34th Annual International Conference of the IEEE EMBS
San Diego, California USA, 28 August - 1 September, 2012

Altering Embryonic Cardiac Dynamics with Optical Pacing

L. M. Peterson, M. McPheeters, L. Barwick, S. Gu, A. M. Rollins and M. W. Jenkins

Abstract - **Several studies have shown that altering blood flow early in development leads to congenital heart defects. In these studies the perturbations to hemodynamics were very gross manipulations (vessel ligation, conotruncal banding, etc.) that would be inappropriate for probing the delicate mechanisms responsible for mechanically-transduced signaling. Also, these perturbations lacked feedback from a monitoring system to determine the exact degree of alteration and the location of its effect. Here, we employed optical pacing (OP) to alter the heart rate in quail embryos and optical coherence tomography (OCT) to measure the resultant shear forces on the endocardium. OP is a new technique utilizing pulsed 1.851 µm infrared laser light to noninvasively capture the heart rate to the pulse frequency of the laser without the use of exogenous agents. To measure shear stress on the endocardium, we extended our previous OCT algorithms to enable the production of 4-D shear maps. 4-D shear maps allowed observation of the spatial and temporal distribution of shear stress. Employing both OCT and OP, we were able to develop perturbation protocols that increase regurgitant flow and greatly modify the oscillatory shear index (OSI) in a region of the heart tube where future valves will develop. Regurgitant flow has been linked with valve development and precise perturbations may allow one to determine the role of hemodynamics in valvulogenesis.**

I. INTRODUCTION

Alterations in blood flow during early cardiovascular development can lead to congenital heart defects (2003; Vermot, Forouhar et al. 2009). Biomechanical forces exerted by the blood likely influence gene expression in surrounding cells (Poelmann, Gittenberger-de Groot et al. 2008). Altered gene expression can then lead to altered cardiac structure and function that can in turn lead back to altered biomechanical forces. This feedback loop is poorly understood due to the absence of proper tools to assess forces on the early looping heart and their resultant effects in cardiogenesis. Even small alterations to how the heart beats can influence blood flow that then results in different levels and patterns of shear stress along the endocardium and potentially lead to abnormal heart looping, trabeculation, valvulogenesis and septation. To elucidate the role of mechanically-transduced signaling in cardiovascular development, a set of tools is needed to consistently perturb hemodynamics in specific ways and precisely monitor the resultant cardiovascular development.

In order to induce changes in cardiac function various methods (conotruncal banding (Reckova, Rosengarten et al. 2003), pharmacology (Vermot, Forouhar et al. 2009), etc.) have been utilized. Unfortunately, common perturbation methods are either invasive, difficult to control or repeat, or affect multiple pathways simultaneously. In 2010, we utilized a pulsed diode laser (1.875 µm) to control the heart rate of embryonic quails (Jenkins, Duke et al. 2010). At low radiant exposures, embryonic quail hearts were reliably paced *in vivo* without detectable damage to the tissue. Optical pacing (OP) has the potential to become a robust tool for perturbing cardiac function as it is noninvasive, highly repeatable and can precisely alter cardiac function.

Optical coherence tomography (OCT) can monitor OP perturbations to assess the exact degree of alteration and the location of its effect. Specifically, OCT has been used to measure maximum shear stress values on the endocardium of the avian heart tube (Jenkins, Peterson et al. 2010). However, in order to properly assess how OP affects shear stresses on the endocardium, a 4-D map of shear forces is needed.

Here, we employ OCT to directly measure the resultant shear forces on the endocardium as a method to optimize OP protocols. OCT allowed us to establish an OP protocol that increased regurgitant flow at the site of future valve development. 4-D Doppler OCT image sets of individual embryonic hearts were acquired while freely beating and, subsequently, during optical pacing. Pulsed Doppler traces revealed a significant increase in the fraction of the cardiac cycle during which regurgitant flow occurred. In order to measure the resultant shear forces on the endocardium, we developed OCT algorithms to measure 4-D shear stress maps and a method to measure oscillatory shear index (OSI), which measures the variation in shear stress. The shear stress and OSI maps revealed increased shear stress due to regurgitant flow over a larger area in the region of future valve development due to OP. Using OP and OCT we are able to precisely perturb the heart and measure resulting biomechanical alterations at the endocardial surface, which may help elucidate mechanisms of valvulogenesis in the future.

II. METHODS

Fertilized quail eggs were incubated in a humidified, forced draft incubator at 100°F. After 48 hours of

---

* The project described was supported by the National Heart, Blood and Lung Institute (RO1HL095717). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Heart, Blood, and Lung Institute or the National Institutes of Health.

L. M. Peterson, M. McPheeters, S. Gu, A. M. Rollins and M. W. Jenkins are with the biomedical engineering department at Case Western Reserve University, Cleveland Ohio 44106 USA

L. Barwick was with Case Western Reserve University, Cleveland, Oh 44106 USA. He is now with Brigham Young University

M. W. Jenkins is the corresponding author. Phone: 216 844-3298; email: mwj5@case.edu

978-1-4577-1787-1/12/$26.00 ©2012 IEEE

development the eggs were taken from the incubator, the eggshell was removed, and the contents were placed in a sterilized 3.5cm Petri dish. These embryos were then placed in an environmental OCT imaging chamber (Jenkins, Watanabe et al. 2011) for imaging while the heartbeat naturally, and again while the heart was optically paced. The OCT system used to collect the data utilizes a buffered Fourier Domain Mode Locked laser (Jenkins, Adler et al. 2007) which operates at a line rate of 117 kHz. The system resolution is ~10μm in air. 4-D Doppler data was collected by imaging over multiple heartbeats at sequential slice locations and reassembled using image-based retrospective gating (Gargesha, Jenkins et al. 2009). A sampling pitch of 1.4 μm was used in the B-scan direction. The Doppler images were unwrapped using a Goldstein algorithm (Jenkins, Peterson et al. 2010). Optical pacing was conducted utilizing an 1851 nm diode laser. The light was delivered through a 400 μm multimode fiber and aimed at the inflow region of the heart tube using an aspheric lens which generated a spot size of ~ 400 μm on the tissue's surface. The heart was paced 50% above the intrinsic rate (4.5Hz) for the duration of the 4-D imaging session (~5min). Following cessation of optical pacing the heart returned to its natural heart rhythm.

In order to analyze how the optical pacing affected the biomechanical forces impinging on the embryonic heart, we calculated 4-D shear stress maps and measured the OSI before and during OP. To calculate shear stress, we manually segmented the endocardium. The resulting surfaces were used to generate surface meshes, which were used to determine the direction normal to the endocardial wall. The segmentations were also employed to determine the centerlines of the heart tube at different time points, which were then used to estimate the absolute blood velocity. Shear stress was calculated using the equation $\tau = \eta du/dn$, where η is the dynamic viscosity of the blood and du/dn is the velocity gradient in the direction normal to the wall of the endocardium. We assumed that the fluid is Newtonian and we approximated the dynamic viscosity to be 5 mPa s (Vennemann, Kiger et al. 2006). It has been shown that the blood flow in the looping avian heart has both a low Reynolds number (0.5) and a low Womersley number, indicating that the flow is laminar and dominated by viscous forces (Vennemann, Kiger et al. 2006). Segmentation and calculations were carried out using Amira and Matlab. After calculating the shear stress at 14 time points in the cardiac cycle we calculated the oscillatory shear index (OSI) which is a measure of the variation of the instantaneous shear stress vector relative to the average direction over time. This measurement was accomplished by using nonrigid registration to match the points of the segmented surface mesh over the 14 time points. The OSI was then calculated as detailed in (Taylor, Hughes et al. 1998). The OSI ranges from 0, if the shear is predominately in the same direction, to 0.5 if the average shear stress over time is zero.

III. . RESULTS

Figure 1 A shows the endocardial surface from a stage 14 quail heart. The yellow square indicates the location where the following pulsed Doppler traces were measured. Panel B and C show pulsed Doppler traces for the outflow tract before (B) and during (C) optical pacing. The 4-D Doppler data that was collected allows us to create pulsed Doppler traces from any location in the heart tube. One particular area of interest is the site of future valve development in the outflow tract of the heart where these traces were measured (Garita, Jenkins et al. 2011). The portion of the trace below the solid line represents regurgitant flow during the heartbeat. This regurgitant flow is both stronger and persists for a longer fraction of the heartbeat in the paced heart. The percentage of the cardiac cycle with regurgitant flow before and during pacing is 17% and 43% respectively.

Figure 2 shows a view of a 3-D shear stress map of the outflow tract of a quail embryo heart both before (left) and during (right) optical pacing. The shear stress map shows the time points corresponding to the highest regurgitant flow. This time point is indicated by a vertical dotted line in Fig 1 (B&C). The shear stress map of the endocardium clearly shows stronger regurgitant flow over a more extensive region during OP compared to the heartbeat during sinus rhythm. The maximum regurgitant shear stress was 3.9 Pa before OP and 7.7 Pa during OP. In Fig. 3, OSI is calculated and mapped to a surface rendering of the outflow tract. OSI

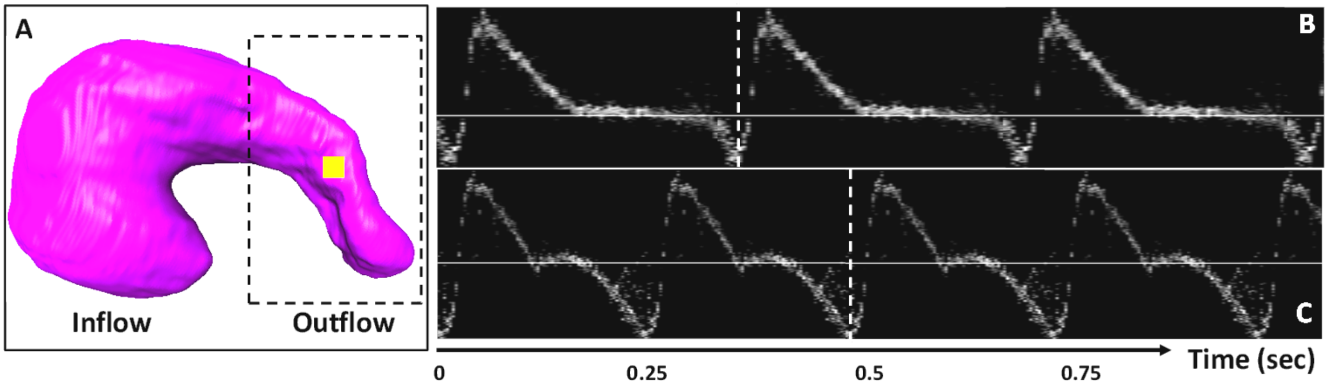

Figure 1 Pulsed Doppler traces. A shows a surface rendering of a segmented volume of a stage 14 quail heart. The inflow and outflow sections of the heart are labeled and the yellow square corresponds to the area where the following pulsed Doppler traces were measured. B&C are pulsed Doppler traces from the outflow tract of a stage 14 quail heart. B corresponds to the heart before optical pacing and C corresponds to the heart during optical pacing. The time of highest regurgitant flow is indicated by a vertical dotted line in both traces.

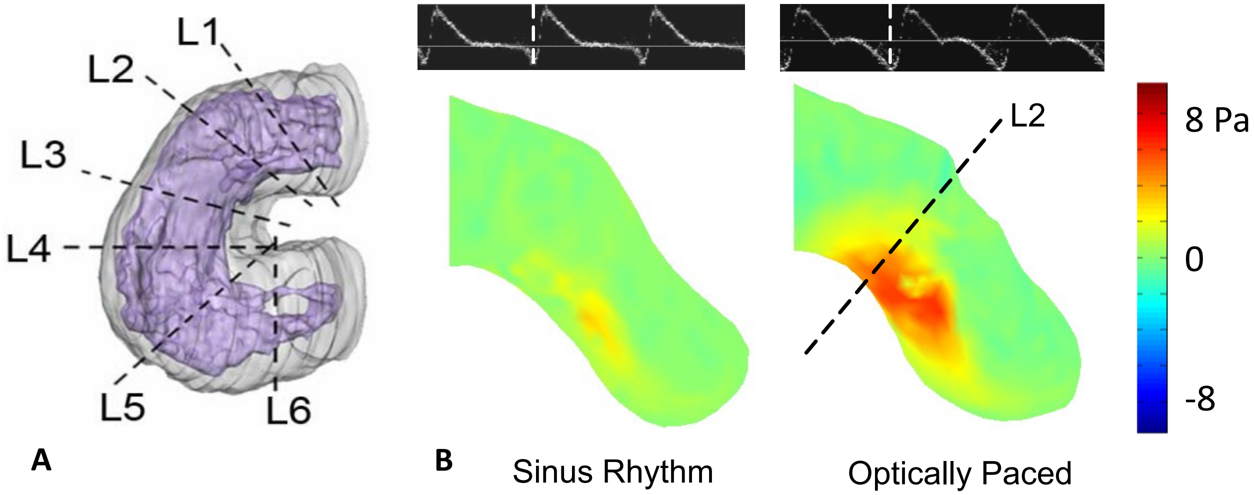

Figure 2 Shear stress maps. A. 3-D OCT reconstruction of the tubular heart. L2 and L6 are sites of future valve development. B. 3-D shear maps of the outflow tract of the heart before (left) and during OP (right). The black dotted line indicates the location of L2. The L2 region is thought to develop into the semilunar valves and cardiac septa. Both shear maps display the time point corresponding to the maximum regurgitant flow for the heart indicated by the white dotted lines in the pulsed traces above. The regurgitant flow during OP generates a stronger shear stress along the endocardial wall. The maximum regurgitant shear stress before and during optical pacing was 3.9 Pa and 7.7 Pa respectively.

is significantly increased during OP. This is due to the increased regurgitant flow caused by pacing the heart well beyond its intrinsic rate. Although regurgitant flow velocities were altered, forward flow remained similar before and during OP. The region of increased regurgitant shear stress and OSI corresponds to the site of future valve development (Garita, Jenkins et al. 2011). Because regurgitant flow may facilitate valve development (Vermot, Forouhar et al. 2009), OSI may be a very useful metric. In the future, investigators can develop OP protocols to specifically alter OSI to varying degrees in the outflow tract to investigate the effects of regurgitant flow on valvulogenesis.

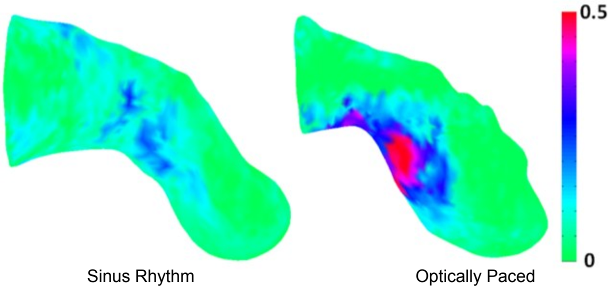

Figure 3 Oscillatory shear index. The above figures display the oscillatory shear index (OSI) in the outflow tract of the heart before and during OP. The OSI was calculated over the course of an entire heartbeat and displayed on the surface mesh of the segmented outflow tract.

IV. CONCLUSIONS

Here, we demonstrate an OP protocol capable of altering forces that may impact valvulogenesis. In order to evaluate our OP protocols, we developed an OCT method to measure shear stress in 4-D and assess OSI in the outflow tract of an avian embryo heart. We show that OCT can be used to optimize OP protocols by measuring the degree and location of altered stresses. Our OP protocol significantly altered shear stress patterns in the developing heart tube. In particular we see significantly increased regurgitant flow, shear stress, and OSI as a result of OP. Our pulsed Doppler traces indicate that the percentage of the cardiac cycle with regurgitant flow increased from 17% to 43% with OP. The maximum shear stress resulting from the regurgitant flow also increases from 3.9 Pa to 7.7 Pa and the affected region is much larger. This is particularly interesting given the fact that valve development has been linked with regurgitant flow in this region (Vermot, Forouhar et al. 2009; Garita, Jenkins et al. 2011). In the future we plan to develop OCT and OP as tools to precisely control cardiac function and deploy them to elucidate the complex interplay between structure and function in the developing heart in vivo.

REFERENCES

[1] Gargesha, M., M. W. Jenkins, et al. (2009). "High temporal resolution OCT using image-based retrospective gating." Opt. Express **17**(13): 10786-10799.

[2] Garita, B., M. W. Jenkins, et al. (2011). "Blood flow dynamics of one cardiac cycle and relationship to mechanotransduction and trabeculation during heart looping." Am J Physiol Heart Circ Physiol **300**(3): H879-891.

[3] Hove, J. R., R. W. Koster, et al. (2003). "Intracardiac fluid forces are an essential epigenetic factor for embryonic cardiogenesis." Nature **421**(6919): 172-177.

[4] Jenkins, M., M. Watanabe, et al. (2011). "Longitudinal Imaging of Heart Development with Optical Coherence Tomography." IEEE Journal of Selected Topics in Quantum Electronics: in press, Article first published online: 30 August 2011.

[5] Jenkins, M. W., D. C. Adler, et al. (2007). "Ultrahigh-speed optical coherence tomography imaging and visualization of the embryonic avian heart using a buffered Fourier Domain Mode Locked laser." Opt. Express **15**(10): 6251-6267.

[6] Jenkins, M. W., A. R. Duke, et al. (2010). "Optical pacing of the embryonic heart." Nat Photonics **4**: 623-626.

[7] Jenkins, M. W., L. Peterson, et al. (2010). "Measuring hemodynamics in the developing heart tube with four-dimensional gated Doppler optical coherence tomography." J Biomed Opt **15**(6): 066022.

[8] Poelmann, R. E., A. C. Gittenberger-de Groot, et al. (2008). "The development of the heart and microcirculation: role of shear stress." Med Biol Eng Comput **46**(5): 479-484.

[9] Reckova, M., C. Rosengarten, et al. (2003). "Hemodynamics is a key epigenetic factor in development of the cardiac conduction system." Circulation Research **93**(1): 77-85.

[10] Taylor, C. A., T. J. R. Hughes, et al. (1998). "Finite Element Modeling of Three-Dimensional Pulsatile Flow in the Abdominal Aorta: Relevance to Atherosclerosis." Annals of Biomedical Engineering **26**(6): 975-987.

[11] Vennemann, P., K. T. Kiger, et al. (2006). "In vivo micro particle image velocimetry measurements of blood-plasma in the embryonic avian heart." Journal of Biomechanics **39**: 1191-1200.

[12] Vermot, J., A. S. Forouhar, et al. (2009). "Reversing Blood Flows Act through klf2a to Ensure Normal Valvulogenesis in the Developing Heart." Plos Biology **7**(11): -.