2(a) is a photograph of the chest post operation via the conventional method, and Fig. 2(b) shows the corresponding optoacoustic image, displayed as a maximum intensity projection (MIP). The reconstruction was optimized by signal amplification at the heart rate frequency, as well as temporal trimming to remove components before the heart. Despite processing of the acquired signals, the heart could not be easily identified. To improve the image quality, modifications of the conventional surgical method were made. Due to their robust nature, black mice have been commonly adopted for cardiac related research. However, the pigmentation in the skin strongly absorbs the illumination photons and attenuates the optoacoustic signals. In the modified method, albino black mice were used to reduce the presence of melanin. Light colored sutures were used to close the ribcage, and the skin was closed using clear glue. Figure 2(c) shows the reduced superficial coloring on the chest of the modified acute infarct method, and Fig. 2(d) demonstrates the significantly improved image of an infarct heart.

Before in-vivo experiments, hair of the mice was removed from the region around the chest. The mice were anesthetized for the imaging session using a ~2% isoflurane-medical air mixture (~0.8 l/min gas flow). For high-frame-rate in-vivo monitoring of cardiac dynamics with enhanced contrast and characterization of the PTT, 100 nmol of indocyanine green (ICG) diluted in 50 μL of saline was injected intravenously.

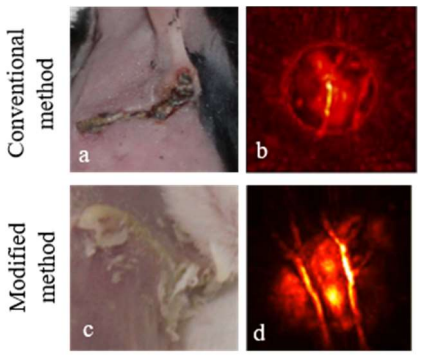

Figure 2. Optoacoustic cardiac imaging of (a-b) conventional and (c-e) modified method. (a, c) Photographs of the chest post operation. (b, c) Optoacoustic images shown as maximum intensity projections (MIP).

Optoacoustic image reconstruction and real-time preview

On-the-fly visualization of three-dimensional optoacoustic images was enabled by an accelerated reconstruction algorithm based on graphics processing units [18]. Live preview providing instant feedback was performed prior to each acquisition with the laser set to a pulse repetition frequency of 10 Hz. The preview software is essential tool during sample positioning for obtaining the optimal view for subsequent data acquisition.

Reconstruction of the acquired sequence was then performed off-line. For this, a deconvolution operation was first performed on the raw acquired signals with the measured electrical impulse response of the detection transducer elements. Then, the signals were band-pass filtered with cut-off frequencies between 0.1 and 7 MHz. A three-dimensional volume of $12\times12\times12$ mm^3 was eventually reconstructed for each time instance containing $120\times120\times120$ voxels.

Heart rate analysis

Fast Fourier transforms (FFT) were performed on the time domain signals of all OA reconstructed voxels. The peak frequency of the sum spectra is determined to be the heartrate, as this parameter is assumed to be the dominant contributor to signal oscillations.

Pulmonary transit time

For a robust estimation of the temporal profile maximum of each OA voxel, a running average over 100 samples was conducted to smooth the signals. The temporal difference between the instances at the peak of RV and LV was taken as the pulmonary transit time. Averaging the rise-time over regions-of-interest (ROI) would yield more reliable results. This required the segmentation of cardiac muscles to remove other features such as blood vessels and scar artifacts. Based on frequency analysis and extraction of the heart beat rate, cardiac muscle can be distinguished from nearby tissue without requiring any manual input for ROI selection. Calculated on a voxel-by-voxel basis, the ratio of the heartbeat frequency amplitude to the spectrum mean is calculated as the frequency fraction map. The higher values correspond to a strong presence of the heart beat rate, and thus a high likelihood of the voxel representing cardiac tissue. A suitable threshold is applied to obtain the binary segmentation mask. With the identification of the left and right ventricle, an averaged rise-time is calculated over a ROI, yielding a reliable measurement of the pulmonary transit time.

3. RESULTS

Figure 3(a) shows temporal snapshots of cardiac flow dynamics, displayed as maximum intensity projections (MIPs) of the reconstructed three-dimensional images. The baseline image at 800 nm is shown at t_o. The ICG bolus is seen entering into the right ventricle (RV) in t_2, and later enters into the left ventricle (LV) at t_4. The late phase image is shown at t_5, near homogenous distribution of ICG in the bloodstream. The high image resolution enables clear identification of anatomical. Specifically, the thoracic vessels (TV1 and TV2) can be clearly seen above the heart, and the heart vessel (HV) becomes visible with the increase of contrast after injection of ICG. Figure 3(b) displays the temporal profiles of the right and left ventricles. The raw signals consists of high frequency oscillations due to heart beat and breathing. The filtered signals indicate reliably the instances of signal peaks, and the difference between RV and LV was taken as the pulmonary transit time (Δt).

The pulmonary transit time of an infarcted mouse was found to be 2.07 s, which is significantly higher than that of a healthy mouse (1.34 s). The heartbeat rate was found to be similar between infarct and healthy mice (318 bpm vs. 309 bpm), indicating the compromised heart function of the infarct mouse.

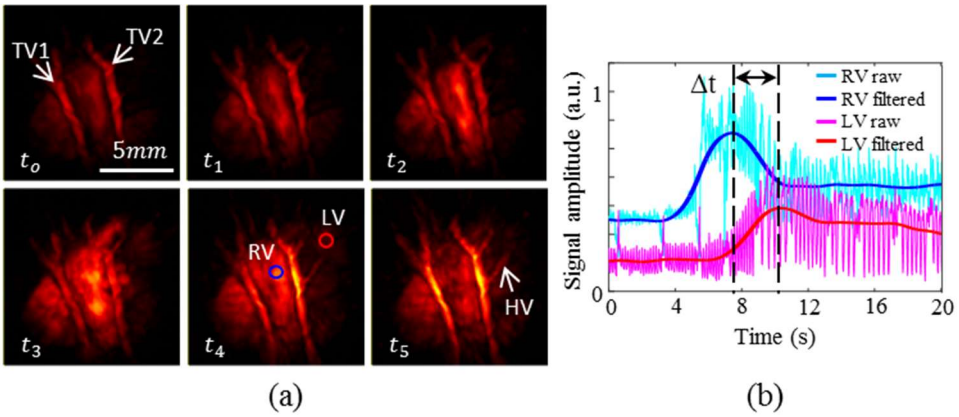

Figure 3. Real-time volumetric optoacoustic imaging to monitor the heart during an indocyanine green (ICG) injection event. (a) Temporal Snapshots shown as MIPs, where t_o shows the baseline image at 800 nm. The ICG bolus is seen entering into the right ventricle (RV) in t_2, and later enters into the left ventricle (LV) at t_4. The late phase image is shown at t_5, near homogenous distribution of ICG in the bloodstream. Other features identified include the thoracic vessels (TV1 and TV2) and heart vessel (HV). (b) Temporal profiles of the right and left ventricles. The raw signals consists of high frequency oscillations due to heart beat and breathing. The signal time-to-peak could be reliably identified by the filtered signals, and the pulmonary transit time (Δt) extracted.

4. DISCUSSION

The presented results indicate the suitability of high-frame-rate three-dimensional optoacoustic tomography to characterize the infarcted mouse heart. Mouse models of myocardial infarction play an important role in investigating the disease mechanisms as well as for the assessment of potential therapeutic interventions. Hence, new methods enabling extracting otherwise unmeasurable functional parameters can significantly advance cardiovascular research. The high temporal resolution of the optoacoustic system employed enables imaging the perfusion of an agent with high contrast in the entire heart, and hence the PTT can be accurately measured. The PTT can serve as an indicator of right ventricular dysfunction or left ventricular hypertrophy, and cannot be measured with MRI or other tomographic imaging methods due to the need of gating-based approaches to render images of the heart.

Parallel to technical developments, an infarct model suitable for optoacoustic imaging was developed in the current study. Black mice are commonly used for cardiac studies with many cardiac disease models established. However, the excess melanin in the skin causes complications when imaged using optoacoustic methods. Strong superficial signals shield important information from deep tissue. Thus, using albino black type ensured compatibility in cardiac surgery, whilst maintaining high image quality. The use of light-colored suture and clear glue for closing of the rib cage and skin was also intended to minimize highly absorbing sources. From the results presented in this work, the compatibility of OA imaging for the infarct model has been demonstrated. In some cases, severe scaring causes strong undesirable artifacts and obstruct the MIP views, as seen e.g. in Fig. 1(c). Yet, the overall data analysis and extraction of physiological parameters were not affected. Therefore, the infarct mouse model described in this work is concluded to be suitable for cardiac optoacoustic imaging.

For the particular biological dynamics presented in this work, an imaging rate of 50 Hz was sufficient for cardiac analysis on a beat-by-beat basis, although a frame rate of 100 Hz is achievable with the current imaging system. Optoacoustics can further provide other functional parameters with multispectral readings. For example, the blood oxygen saturation can be characterized from optoacoustic images taken at multiple optical wavelengths. Five dimensional imaging (multispectral real-time three-dimensional) has been enabled with fast tuning lasers [15], and the use of multiple laser sources further enables multispectral imaging of fast-moving objects [19]. On the other hand, image quality can also be enhanced with more advanced tomographic reconstruction algorithms. Herein, image reconstruction was done using back projection due to its high speed. A model-based algorithm can alternatively be

used [20], which provides a higher contrast to noise ratio (CNR) by accurately accounting for the transducer effects in the signals. Another important issue to consider in the reconstruction is the presence of acoustic reflection artefacts [21], originating from the lungs. Improvements in image CNR and reduction of artefacts may facilitate the acquisition of information from deeper regions.

5. CONCLUSION

With the establishment of a suitable infarct mouse model for cardiac optoacoustic imaging, a proper comparison of the imaged healthy and infarcted hearts could be performed. . Infarcted hearts could be differentiated from healthy controls based on a significantly higher pulmonary transit time (PTT: infarct 2.07 s vs. healthy 1.34 s), while no statistically significant difference was observed in the heart rate (318 bpm vs. 309 bpm).

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