Live imaging of rat embryos with Doppler swept-source optical coherence tomography

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Abstract. The rat has long been considered an excellent system to study mammalian embryonic cardiovascular physiology, but has lacked the extensive genetic tools available in the mouse to be able to create single gene mutations. However, the recent establishment of rat embryonic stem cell lines facilitates the generation of new models in the rat embryo to link changes in physiology with altered gene function to define the underlying mechanisms behind congenital cardiovascular birth defects. Along with the ability to create new rat genotypes there is a strong need for tools to analyze phenotypes with high spatial and temporal resolution. Doppler OCT has been previously used for 3-D structural analysis and blood flow imaging in other model species. We use Doppler swept-source OCT for live imaging of early postimplantation rat embryos. Structural imaging is used for 3-D reconstruction of embryo morphology and dynamic imaging of the beating heart and vessels, while Doppler-mode imaging is used to visualize blood flow. We demonstrate that Doppler swept-source OCT can provide essential information about the dynamics of early rat embryos and serve as a basis for a wide range of studies on functional evaluation of rat embryo physiology. [DOI: 10.1117/1.3241044]

Keywords: rat; optical coherence tomography; Doppler; embryo culture; live embryo imaging; blood flow.

Paper 09197LR received May 15, 2009; revised manuscript received Aug. 10, 2009; accepted for publication Aug. 11, 2009; published online Oct. 1, 2009.

1 Introduction

The rat is a classic mammalian animal model for a wide range of embryonic studies aimed to address congenital abnormalities. Rat physiology is considered to be more complex and informative than in the mouse, providing a closer animal model to humans for neurobehavioral, toxicological, and organ manipulation studies. Until recently, the use of rats was hampered by the lack of genetic manipulation techniques, but derivation of rat embryonic stem (ES) cells by Buehr et al.[1] and Li et al.[2] provides a basis for a wide range of direct genetic alterations to create disease models with more relevance to human disorders, including better models of congenital cardiovascular defects. Together with advances in genetic manipulation, higher resolution imaging methods such as optical coherence tomography (OCT) have emerged as improved phenotyping tools. OCT is a 3-D optical imaging technique with a spatial resolution of 2 to 20 μm and an imaging depth of 2 to 3 mm in tissue. It does not require the application of contrast agents and can be combined with Doppler OCT detection of velocity at the same spatial resolution.

OCT has been successfully used by several groups for live embryonic imaging of *Xenopus laevis*,[3,4] quail,[5] and chick.[6] Recently, our group optimized protocols for static mouse embryo culture for OCT imaging, demonstrating that OCT and Doppler OCT can be successfully applied to live embryonic 3-D structural imaging and cardiovascular and hemodynamic analysis[7] at early, postimplantation embryonic stages E7.5 to E10.5. Our recent work has shown that this method is a highly sensitive means to image blood flow as it is possible to make velocity measurements from individual blood cells during early stages of circulation in mouse embryos.[8] While OCT has proven to be an extremely useful modality for live embryonic imaging in different species, its capabilities have never been tested in the rat model.

In this study, we have used OCT to image dynamic cardiovascular development in the rat model at the embryonic stages E10.5 to E11.5, equivalent to E8.5 to E9.5 in mouse embryos. We performed 3-D structural imaging of live rat embryos, dynamic heart imaging, and Doppler OCT analysis of the beating heart. Here, we report the first live dynamic imaging of the rat embryo at cellular resolution, opening the door for a wide range of embryonic physiological studies in the rat model.

2 Materials and Methods

2.1 *Imaging System*

The experimental system used in these studies is Doppler swept-source OTC (SS-OTC), which is customized from the Thorlabs SL1325-P16 system and described in more details in Ref. 7. Briefly, it utilizes a broadband swept-source laser with a central wavelength of $\lambda_0 = 1325$ nm and a bandwidth of $\Delta\lambda = 110$ nm; output power is $P = 12$ mW; the A-line scanning frequency 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 14-bit digitizer. A Mach-Zehnder interferometer (MZI)-based optical frequency clock is used to calibrate the OCT interference signals from the optical time-delay domain in the frequency domain before application of fast Fourier transform (FFT) algorithms. A FFT is used to reconstruct an in-depth profile (A-scan) of OCT intensity from a single scan over the operating wavelength range. By 2-D mechanical scanning over the area of up to 10×10 mm, the 3-D structure of the embryo can be revealed. The Doppler phase shift induced by moving blood cells and heart wall was measured between adjacent A-scans, resulting

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1083-3668/2009/14(5)/050506/3/$25.00 © 2009 SPIE

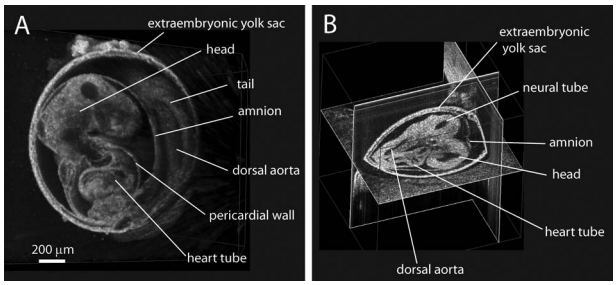

Fig. 1 Live 3-D imaging of early rat embryos with SS-OCT. (a) 3-D rendering view of a live E10.5 embryo cultured on the imaging stage. The clipping plane on the reconstruction is at a 60-deg angle to the direction of the OCT laser beam. (b) A cross-section view of a typical 3-D reconstruction of an E10.5 embryo. Major embryonic structures are clearly distinguishable on the reconstructions. Both data sets consist of 512 × 512 A-scans.

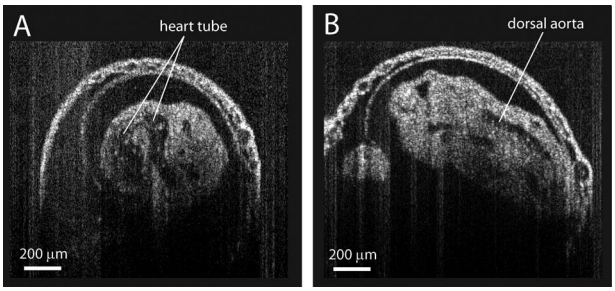

Fig. 2 Dynamic imaging of live rat embryos. Representative frames out of time lapse (a) through embryonic heart and (b) along the embryonic body. The time lapses are acquired at about 28 frames/s. Individual circulating blood cells and small groups of cells are distinguishable in the heart tube (a) and dorsal aorta (b).

in velocity maps of the same resolution as structural SS-OCT images. The flow sensitivity of the current system is approximately 200 μm/s. The refractive index was assumed as $n = 1.4$. The scanning head was positioned inside the 37 °C, 5% CO_2 tissue culture incubator during the imaging sessions.

2.2 *Embryo Manipulations*

Wistar Kyoto (WKY/N) male and female rats (Charles River Laboratories, Wilmington, Massachusetts) were mated overnight. Females were examined for vaginal plugs daily and the presence of a plug was taken as E0.5. Embryos were dissected with the yolk sac intact at E10.5 and E11.5 in dissecting medium preheated to 37 °C. The dissecting medium consisted of 89% DMEM/F12, 10% FBS, and 1% 100X Pen-strep solution (Invitrogen, Grand Island, New York). The dissection station was heated and maintained at 37 °C using a custom-made heater box. The dissected embryos were transferred to a 37 °C, 5% CO_2 incubator for at least 1 h for recovery and imaged within 8 h after the dissection.

3 Results and Discussion

3.1 *Live Embryo Culture for Imaging*

We focused our rat embryo studies on the developmental stages E10.5 and E11.5 (which correspond to E8.5 and E9.5 in the mouse species). To perform live imaging of rat embryos, we adopted embryo manipulation protocols that we previously developed for mouse embryo imaging, as described in Refs. 7 and 8. Rat embryos in a static culture exhibited strong and stable heartbeats and blood circulation during the imaging session (up to 8 h). The average measured heart rate (from SS-OCT time lapses) was 75 beats per minute (bpm) for E10.5 and about 110 bpm for E11.5 embryos. These values correlate well with previously published heart rate data at these embryonic stages in rat embryos,[9] suggesting that the embryos in static culture were exhibiting normal physiological behavior.

3.2 *Structural 3-D SS-OCT Imaging*

Figure 1 shows 3-D reconstructions of the embryos with extraembryonic yolk sacs at E11.5. All major structures are clearly outlined on the reconstructions. Even though the rat and the mouse are both rodents, their embryonic development differs noticeably. Rat embryos are about 30% larger than the mouse embryos at the corresponding developmental stage, they develop slower, and the gestation period is longer than in the mouse. Despite the larger size, the rat embryos at the studied stages are within the imaging depth of the SS-OCT system and the embryonic structures are resolved better than the ones of the mouse at the corresponding developmental stages due to their larger size. At later time points, high-resolution OCT imaging can be complimented with high-frequency ultrasound imaging, which enables deeper tissue imaging with the expense of lower resolution (30 to 50 μm).

3.3 *Dynamic Structural Imaging*

To test the potential of SS-OCT to analyze dynamic structural changes in rat embryos, we acquired 2-D time-lapse sequences. Figure 2(a) shows a representative frame taken from a time lapse acquired of the beating embryonic heart tube. The time lapse was acquired at 512 A-scans per frame and 28 frames/s. The walls of the beating heart are nicely visualized, providing an opportunity to observe heart dynamics during the heartbeat. Figure 2(b) is a frame from a time lapse taken through the embryo along the dorsal aorta. Individual circulating erythrocytes are clearly detectable within the heart tube [Fig. 2(a)] and the dorsal aorta [Fig. 2(b)].

3.4 *Doppler SS-OCT Imaging of Cardiodynamics and Hemodynamics*

We performed Doppler SS-OCT imaging to visualize hemodynamics within rat embryos. Figure 3 shows a structural SS-OCT image through the embryonic heart and a series of color-coded Doppler images of the same area taken from a time lapse and representing different phases of the heartbeat cycle. The detected Doppler shift signal was generated by the velocity component, which is parallel to the OCT laser beam. The blue shift corresponds to movement toward the detector, while the red shift corresponds to movement away from the detector. As one can see from the figure, the Doppler shift was detected from the circulating blood cells inside the heart (labeled as b) as well as from the moving heart wall (labeled as hw). Even though the heart at the studied stages is within the imaging depth of the SS-OCT system and the structure is well resolved, hemodynamic analysis in the heart is complicated by the Doppler shift phase wrapping caused by the high flow velocities and the difficulty of mapping of the flow direction

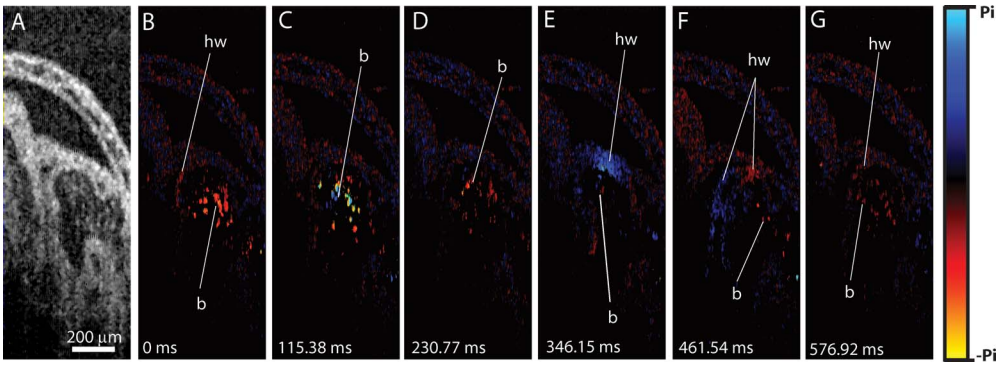

Fig. 3 Doppler SS-OCT velocity imaging in the live embryonic heart. (a) Structural image acquired from the primitive ventricle of the beating embryonic heart at E10.5 and (b) to (g) the corresponding representative Doppler color-coded maps from the same area taken out of a time lapse. Doppler imaging was performed at about 26 frames/s at 512 A-lines per frame. (Color online only.)

required for the velocity calculation. While several groups are developing and optimizing algorithms to overcome these limitations,[6,10] this paper demonstrates that Doppler shift maps can be acquired from the beating rat embryonic heart for live hemodynamic analysis.

4 Conclusions

For the first time we demonstrated live imaging of rat embryos with resolution at the single cell level. We performed (1) 3-D imaging of live embryos cultured on the imaging stage, which provides a tool for structural analysis of embryo morphology; (2) dynamic 2-D imaging; and (3) dynamic Doppler shift velocity imaging of a beating heart. Our results show that OCT imaging of cultured live rat embryos is a useful tool for studying morphological changes during development as well as for analyzing dynamic physiological processes, such as heartbeat and blood flow. Comparative studies between rat and mouse embryos indicate differences between these rodent species, potentially highlighting processes that may benefit from the generation of new mutant rat models.

Acknowledgments

The study is supported, in part, by postdoctoral fellowship from the American Heart Association (AHA) (IVL) and grants from the National Institutes of Health (NIH) (HL077187 MED) and the Department of Defense Office of Naval Research Young Investigator Program (DOD ONR YIP) (KVL).

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