Optical Coherence Tomography
A New High-Resolution Imaging Technology to Study Cardiac Development in Chick Embryos

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Background—Optical coherence tomography (OCT) is a depth-resolved, noninvasive, non-destructive imaging modality, the use of which has yet to be fully realized in developmental biology.

Methods and Results—We visualized embryonic chick hearts at looping stages using an OCT system with a 22 μm axial and 27 μm lateral resolution and an acquisition rate of 4000 A-scans per second. Normal chick embryos from stages 14 to 22 and sham-operated and cardiac neural crest-ablated embryos from stages 15 and 18 were scanned by OCT. Three-dimensional data sets were acquired and processed to create volumetric reconstructions and short video clips. The OCT-scanned embryos (2 in each group) were photographed after histological sectioning in comparable planes to those visualized by OCT. The optical and histological results showing cardiovascular microstructures such as myocardium, the cardiac jelly, and endocardium are presented.

Conclusions—OCT is a powerful imaging modality which can provide new insight in assessing and understanding normal and abnormal cardiac development in a variety of animal models. (Circulation. 2002;106:2771-2774.)

Key Words: imaging ■ morphogenesis ■ tomography ■ cardiac volume ■ heart defects, congenital

The study of heart development has undergone a renaissance in research in recent years. Various technologies, including video light microscopy, ultrasound, confocal microscopy, high resolution MRI, and optical coherence tomography (OCT) have been applied in the past to visualize embryonic hearts to gain more insight into the complex developmental process of cardiovascular development.

OCT is an echo-based imaging modality that measures the time-of-flight of back-reflected light using low-coherence interferometry. Through the use of broadband near-infrared light sources, OCT achieves resolutions of ~10 to 30 μm, with depth penetrations of a few millimeters. Since its introduction in 1991, OCT has been used in the imaging of semi-transparent tissues (eg, anterior segment and cornea of the eye, Xenopus laevis tadpoles) and in highly light-scattering tissues (eg, retina, subluminal structures in the gastrointestinal tract). Further, current-generation systems are capable of video-rate imaging. Catheter-based OCT systems are being developed to image atherosclerotic plaques clinically. Proof-of-principle experiments in basic research have imaged the atrioventricular node and used color Doppler OCT to quantify flow dynamics in the Xenopus laevis heart.

In this study, we demonstrate three-dimensional OCT imaging of the chick embryo heart during looping. Using this three-dimensional data, we compared OCT images with histological sections and generated volumetric reconstructions of the early heart tube in normal and cardiac neural crest (CNC)-ablated embryos.

Methods

OCT

The principles and physics of OCT have been described in detail, but will be briefly summarized here. OCT and ultrasound perform B-scan imaging in analogous manners; a 2-dimensional image (ie, B-scan) is composed of a series of 1-dimensional line scans (ie, A-scan) acquired as the probe beam is laterally scanned across the sample. The high-speed OCT system used in this study is described in reference 15 (Figure I). This system has a 22 um axial and 27 um lateral resolution and can acquire 4000 A-scans per second. For this study, we set the system to acquire 8 B-scans per second. Thus, each B-scan was composed of 500 A-scans. Note that higher imaging rates can be achieved by using fewer A-scans per B-scan.

Embryo Preparation and Imaging

Fertilized Hubert Ross chicken eggs (Gold Kist Hatchery, Siler City, NC) were incubated at 37°C and 97% humidity in a forced-draft
incubator. At Hamburger-Hamilton (HH) stages 14/15, 16, 18, 20, and 22, the embryos were placed in 1.8% buffered potassium chloride solution until the hearts stopped beating in diastole. Sham-operated and CNC-ablated embryos, prepared as described previously, were collected similarly and scanned at stages 15 and 18. One living stage 15/16 embryo was used to illustrate dynamic motions that can be acquired by OCT.

OCT volume scans were performed by acquiring B-scans parallel to the x-y plane at 10 mm intervals along the z-axis (Figure 1A). Image acquisition time was 56.25 sec per 50 images, and our samples consisted of 50 to 100 images; 3-dimensional datasets were ~25 MegaVoxels in size. The total imaged volume was 1 to 2 cubic millimeters. Using volume scan data, 3 orthogonal plane images can be formed: frontal plane (B-scan), sagittal plane, and transverse plane (Figure 1B). We represented this data in two formats, “flip-book” movies where B-scans are displayed in their order of acquisition, and volumetric renderings of the early heart tube focusing on the outflow tract using 3-dimensional imaging software. The movies were created in Matlab (The MathWorks) and the 3-dimensional images and slices were created in Slicer Dicer (PIXOTEC, LLC).

After scanning, the same embryos were paraffin-embedded and sectioned in an appropriate plane to compare cardiac anatomy (Figure II). Correspondence was determined by the best match between OCT images and histological sections.

Results
To validate our morphological impressions from the OCT images, we used histological sections from the same embryos embedded in the appropriate planes. In Figure 1D through 1G, data from 2 planes are shown from 2 HH-stage 15 chick hearts. The images demonstrate a strong correlation between OCT images and the corresponding histological sections (Figure 1D and 1E and 1E and 1F, respectively). Figure 1D and 1E are transverse sections of the cardiac outflow limb. The preparation artifacts frequently present in histological sections, such as dehydration and retraction of tissue, lead to morphological changes, as demonstrated by greater area of lumen of the outflow limb in Figure 1D and 1F compared with the OCT images in Figure 1E and 1G, where the endocardial cell layers are very close. Figure 1F and 1G show frontal sections through the outflow limb and presumptive right ventricle.

Figure 2 shows 3-dimensional reconstructions of the chick heart of sham-operated embryos at HH-stages 15 (A) and 18 (B). Figure 2C and 2D show 3-dimensional reconstructions of the heart of HH-stage 15 sham and CNC-ablated chick embryos, respectively, from the right lateral view. Figure 2E demonstrates a cutaway through the straight outflow limb of the early heart tube and reveals further internal structural detail in the experimental embryo. Figure 2F shows the same heart from the left lateral view.

Several flip-book movies, rotational three-dimensional reconstruction images of the early heart tube are demonstrated in the Data Supplement. Still frames of a movie showing dynamic motions of a HH-stage 15/16 heart are presented at the bottom of Figure 1H through 1K.
Discussion

We have demonstrated images of the looping chick heart in a variety of different planes generated with OCT, and shown that there is a strong correlation at the micron-scale level between histology and OCT. Further, we demonstrate that OCT has the ability to assess cardiovascular anatomy in 3 dimensions, as shown in Figure 2, and we also show that it not only can identify significant morphological differences between a normal and abnormal specimen but can also detect growth and further structural differentiation between stages, which makes it a powerful tool for studies in cardiac development.

The study of heart development in animal models, using the powerful technologies of molecular and cellular biology, has been pursued aggressively to understand the mechanisms of congenital cardiac malformations. However, the limitations of current imaging methods for assessing cardiac structure in these animal models frequently force investigators to analyze phenotypes with postmortem histopathology. Morphological abnormalities frequently cannot be clearly identified or appreciated in 2 dimensions, particularly those involving misorientation of cardiovascular structures, because of our inability to acquire reliable data in 2 dimensions from a 3-dimensional structure with curves and loops. Furthermore, histological images have artifacts due to tissue dehydration, shrinkage, and stretching during processing, and high quality histology is often difficult to obtain, costly, and time-consuming for small and fragile specimens. Thus, it is generally impractical to histologically prepare the large numbers of specimens typically needed to track developmental changes in these studies.

Three-dimensional reconstructions can extend the sensitivity of these studies. With a 3-dimensional data set in hand, the user has a tremendous level of flexibility in representing anatomic information, ranging from sectional images along arbitrary anatomical planes to volumetric reconstruction of organs. OCT offers the possibility of generating and cataloging high-resolution 3-dimensional images of embryonic development and could allow new insight into assessing and understanding normal and abnormal cardiac development in established animal models such as the chick or mouse.

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References

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