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Quantification of the detailed cardiac left ventricular trabecular morphogenesis in the mouse embryo

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ABSTRACT

During embryogenesis, a mammalian heart develops from a simple tubular shape into a complex 4-chamber organ, going through four distinct phases: early primitive tubular heart, emergence of trabeculations, trabecular remodeling and development of the compact myocardium. In this paper we propose a framework for standardized and subject-independent 3D regional myocardial complexity analysis, applied to analysis of the development of the mouse left ventricle. We propose a standardized subdivision of the myocardium into 3D overlapping regions (in our case 361) and a novel visualization of myocardial complexity, whereupon we: 1) extend the fractal dimension, commonly applied to image slices, to 3D and 2) use volume occupied by the trabeculations in each region together with their surface area, in order to quantify myocardial complexity. The latter provides an intuitive characterization of the complexity, given that compact myocardium will tend to occupy a larger volume with little surface area while high surface area with low volume will correspond to highly trabeculated areas.

Using 50 mouse embryo images at 5 different gestational ages (10 subjects per gestational age), we demonstrate how the proposed representation and complexity measures describe the development of LV myocardial complexity. The mouse embryo data was acquired using high resolution episcopic microscopy. The complexity analysis per region was carried out using: 3D fractal dimension, myocardial volume, myocardial surface area and ratio between the two. The analysis of gestational ages was performed on embryos of 14.5, 15.5, 16.5, 17.5 and 18.5 embryonic days, and demonstrated that the regional complexity of the trabeculations increases longitudinally from the base to the apex, with a maximum around the middle. The overall complexity decreases with gestational age, being most complex at 14.5. Circumferentially, at ages 14.5, 15.5 and 16.5, the trabeculations show similar complexity everywhere except for the anteroseptal and inferolateral area of the wall, where it is smaller. At 17.5 days, the regions of high complexity become more localized towards the inferoseptal and anterolateral parts of the wall. At 18.5 days, the high complexity area exhibits further localization at the inferoseptal and anterior part of the wall.

1. Introduction

The development of the myocardium appears to be similar in many vertebrates. In particular, before the intramural cardiac vessels appear, the ventricular walls consist mainly of trabeculations. Those early trabeculations increase myocardial surface area and serve to increase myocardial oxygenation and nutrient delivery by diffusion, while sufficient tissue volume builds up for myocardium development at the later stages. However, the decrease in trabecular complexity does not lead to a disappearance of the trabeculations. A number of animal studies suggest that smooth ventricles lead to severe heart failure and elevated embryonic lethality (Captur et al., 2015). On the other hand, it was observed that excessive trabeculations can lead to heart failure, atrial

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and ventricular arrhythmias and thromboembolic events (stroke) (Ritter et al., 1997; Oechslin et al., 2000; Kovacevic-Preradovic et al., 2008; Penela et al., 2013; Oechslin and Jenni, 2011). Trabeculated myocardium can be seen in normal hearts and can be frequently seen at younger ages, diminishing with age, with some gender differences (Dawson et al., 2010). A high proportion of young athletes, especially of African/Afro-Caribbean origin, also exhibit an increased amount of trabeculations (Gati et al., 2013).

One of the animal models to investigate the developing heart and related pathologies is the mouse embryo, where genetic alteration have been found to affect normal cardiac development, leading to either decreased or increased trabeculations. The trabeculae first appear at gestational age (GA) of about 9.0–9.5 embryonic days, at the end of cardiac looping. By GA14.5 ventricular septation is complete and a dense trabecular meshwork is established. At this moment, trabecular meshwork starts to become less complex and intertrabecular spaces seem to transform into the vessels (Captur et al., 2016). At the same time, papillary muscles, the moderator band, and effective arterial valves are starting to form.

Recently, a quantification of myocardial complexity of mice embryos was presented (Captur et al., 2016). The authors proposed to use fractal dimension (FD), measured on the myocardial contours in 2D short axis and 2-chamber MRI image stacks. The authors demonstrated that trabecular profiles increase from base towards the mid/apical region and decrease towards the apex. They also demonstrated that during later development, from the establishment of the interventricular septum at GA14.5 to GA18.5, trabecular complexity reduces and its decline is not proportional to the increase in compact wall volume. Even though the 2D FD measurement correlates with the visual perception of complexity, it has several drawbacks when applied to non-linear fractal structures such as natural fractals. The first and the largest problem in FD calculation is a lack of consensus with respect to the choice of its parameters. For example, in the typically used box counting, one has to decide on the minimum and maximum block size, the step for the block shift, how to perform the block shifts as well as how many to do, and finally, how to fit a line to the box counts (Foroutan-Pour et al., 1999; Jelinek et al., 2006; Karperien and Jelinek, 2016). Any variation in these parameters can significantly affect the FD. Another problem with 2D FD for analysis of myocardium is that the calculated values do not represent the 3D structure, and 2D slice-by-slice analysis inherently restricts the applicability of a larger range of methods.

In this paper, we propose a framework for 3D myocardial complexity assessment in terms of 3D FD and myocardial surface area to volume ratio. Such ratio represents an important parameter in diffusion processes and is widely used in chemistry, physics, biology and physiology (Latour et al., 1993; Yuan et al., 2010). To asses the myocardial complexity in each region, we propose to use a moving window (encompassing the neighboring regions), calculating the volume of the myocardium within the window and the area of the myocardial surface in contact with the blood. The surface area is expected to increase with the amount of blood cavities within the myocardium, while the volume increases with the amount of tissue filling the region. Therefore, highly trabeculated areas will have high area and low volume measures, while compact tissue will have low area and high volume. In our experiments, we demonstrate that the regional ratio of myocardial surface area to volume increases with myocardial complexity.

The proposed methodology was used to study the development of cardiac trabeculations in the left ventricle (LV) of mouse embryos across different gestational ages. The framework is based on the recently introduced myocardium reparameterization (Paun et al., 2017), where an individual shape-independent method for establishing point-to-point correspondence between the cardiac ventricles is proposed. Consequently, that method provides a way of subdividing the ventricles into 3D regions in a standardized way for inter-individual comparison of structural and shape properties as well as studying temporal development. The myocardial complexity can then be estimated on a per region basis with regions of any desired size. The study was performed on the set of 50 mouse embryos, acquired at different gestational ages: from GA14.5 embryonic days, when the ventricular septation is complete and a dense trabecular meshwork is established within the ventricular cavities, through GA15.5, GA16.5, GA17.5, to GA18.5 embryonic days, during the simultaneous processes of decrease in trabecular complexity and continued growth of the compact myocardial layer. The myocardium was subdivided into 361 regions (9 slices in longitudinal direction (apex-base) and 40 in circumferential direction (septal–inferior–lateral–anterior) plus apex). In each of these regions we calculated the 3D FD, myocardial volume, myocardial surface area, and surface to volume ratio. We show how these measures represent the myocardial complexity for each gestational age and how they do evolve from one age to another, emphasizing the differences. Finally we discuss the advantages and disadvantages of each of the measures.

2. Data

All specimens were handled in compliance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health and with the approval of the MRC National Institute of Medical Research Ethical Review Panel. Mouse (*Mus musculus*) embryos were obtained from NIMR:Parkes (a robust outbred strain maintained at the MRC National Institute of Medical Research). For approximate embryo staging, detection of a vaginal plug was taken as gestation day 0.5 (GA0.5).

In order to minimize retention of blood in embryo hearts, harvested embryos were first agitated in phosphate buffered saline (PBS) solution at 37 °C containing heparin for approximately 15 min, while all umbilical vessels being repeatedly clipped to allow blood to be pumped out. Potassium chloride was then added (final 50 mM) to ensure that hearts arrested in diastole. Hearts (including attached lungs and thymus) were then isolated, washed briefly in fresh PBS and after removal of at least one lung lobe, samples were fixed for 30 min in fresh 4% paraformaldehyde at 4 °C. To remove remaining blood within the heart chambers, samples were then washed in repeated changes of distilled water over $30-60$ min at room temperature with constant agitation (roller). The resulting osmotic shock lysed any remaining blood within the heart chambers without any alteration to heart structure as assessed by histology. After overnight fixation in 4% paraformaldehyde (4 °C), hearts were dissected away from associated lung, thymus and pericardial tissue prior to dehydration and embedding in methacrylate resin (Mohun and Weninger, 2012). Samples were similarly positioned during embedding to ensure relatively reproducible base-to-apex sectioning during the high resolution episcopic microscopy (HREM) imaging process.

The prepared samples were then used for the HREM analysis as described in Weninger et al. (2006). Briefly, HREM uses block-face imaging to produce perfectly registered digital image stacks capturing the 3D architecture of the embryonic heart at high resolution. Resulting datasets comprise 1000–2000 digital, short-axis images, produced by repeated removal of 2 μm (GA14.5–GA16.5) or 3 μm (GA17.5, GA18.5) sections (base-to-apex direction). The volumetric visualizations of the datasets from each GA can be seen in Fig. 1.