

## Ultrasonic observation of 3 dimensional arterial bifurcation geometry using a chick chorioallantoic membrane model

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### 1. Introduction

The arterial bifurcation geometry significantly affects the local changes in wall shear stress distribution, which is closely related to the formation and development of atherosclerotic plaques. However, variation of the arterial bifurcation geometry caused by pulsatile blood flow is not yet fully understood especially in 3 dimensional (3-D) motion due to the difficulties in selecting a suitable *in vivo* experimental model and methodology. The objective of this research is to establish an *in vivo* chick chorioallantoic membrane model to investigate the cyclic variation of the arterial bifurcation geometry by reconstructed 3-D ultrasound images. Chick embryos at an 11 day of development were used to visualize the vessel geometry by a microscope and to obtain cross-sectional ultrasound images to reconstruct the 3-D morphology of arterial bifurcation geometry at peak systolic and diastolic phases. The expansion ratios of cross-sectional area and translational distances between peak systolic and diastolic phases were computed in 3 branches of the arterial bifurcation. Bifurcation angle and tortuosity at peak systole and diastole were also calculated from the reconstructed 3-D ultrasound images of chick embryo arterial bifurcation.

### 2. Methods and Materials

In a chick chorioallantoic membrane model to culture shell-less chick embryos on a petridish after 3 days in the shell in a digital incubator (RCOM PRO 50, Autoalex Co., Korea), the shell-less embryos died easily compared with the ones in the shell. In our experiments, a survival rate was reached to 10% at an 11 day of the embryo development (4 samples among 40 embryos). All experimental procedures were approved by the Ethics Committee of Jeju National University. A personal computer based high frequency ultrasonic system with a 35 MHz broadband acoustic transducer (PCB v4.2, 35<sub>T1</sub>MHz, Capistrano Labs Inc., San Clemente, CA, USA) was used for imaging.

A total of 40 slices of the cross-sectional arterial images in the bifurcation geometry including a mother and daughter branches were acquired along the scan direction with a 0.1 mm interval to reconstruct 3-D images in each embryo sample.

After identifying peak systolic and diastolic phases in a series of the cross-sectional images, the lumen boundaries were manually detected in the cross-sectional images and the 3-D ultrasound images of the chick embryo arterial bifurcation were reconstructed using an image processing software, Amira (FEI visualization sciences group, Dahlem, Berlin, Germany). The details of experimental protocol and data analysis are in [1].

### 3. Results and Discussion

The 3-D arterial bifurcation images and the extracted centerlines at peak systolic and diastolic phases are superimposed on a single frame for two chick embryos as shown in Fig. 1. Translational motion, longitudinal variation of the wall displacement, and the radial wall motion are well recognized in 3 branches of the arterial bifurcation. The translational motion was dominant for sample #1, and this was confirmed by the overlapped centerlines at peak systolic and diastolic phases.

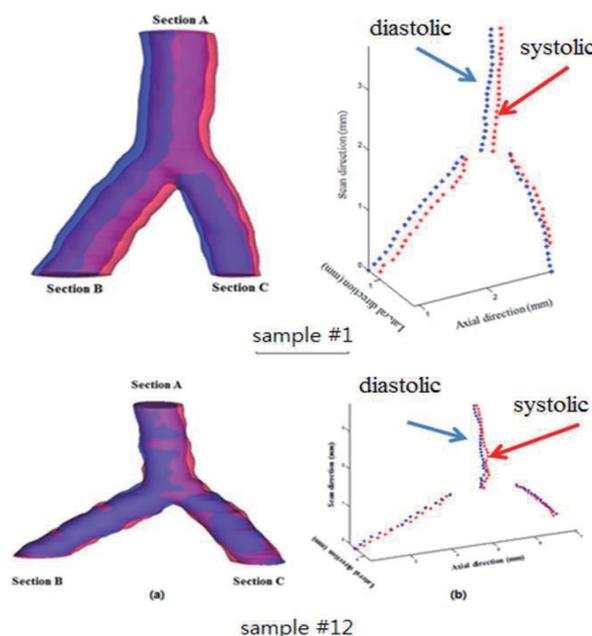


Fig. 1 The overlapped 3 dimensional images of the arterial bifurcation geometry and the extracted centerlines at peak systolic and diastolic phases for 2 chick embryo samples.

The movement of the centerline was different at different branches. Regional variation was shown in

terms of magnitude and direction of the wall movement depending on branch sections and its curvature geometry. As for sample #12, the translational movement was small but we could observe the different amplitude and variation on the different sections of the branches. Through these results in 3-D observation, we confirmed the previous asymmetric variation of the vessel motion in longitudinal images [2,3].

**Table I** summarizes the quantified parameters from the reconstructed 3-D images at peak systolic and diastolic phases for two chick embryo samples. The expansion ratios of cross-sectional area for mother branch (Section A) and daughter branches (Sections B & C) are 14.8%, and 18.8%, 14.6%, and 17.2%, 11.8%, 27.9% for the 1<sup>st</sup> and 2<sup>nd</sup> samples, respectively. However, the translational distances of the centerlines at mother and daughter branches are much larger (0.126 mm, 1.162 mm, and 0.108 mm) for the 1<sup>st</sup> sample, than the ones (0.054 mm, 0.018 mm, and 0.90mm) for the 2<sup>nd</sup> sample. Therefore the 1<sup>st</sup> sample has larger translational motion while the 2<sup>nd</sup> sample is expanding larger during systole. Bifurcation angles of the 2<sup>nd</sup> sample were almost twice ( $\sim 100^\circ$ ) of the ones of the 1<sup>st</sup> sample ( $\sim 52^\circ$ ) but the angles were not much changed at systole and diastole. Tortuosity of the 2<sup>nd</sup> sample was larger than the one of the 1<sup>st</sup> sample for both branches as we expected from 3-D images. Tortuosity of the section A to B was much larger than that of the section A to C but the difference at systole and diastole was little for the 2<sup>nd</sup> sample. Even though there are differences between two samples, the location and geometry of the arterial bifurcation are not the same, so these differences are just representative results and cannot be interpreted as inter-sample differences alone. The similar differences are expected to exist even at different arterial positions of the same chick embryo sample. The differences in the arterial positions in the same chick embryo sample and inter-sample differences of the arterial bifurcation at the same location need to be further investigated in the future.

#### 4. Conclusions

Parameters	Sample (#)	Phase	Section A	Section B	Section C
Expansion ratio of cross-sectional area (%)	Case 1	Systolic to diastolic	14.8	18.8	14.6
	Case 2	systolic to diastolic	17.2	11.8	27.9
Displacement of center point (mm)	Case 1	Systolic to diastolic	0.126	0.162	0.108
	Case 2	systolic to diastolic	0.054	0.108	0.090
Bifurcation angle ( $^\circ$ )	Case 1	Diastolic	51.95 $^\circ$		
		Systolic	52.09 $^\circ$		
	Case 2	Diastolic	100.41 $^\circ$		
		Systolic	96.32 $^\circ$		
			A-B	A-C	
Tortuosity	Case 1	Diastolic	0.0715		0.1001
		Systolic	0.0952		0.0759
	Case 2	Diastolic	0.2745		0.1579
		Systolic	0.2773		0.1644

Table I The quantified geometrical parameters of arterial bifurcation of 2 chick embryo samples.

In the present research, we developed an *in vivo* chick chorioallantoic membrane model in order to observe the variation of 3-D arterial bifurcation geometry during a cardiac cycle using high frequency ultrasound imaging. The results show that the artery has a complex movement including translational motion and asymmetrical expansion and contraction during a cardiac cycle. These 3-D ultrasonic observations of the dynamic arterial motion may be useful to the estimation of local changes in WSS distribution and therefore the development and etiology of atherosclerosis and plaques in the arterial bifurcation. This chick embryo model may provide a new animal model to study arterial pathophysiology.

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