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.

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

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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. and Li et al. 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, quail, and chicken. 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 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. 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 OCT (SS-OCT), which is customized from the Thorlabs SL1325-P16 system and described in more details in Ref. Briefly, it utilizes a broadband swept-source laser with a central wavelength of λc = 1325 nm and a bandwidth of Δλ = 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. The Doppler interference is derived from the phase shift induced by moving blood cells 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 in...
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 of the current system is approximately 1.4. The scanning head was positioned inside the 37 °C, 5% CO₂ 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% CO₂ 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 normal physiologic behavior. 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)].

3.2 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.
required for the velocity calculation. While several groups are
developing and optimizing algorithms to overcome these
limitations,4 this paper demonstrates that Doppler shift
tests 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 em-
byros 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 Dop-
pler 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 devel-
opment as well as for analyzing dynamic physiological pro-
cesses, 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.

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