ARC'18
مؤتمر مؤسسة قطر السنوي للبحوث
QATAR FOUNDATION ANNUAL RESEARCH CONFERENCE
البحث والتطوير: التركيز على الأولويات، وإحداث الأثر
R&D: FOCUSING ON PRIORITIES, DELIVERING IMPACT
19-20 مـــارس
19-20 MARCH


## Health and Biomedical - Poster Display

http://doi.org/10.5339/qfarc.2018.HBPD769

# Generating an imagingbased approach for enhanced structural and functional analysis of zebrafish cardiovascular systems

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Cardiovascular diseases (CVDs) have latterly become one of the leading cause of death and impairment worldwide. According to the World Health Organization (WHO), data estimation in 2008 were about 17.3, 7.3 and 6.2 million death cases for CVDs, heart attacks, and strokes, respectively [1]. Congenital heart defects (CHDs) are important forms of CVDs affecting about 1% of the population. According to Dr. Ahmad Sallehuddin (Consultant and Chief of Pediatric Cardiac Surgery at HMC), CHD incidence in Qatar is about 6-8 in every 1,000 births and about 100 new patients with CHDs require surgery every year [4]. Both genetic and environmental factors were shown to contribute to CVDs. Human genetic studies are not sufficient alone to explain the genetic basis of CVDs due to disease heterogeneity, inconsistent penetrance, and predominantly a delayed onset of symptoms. Therefore, animal models are necessary to investigate and distinguish novel genes that contribute to the pathology of CVDs and also to unravel environmental factors that play role [5]. More recently, the Danio rerio (Zebrafish) has emerged to become an intriguing vertebral model in medical science research. This model has several advantages, such as an almost entirely sequenced genome that is highly preserved with humans: approximately 70% of the human genes are estimated to have orthologue genes in the zebrafish genome [2,8]. The features of the zebrafish over mammals as a vertebral model include its simplicity of genetic manipulation, a large quantity of offspring, and their external and fast development [5,8]. Transparency of zebrafish embryos provide precise observation of the heart beats, heart chambers and circulating blood in vivo via light microscopy [5]. Additionally, passive diffusion is sufficient for oxygen delivery during early stages of zebrafish




Cite this article as: Yalcin H et al. (2018). Generating an imagingbased approach for enhanced structural and functional analysis of zebrafish cardiovascular systems. Qatar Foundation Annual Research Conference Proceedings 2018: HBPD769 http://doi.org/10.5339/qfarc.2018.HBPD769.

embryos development because they are independent of circulatory system [7]. Therefore, embryos having intensive cardiac faults survive early development, which enables investigation of mutations whereas mammalian models are not appropriate. There are many genetic cardiovascular models for zebrafish that allow researchers to investigate cardiovascular diseases. Novel techniques are needed for these models to assess comprehensive and quantitative phenotyping of mutant hearts and blood vessels. These analyses involve determination of the cardiac atrial and ventricular shortening fractions, examination of the blood flow velocities and recording of the electrocardiograms. In addition to genetic studies, cardiotoxicity investigations for drugs or teratogens necessitate heart function and morphology assessment. For the current study, the aim is to adapt previously established experimental and image analysis techniques to zebrafish studies, which will enhance structural and functional analysis of the zebrafish cardiovascular systems in biomedical research Methodology: This project involves generation of two main protocols, which are for structural and functional analysis of zebrafish embryos. Both these analyses are required to be performed to assess the severity of induced heart defects in zebrafish embryos. For the structural analysis, we will perfuse zebrafish (3 -5 days post fertilization (dpf)) hearts with microfilm, a CT- dense agent. Once it polymerises, a cast is generated for heart lumen. The embryos are then fixed with paraformaldehyde and scanned via micro-CT. This procedure provides 3D images for the cardiovascular systems of the animals enabling measurement of the volumes of the heart chambers. This technique enables us to measure the effect of induced heart mutations on the heart morphology during the embryonic growth of the animal. We have previously used this technique for visualization of cardiac chambers for embryonic chicken successfully [3,10]. The approach is adapted for zebrafish studies in the current work. For the functional analysis, image sequences of the beating ventricles and the red blood cell (RBC) movements of the zebrafish larvae (3 -5 dpf) are recorded at a camera speed of 70 fps and above. This imaging is done by taking videos using Micromanager Program that is connected to a stereomicroscope. To take image sequences, zebrafish larvae are placed on its lateral side, in which the right side will be on top. A variety of measurements and calculations are performed using Image J Program to assess heart functions. The parameters that are calculated include myocardial wall velocities for assessment of severity of induced heart muscle defects, and measurement of RBC blood flow velocities for assessment of rhythmic beating defects of the heart. Other parameters are fractional area change, fractional shortening, heart rate, stroke volume, cardiac output and ejection fraction [9]. Stroke volume, is the blood volume that is ejected from the heart in each beat, cardiac output, is the amount of circulating blood across the heart in each minute, and ejection fraction, is the measurement of blood that is ejected from the ventricle with each beat. This will provide more precise and accurate evaluation on the heart function for genetically mutated animals to be compared with the normal ones. Several zebrafish mutants display phenotypic features resembling human cardiac diseases. These mutants whose molecular damages have been determined like mutations in regulatory myosin light chain, titin, cardiac troponin T, essential myosin light chain, and beta-myosin heavy chain, have been linked with cardiomyopathy in humans [6]. Therefore, reduced contractility, myocardial wall velocities, and cardiac output can be studied as a sign of having cardiomyopathy. Accordingly, opening ways to apply drugs for morphological and structural evaluation of the heart. In conclusion, in this study, we are developing imaging-based techniques for accurate structural and functional analysis of the zebrafish hearts through adapting previously established methods on another animal systems. Once we establish our approach, analysis methods will be readily available for other Zebrafish researchers.