Diffusion Tensor MRI Revealed Developmental Changes of Cardiomyocyte Architecture in Pig Hearts

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Target Audience: Scientists and students who are interested in the heart development, electrophysiology, and mechanics.

Purpose: Cardiomyocyte architecture is a critical determinant of coordinated cardiac contractile function. The heart undergoes substantial structural and functional changes to accommodate the rapid switching from fetal to neonatal circulation immediately after birth. Because of the acute opening of the pulmonary circulation, the relative workload of the left ventricle (LV) and right ventricle (RV) changes substantially immediately after birth. We hypothesized that three dimensional cardiomyocyte architecture might be required to adapt rapidly to accommodate programmed perinatal changes of cardiac function.

Methods: Isolated fixed hearts from: (1) fetal pigs at mid-gestation (MG) and pre-born (PB); (2) new-born pigs at postnatal day 1 (P1), P5, P14; (3) and adult pigs (n = 5 for each group) were prepared. Diffusion-weighted magnetic resonance imaging (DTMRI) were acquired on a 4.7 Varian INOVA system with following paramteres: TR 2s; TE 34ms; b-value, 0 and 784 s/mm^2; slice thickness, 0.5 mm for fetal hearts and 1mm for all other hearts; in plane resolution 937 x 937 μm^2 for adult hearts and 156 × 156 μm^2 for all other hearts. DTMRI measured cardiomyocyte architecture was visualized by 3D fiber tracking and was quantitatively evaluated by the measured helix angle. Upon the completion of MRI, hearts embedded in paraffin and sectioned at 5μm thickness along the short-axis for immunohistochemistry. All data are presented as mean±standard deviation and compared using two-way analysis of variance (ANOVA).

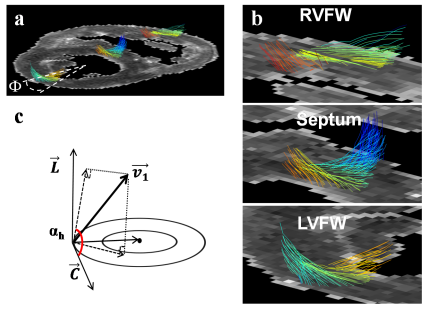
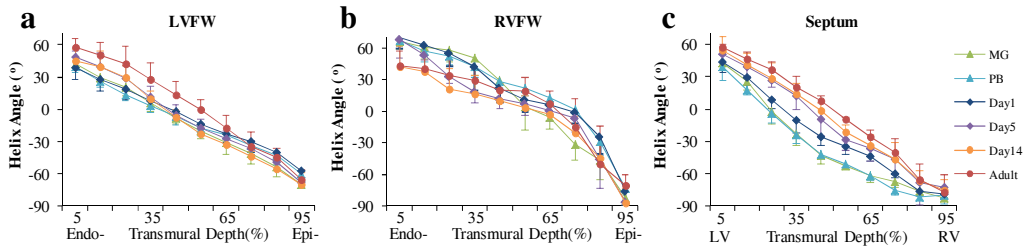


Figure 1. a-b) the transmural shift of cardiomyocyte orientation in the RVFW, septum, and LVFW on a short-axis slice of a P1 pig heart. **c)** The definition of helix angle (α_h) as the angle between circumferential orientation (C) and the projection of cardiomyocyte orientation (v1) on the tangential plane,

Results: In all hearts, the characteristic transmural shift of cardiomyocyte orientation, i.e. formed a left handed helix in the sub-epicardium and a right handed helix in the sub-endocardium (**Fig. 1**), was observed in the septum, left ventricular free wall (LVFW), as well as the right ventricular free wall (RVFW). Quantitativel analysis showed the cardiomyocyte fiber architecture in the RVFW and septum underwent rapid remodeling after birth. In the sub-endocardium of RVFW (i.e. 0 - 10% wall depth), the cardiomyocyte helix angle was ~70° in prenatal (i.e. MG and PB) hearts and P1 hearts. It rapidly decreased to 42°±20° in P14 hearts ($P < 0.05$ compared to MG, PB, and P1), which is similar to that in adult hearts (43°±14°, P = N.S. compared to P14). In the septum, the transmural location of 0° helix angle, reflecting cardiomyocytes that were oriented circumferentially parallel to the short axis plane, rapidly shifted from ~25% wall depth in prenatal hearts to ~30% transmural depth at P1, and further to ~40%, 45% and 50% wall depth at P5, P14, and adulthood, respectively. In the LVFW, no statistically significant changes in cardiomyocyte architecture was observed from MG to P14. Proliferative cells were detected by Anti-Ki67 staining in both LVFW and RVFW in P1, P5, and P14 hearts.

Discussion: The helical architecture of LV cardiomyocytes was developed as early as mid-gestation period, After birth, cardiomyocytes architecture in RVFW and septum changed rapidly from P1 to P14, which may associate with the detected transient cardiac cell proliferation in newborn hearts.

Conclusion: Our results illustrated the plasticity of cardiomyocyte architecture in response to the new demands of LV and RV function after birth. Since the cardiomyocyte reorganization was mostly completed within 14 day after birth, the first two weeks after birth is a critical period for postnatal heart development.



d

LVFW	MG	PB	P1	P5	P14
PB	ns				
P1	ns	ns			
P5	ns	ns	ns		
P14	ns	ns	ns	ns	
Adult	**	**	**	**	**

e

RVFW	MG	PB	P1	P5	P14
PB	ns				
P1	ns	ns			
P5	ns	*	*		
P14	**	**	**	ns	
Adult	ns	*	*	ns	ns

f

Septum	MG	PB	P1	P5	P14
PB	ns				
P1	**	**			
P5	**	**	**		
P14	**	**	**	ns	
Adult	**	**	**	**	**

Figure 2. a-c) Transmural distribution of helix angle in the LVFW, RVFW, and septum. In the LVFW and RVFW, data were analyzed from the endocardial surface (5% wall depth) to the epicardial surface (95% wall depth). In the septum, data were analyzed from the LV endocardial surface (5%) to the RV endocardial surface (95%). **d-f)** Post hoc comparison of transmural distribution of helix angle between different age groups in the LVFW, RVFW, and septum , *, $P < 0.05$; ** $P < 0.001$; ns, not significant.