Non-Destructive, High-Resolution 3-Dimensional Visualization of a Cardiac Defect in the Chick Embryo Resembling Complex Heart Defect in Humans Using Micro-Computed Tomography

Double Outlet Right Ventricle With Left Juxtaposition of Atrial Appendages

Christoph M. Happel, MD, PhD*; Christian Klose, ME*; Gabriele Witton; Gian L. Angrisani, ME; Soenke Wienecke, ME; Stephanie Groos, MD; Friedrich-Wilhelm Bach, ME, PhD; Dirk Bormann, ME, PhD; Jörg Männner, MD, PhD; T. Mesud Yelbuz, MD, PhD

Nondestructive, high-resolution 3-dimensional (3D) imaging of the embryonic heart remains a challenge in cardiovascular development research. In the past, several imaging techniques (eg, magnetic resonance microscopy, optical coherence tomography) were tested for their suitability to visualize the 3D morphology of embryonic hearts. Most of these imaging tools have their drawbacks with respect to resolution and depth penetration. Here we...
present, to the best of our knowledge, the first high-resolution 3D images of normal and malformed embryonic chick hearts at the 10 μm level generated by microcomputed tomography (micro-CT) examination of critical point-dried heart specimens. Cardiac anatomy is demonstrated in great details with respect to myocardial fiber arrangement and trabeculations as well as atrioventricular (AV) and semilunar valves. Positions of great vessels with associated ventricular septal defects are visualized in high quality in malformed hearts.

Figure 1A shows the normal 4-chambered heart of a day 9 chick embryo with correct positioning of the heart chambers and great vessels. Figure 1B depicts an image of a malformed embryonic chick heart in which both great arteries are significantly shifted toward the right side with extreme dextroposition of the aorta, and both great arteries arise from the right ventricle (double outlet right ventricle [DORV]). Note also that both atrial appendages are located to the left of the great vessels (left juxtaposition of atrial appendages [LJAA]). The cardiac defect that we present here in the chick embryo resembles a rare complex heart defect known in humans, as depicted in a classic drawing by Frank Netter, MD, in the Netter Collection of Medical Illustrations – Heart (Figure 1C, reproduced with permission from netterimages.com).

DORV generally occurs as an isolated entity, but it is also seen in association with other heart defects, such as total anomalous pulmonary venous return, AV septal defect, mitral stenosis or atresia, juxtaposition of the right atrial appendage, or a persistent left superior caval vein. Juxtaposition of the atrial appendages itself is almost always associated with complex congenital heart disease.1,2 The condition in which both atrial appendages lie to the left side of the great vessels (LJAA) is more common than that in which the appendages lie to the right of the arteries (RJAA).1 Further, more complex and morbid anomalies are seen in those with LJAA than in those with RJAA.3 The developmental basis of LJAA remained unclear for a long time until an animal model for LJAA in the chick was demonstrated for the first time in 2003.4

Figure 2 demonstrates the dimensional relations of a malformed chick heart with DORV and LJAA (A) and a transverse image plane of a high-resolution 3D micro-CT reconstruction of such a heart showing details of cardiac anatomy of AV and semilunar valves at micrometer level (B). Figures 3 and 4 show separate sections of 3D micro-CT reconstructions of a normal chick heart versus the DORV heart with LJAA to demonstrate the relationship of the great vessels in both hearts by comparison as well as the significant differences at atrial, AV and chamber level.

Note the quality of resolution for both AV and semilunar valves in the normal (Figure 3C, E, G and Figure 4A, C) as well as in the DORV heart with LJAA (Figure 3D, F, H and Figure 4B, D) demonstrating fine details of the anatomic architecture. The high resolution of the micro-CT pictures allows the recognition of small structures such as the lumen of the coronary artery (Figure 2B) or the dysplastic aortic valve with a small fourth leaflet in the experimental heart (Figure 4B). The Data Supplement demonstrates further details of these hearts as fly-through movies (Movies I and II showing a fly-through of the right and left side of the normal heart, respectively, and Movie III demonstrating the relevant findings in detail as a fly-through in the DORV heart with LJAA).

In summary, we have shown that micro-CT facilitates nondestructive visualization of the anatomy of normal and malformed embryonic hearts at high resolution when the heart specimens have been dried by the critical point method.
(Details of embryo preparation and the micro-CT unit together with images of the critical point dried specimens are presented in the Data Supplement (Figure I and Figure II, respectively). Micro-CT might be a valuable tool not only for nondestructive visualization of the 3D morphology of congenital heart defects at early stages of development but, additionally, might be used for exact volumetric measurements or for the determination of mechanical properties of the embryonic and fetal myocardium.

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Disclosures

None.

References


Figure 4. 3D images of a normal (A, C and E) and a malformed day 9 embryonic chick heart (DORV with LJAA; B, D and F) that were generated from our micro-CT data. Hearts are shown in cross-sectional views at the level of the semilunar valves (A and B; view from above), the atrioventricular valves (C and D; view from below), and the apical part of the ventricles (E and F; view from above). These separate sets of images demonstrate striking differences in the positional relationship of the great arteries and atrioventricular valves between the two heart specimens. Note that, in the normal heart (A), the aortic valve is wedged between both atrioventricular valves and is in fibrous continuity with the MV. In the malformed heart (B and D), both great arteries are shifted to the right with extreme dextroposition of the aorta and lack of aorto-mitral fibrous continuity; additionally, a large mal-alignment VSD is clearly seen in D. Note the quality of resolution for the cardiac valves in the normal heart as well as in the DORV heart demonstrating fine details of the anatomic architecture, eg, dysplastic AoV with a small fourth leaflet in the malformed heart (B). The images in C and E show the fully intact interventricular septum of the normal heart. The image in F shows the interventricular septum of the DORV heart below the level of the malalignment VSD. Note here, too, that in all images of the malformed heart the size of the bar is smaller, indicating that the malformed heart is slightly bigger than a normal heart due to dilatation of the chambers and atria. Further details of both hearts are demonstrated in the online-only Data Supplement (Movies I and II showing a fly-through of the right side and left side of the normal heart, respectively, and Movie III demonstrating the findings as a fly-through in the DORV heart with LJAA). AoV indicates aortic valve; D, dorsal; L, left; LV, left ventricle; MV, mitral valve; PV, pulmonary valve; R, right; RV, right ventricle; TV, tricuspid valve; V, ventral; VSD, ventricular septal defect. * denotes the dysplastic leaflet of the quadricuspid AoV in the malformed heart. Bar=500 μm.
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SUPPLEMENTAL MATERIAL
Supplemental Methods

Embryo preparation and micro-CT scanning

White leghorn chick embryos grown in shell-less culture were removed from culture and dissected in a Petri dish with Lockes’ solution at incubation day 9. Their still beating hearts were perfused with Lockes’ solution until all visual signs of blood were removed from the heart chambers and great vessels. Hearts then were arrested in general dilation using a calcium free Lockes’ solution containing 20mmol manganese chloride and fixed in a 2% glutaraldehyde solution followed by post-fixation in Bouins’ solution. The fixed specimens were dehydrated and subjected to critical point drying (CO₂) to enhance contrast in the micro-CT.

For the induction of cardiac malformations, normal chick embryos grown in shell-less culture were treated on incubation day 3 (HH-stage 15) with 1,5µg Bis-diamine and reincubated until incubation day 9. Hearts then were harvested for fixation and critical point drying (see above).

For micro-CT analysis, the dried heart specimens were put on a 20,5 mm specimen holder covered with a thin plastic tube (resulting nominal measuring accuracy 10 µm) and scanned within a Scanco Medical µCT 80 workstation (SCANCO Medical, Zürich, Switzerland; Figure S1). The settings for acquisition were chosen to achieve best resolution images: tube voltage: 45kVp, anode current: 88 µA, resolution and spacing 2048x2048 px, 1000 projections/180°, 10 µm, frame averaging: 6, integration time: 800ms. Generated images were analyzed using the OsiriX Software Package (v.3.7.1. 64-bit), creating 16-bit cLUTs for visualization and using volume rendering techniques for 3D reconstruction.
Supplemental Figures

**Figure I:** The heart specimens were scanned within a Scanco Medical CT 80 workstation (SCANCO Medical, Zürich, Switzerland) as shown in the Figure.

**Figure II:** Image of critical point dried day 9 embryonic chick hearts positioned on plastic rods in original size before scanning (both hearts outlined by a yellow circle with the apex of hearts showing to the top of image). Left: normal heart. Right: DORV with LJAA. Note that the malformed heart is significantly larger due to dilatation of both atria and chambers.