Live imaging of blood flow in mammalian embryos using Doppler swept-source optical coherence tomography

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Abstract. Studying hemodynamic changes during early mammalian embryonic development is critical for further advances in prevention, diagnostics, and treatment of congenital cardiovascular (CV) birth defects and diseases. Doppler optical coherence tomography (OCT) has been shown to provide sensitive measurements of blood flow in avian and amphibian embryos. We combined Doppler swept-source optical coherence tomography (DSS-OCT) and live mouse embryo culture to analyze blood flow dynamics in early embryos. SS-OCT structural imaging was used for the reconstruction of embryo morphology and the orientation of blood vessels, which is required for calculating flow velocity from the Doppler measurements. Spatially and temporally resolved blood flow profiles are presented for the dorsal aorta and a yolk sac vessel in a 9.5-day embryo. We demonstrate that DSS-OCT can be successfully used for structural analysis and spatially and temporally resolved hemodynamic measurements in developing early mammalian embryos. © *2008 Society of Photo-Optical Instrumentation Engineers.* [DOI: 10.1117/1.3046716]

1 Introduction

Abnormalities in cardiovascular (CV) development are the most common and lethal congenital human birth defects. The mouse is an excellent model to study CV development, and over the past 10 years, there have been astounding developments in mouse genomics to identify genes with novel roles in development and disease. Now, there is a pressing need for better tools for phenotyping mutant embryos to reveal primary, early defects that lead to cardiac failure and long-term disorders. Optical coherence tomography (OCT) is emerging as an exciting imaging tool because of the capability to image 2 to 3 millimeters into soft tissue and still maintain a reasonably high spatial resolution ($\sim$2 to 20 μm).[1,2] Implementation of Doppler measurements into the OCT facilitates the acquisition of both structural and velocity information at the same spatial and temporal resolution.[3] OCT offers significant advantages over other methods such as laser scanning microscopy, which has limited depth penetration, or ultrasound, which offers low spatial resolution, and it could be an ideal modality for studying early embryonic mouse development and physiology.

Recently, several research groups have successfully applied OCT for live imaging of CV dynamics and blood flow in Drosophila,[4] *Xenopus laevis*,[5,6] and avian[7] embryos. Despite the critical need to understand mammalian embryonic development, studies on OCT imaging of mammalian cardiovascular dynamics are very limited. Jenkins et al. applied OCT for imaging of extracted mouse embryonic hearts at 12.5 (days post coitum) dpc and 13.5 dpc.[8] The same group reported 3-D OCT images of 13.5-dpc beating, embryonic mouse hearts that were excised and externally paced.[9] Likewise, Luo et al. imaged beating 10.5-dpc hearts in embryos that were maintained outside the uterus, but with dramatically slower than normal heart beats.[10] While these exciting studies have revealed the potential of OCT for imaging embryonic mouse development, further studies have been limited by the challenge of maintaining normal physiology. There are currently no reports describing the evaluation of normal blood flow in mouse embryos using OCT.

Our group has previously defined conditions for the growth and maintenance of 5.5 to 10.5-dpc mouse embryos in culture to facilitate optical imaging.[11] Using confocal microscopy and transgenic embryos with Green Fluorescent Protein (GFP)-labeled blood cells, we have been able to measure shear stress changes in the embryonic yolk sac[12] and to identify early defects in cardiac function in mutant embryos.[13] However, the shallow imaging depth of confocal microscopy (less that 200 μm) was insufficient to make images of the entire 3-D volume of the beating heart or to acquire blood flow data from vessels deep within the embryo. In the work presented here, we used a swept-source OCT (SS-OCT) system[14] enhanced with a Doppler module for *in vivo* structural imaging and hemodynamic measurement in early mouse embryos cultured outside the uterus. Spatial and temporal blood flow velocity profiles are acquired from the dorsal aorta and a yolk sac vessel. Yolk sac measurements made with this system were similar to values previously measured by fast scanning confocal microscopy, while the dorsal aorta measurements show the advantage of the Doppler SS-OCT (DSS-OCT) over the confocal microscopy to detect blood flow deep in tissue. Our results demonstrate that OCT can be used for the morphological analysis of live mammalian embryos as well as to measure hemodynamic changes during development.

2 Materials and Methods

2.1 DSS-OCT System

The experimental system employs a broadband swept-source laser (Thorlabs, SL1325-P16) with output and incident on the

sample power of 12 and 4 mW, respectively, at central wavelength $\lambda_0 = 1325$ nm, SNR more than 110 dB, and spectral width $\Delta\lambda = 100$ nm, which translates to approximately 8 μm in-depth resolution in air. The scanning rate over the full operating wavelength range is 16 kHz. An interferogram is detected by a balanced-receiver configuration that reduces source intensity noise as well as autocorrelation noise from the sample (Thorlabs, PDB140C) and is digitized using a 14-bit digitizer. A Mach-Zehnder interferometer (MZI)–based optical frequency clock is used to recalibrate the OCT interference signals into k-space before application of fast Fourier transform (FFT) algorithms. FFT reconstructs an OCT intensity in-depth profile (A-scan) from a single scan over the operating wavelength range, resulting in the A-scan acquisition and processing rate of 16 kHz. The system is described in more details in Ref. 5).

2.2 Doppler Measurements

Blood flow profiles were reconstructed using the formula:

$$v = \Delta\varphi/[2n\langle k\rangle\tau\cos(\beta)], \qquad (1)$$

where $\Delta\varphi$ is a Doppler phase shift between the successive A-scans, n is a refractive index, $\langle k\rangle$ is the average wave number, τ is time between A-scans, and β is an angle between the flow direction and the laser beam. An angle β was calculated from structural 2-D and 3-D data sets acquired from the embryos; refractive index was assumed as $n = 1.4$. Doppler measurements were validated by measuring flow of milk controlled by a syringe pump through a flow chamber in the range from 0 to 7.0 mm/s with the standard deviation of ± 0.1 mm/s (data not shown).

2.3 Embryo Manipulations

Wild-type CD-1 male and female mice (Charles River Laboratories, Wilmington, Massachusetts) were mated to produce staged embryos. Embryos were dissected with the yolk sac intact at 8.5 to 10.5 dpc in the preheated to 37 °C dissecting medium, consisting of 89% DMEM/F12, 10% FBS, and 1% 100× Pen-strep solution (Invitrogen, Grand Island, New York). The dissection and imaging stations were maintained at 37 °C using a custom-made heater box and conventional heater. Dissected embryos were transferred to a 37 °C, 5% CO_2 incubator for at least 1 h for recovery and kept in the incubator until imaging (up to 4 h after the dissection).

3 Results and Discussion

The advantage of Doppler OCT is that blood flow velocity measurements can be spatially and temporally resolved and correlated with the 3-D structure of the embryo. Structural imaging is important not only for morphological analysis and identification of vascular structures, but also for determining the angle between the direction of blood flow and the laser beam (based on 3-D vessel orientation), which is required for flow velocity calculation. Figure 1(a) shows a typical image acquired from the heart region of the live embryo at the embryonic day 8.5. The acquisition was performed at 512 A-scans per frame without averaging, which resulted in the acquisition rate of 28 frames per second. As one can see from the image, the internal structure of the heart as well as other details of the embryo are clearly outlined on the image; in
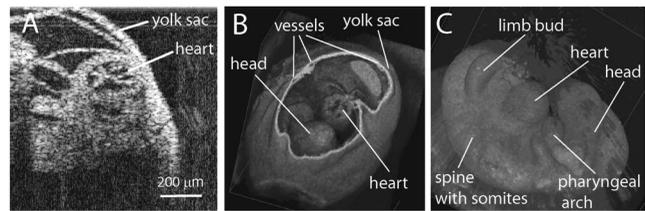

Fig. 1 Structural imaging of early embryos with SS-OCT. (a) Live imaging of 8.5-dpc embryonic heart at 28 fps. (b) and (c) 3-D reconstructions of 9.5-dpc embryo with and without the yolk sac, respectively.

addition, the frame rate can be increased by decreasing the number of A-scans per frame and limiting the field of view precisely to the area of interest to allow following heart wall or vessel wall movements during heartbeat. By 2-D mechanical scanning, the 3-D structure of the embryo can be revealed. Figures 1(b) and 1(c) shows examples of 3-D reconstructions of 9.5-day embryos with and without the yolk sac, respectively. Thus, OCT is very effective as a method to produce 3-D reconstructions of entire embryos, similar to ultrasound, but with cellular resolution.

Figure 2 shows an example of SS-OCT Doppler imaging of blood flow velocity in a 9.5-day mouse embryo. Structural [Fig. 2(a)] and Doppler [Fig. 2(b)] images of part of the yolk sac and the embryonic trunk were taken at 256 A-scans per frame. Strong Doppler signals were detected from two regions in the shown field of view [Fig. 2(b)]: a yolk sac vessel (blue) and the dorsal aorta within the embryo (red). Different colors indicate opposite directions of the flow in these structures.

The Doppler phase shift was measured and blood flow velocity profiles were reconstructed along the lines shown in Fig. 2(a) corresponding to the yolk sac vessel and the dorsal aorta (Fig. 3). The angle between the direction of the flow and the scanning beam required for the velocity calculation was determined from the structural data sets acquired from the embryo. The velocity profiles across the vessel have a parabolic shape, as expected. The peak flow velocity across the vessels was plotted versus time [Figs. 3(b) and 3(d)] and re-
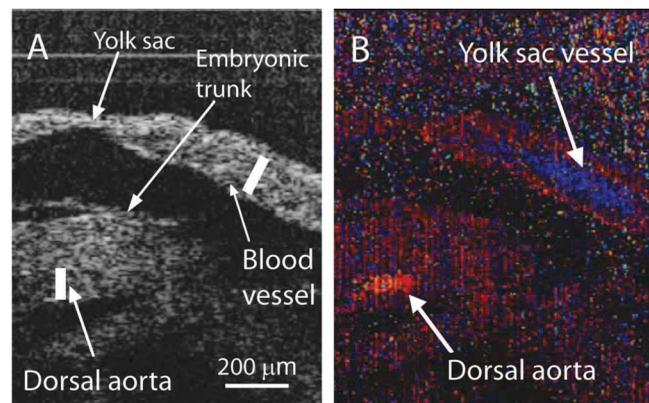

Fig. 2 SS-OCT Doppler measurement of blood flow in the live embryo culture. (a) Structural image of 9.5-dpc embryo showing fragments of a yolk sac and an embryonic trunk. (b) Corresponding color-coded Doppler image showing strong signals produced by blood flow in the yolk sac vessel and a dorsal aorta. (Color online only.)
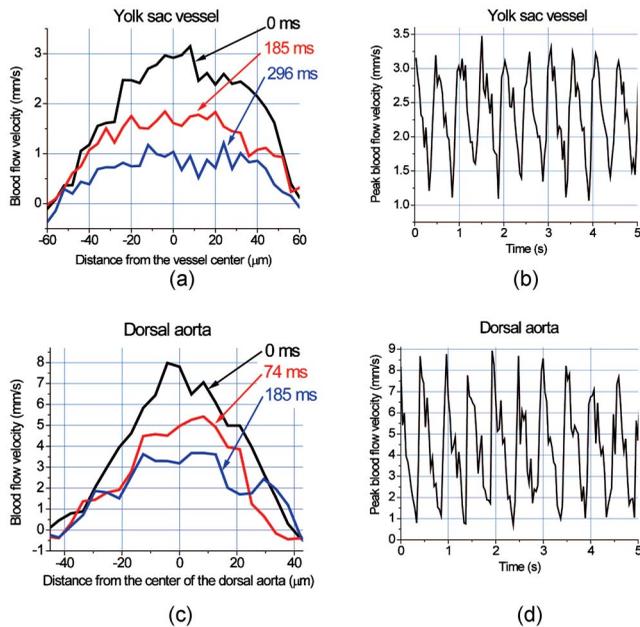
Fig. 3 Blood flow measurement in the live embryo. Blood flow velocity profiles [(a) and (c)] and dynamics of the peak blood flow velocity [(b) and (d)] in a vessel of the yolk sac and the dorsal aorta, respectively, measured along the lines shown in Fig. 2(a) at different phases of the heartbeat cycle.

veals the periodicity of the cardiac cycle, which can be used to calculate the heart rate of the embryo as well as to study hemodynamic changes during the heartbeat.

The values of the peak blood flow velocity and the heart rate measured in the embryonic yolk sac vessel correlate well with previously reported values at this developmental stage using confocal microscopy.[12] We observed a similar heart rate (2 beats per second)[12] and similar peak blood flow velocities (3.2 mm/s compared to about 2 mm/s for vessels of similar size), showing that DSS-OCT is an effective way to make blood flow measurements in early embryos. Even though embryo manipulation and static culture protocols have been developed and successfully applied for confocal microscopy, this work is the first demonstration that these protocols can be adopted for OCT imaging, providing a solid ground for a wide range of live mammalian embryo studies to assess cardiovascular function. The embryonic heart is within the OCT imaging distance; however, hemodynamic analysis in the beating heart with Doppler OCT is challenging due to the difficulty of mapping the flow direction and high blood flow velocities, which require application of phase unwrapping algorithms. Our future studies will focus on characterizing early embryonic hemodynamics at different embryonic stages in normal and mutant mice to develop a model of hemodynamics.

4 Conclusions

Here, we have presented structural SS-OCT images acquired from live mouse embryos as well as spatial and temporal profiles of blood flow velocity in the embryonic yolk sac and in the embryo proper acquired with Doppler SS-OCT. Measurements of blood flow velocity and embryonic heart rate correlated with previously reported values,[12] indicating that DSS-OCT is an effective way to make blood flow measurements in early embryos but can image blood flow in deeper vessels than previously measured with confocal microscopy. These results show that SS-OCT and DSS-OCT imaging of live embryos in culture is a valuable and exciting tool that can be used to characterize normal development, to analyze cardiovascular defects in mutant mice, or to study the effects of pharmaceutical agents.

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