NON-INVASIVE TOOL TO ASSESS HEART RHYTHM IN ZEBRAFISH EMBRYOS

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INTRODUCTION

In the last years the zebrafish (Danio rerio) has emerged as model organism for cardiac research, in spite of the morphological differences with the human heart. In consequence of the similarity to humans in the early function, the zebrafish embryo has been suggested as an ideal model i) to study the molecular mechanism of cardiac development, and ii) to identify genes related to congenital cardiac defects in human [1]. The overall similarity of zebrafish embryos and human, in responses to human cardiotoxic drugs, was demonstrated, for example, in drug-induced cardiac arrhythmia [2]. For this reason, several methods have been developed to assess cardiac functions in zebrafish embryos [3,4]. Unfortunately, all these techniques suffer from drawbacks (time consuming, skillful operator are ended to perform the experiments) which limit their applications for large scale studies. The development in digital imaging has recently made analysis of cardiac functions in genetically modified transparent zebrafish embryos easier. This allowed to assess non-invasively heart rate variability in zebrafish embryos from videos of beating heart, but without measuring heartbeat rhythm, an important indicator of the cardiac function (heartbeat regularity is associated with cardiotoxicity in humans [1]), from power spectrum of heart signal. In the present study, we present a simple, non-invasive method that, by video-recording embryo images using confocal microscopy, and integrating image processing and power spectral analysis, allows to measure the heartbeat rhythm in zebrafish embryos heart chambers (atrium, ventricle, bulb) (Figure 1). The reliability of the herein proposed method was verified. Some embryos undergone treatment by tricaine, a cardiac anaesthetizing drug, in consequence of which a decrease of the heart rate is expected: the heartbeat regularity in tricaine-treated embryos determined from power spectral analysis decreased as compared to no-treated embryos. The results demonstrated that our method is able to assess the cardiac physiology, in term of heart rhythm, in zebrafish embryos.

METHODS

Zebrafish embryos at the developmental stage of 96 hpf were mounted in 0.5% agarose (dissolved in embryo medium) inside of a viewing chamber and analyzed with a Leica confocal laser-scanning microscope (model TCS SP5) with a zoom factor of 2,08. In this study, we used the zebrafish transgenic line Tg(flk1:GFP)s843 and Tg(gata1:dsRed)sd2, which express green fluorescent protein (GFP) in the vasculature [5], and dsRed (red fluorescent protein) in blood cells, respectively. Treated and no-treated embryos were investigated: treated embryos were anesthetized with a tricaine solution at the final concentration of 4mg/ml, in order to depress the cardiac rhythm. Images (20× magnification) including atrium, ventricle, and aortic bulb (Fig. 1) were captured (4 s image recordings) in bidirectional mode at a 71 fps sampling frequency. Recordings were grabbed and stored in TIFF format.

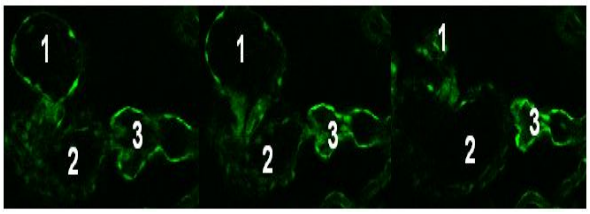

Figure 1: the images (green channel of the RGB images) show three different phases of the cardiac cycle. The three chambers, and their dynamics (atrium(1), ventricle(2), bulb(3)) are clearly distinguishable.

A custom made software was developed in Matlab® environment to perform digital analysis on the acquired images. Technically, a frame-grabber was used to grab video frames. To monitor the heart beat, only the red scale of each RGB frame was considered. Images of embryo's ventricle, atrium, and bulb, respectively were acquired. Considering that i) a 8 bits image intensity colour range is [0-255], and that ii) the value of static pixels located in non-moving areas was zero, to reduce noisy artefacts we fixed a threshold value of 100 in pixel intensity over each frame, and the amount of static over-threshold pixels was counted, and expressed as percentage to total number of the pixels (200 x 512) of each frame.

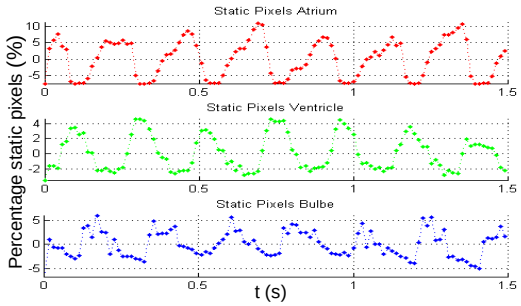

Figure 2: A: typical example of the static pixels signal: the three curves are relative to the same acquisition; we observed that atrium peaks (upper panel) anticipated, in time, the ventricle peaks (mid panel), and those peaks anticipated the bulb peaks (lower panel).

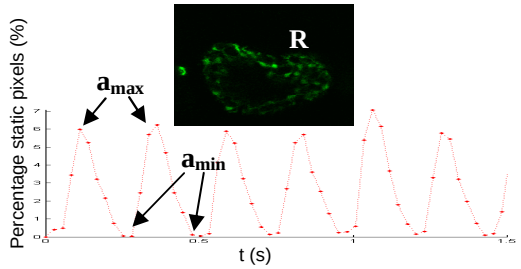

Figure 3: Example of the time history of static pixels extracted from images of the bulb, shown in the inset (a_{max}: maximum values of static pixels; a_{min} : minimum values of static pixels) The heart beat is given by the time interval between two consecutive peaks

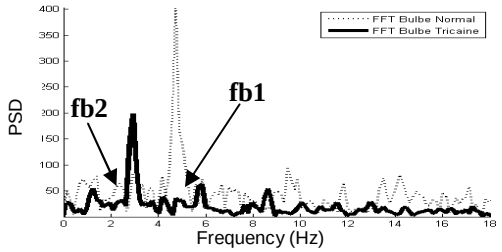

Figure 4: Power spectrum obtained from the bulb analysis of a no treated and a sedated embryo. Arrows indicate the main frequency components fb1 end fb2.

The percentage values of the over-threshold static pixels within each frame were plotted versus the time of acquisition. An example of the corresponding waveforms as extracted from the three heart chambers of the not treated embryo, is depicted in figure 2. The automated evaluation of the heart rhythm of zebrafish embryos was performed by means of a spectral analysis: the Fast Fourier Transform (FFT) algorithm was applied for the calculation of the power spectral density (PSD).

RESULTS AND DISCUSSION

The recorded videos allowed to appreciate that flow of blood cells was clearly seen inside blood vessels in zebrafish embryos. This flow was pulsatile, with a rhythm of fast and slow movement. Our image analysis allowed to record this oscillatory movement of blood cells and to map it into a time history. It can be noticed from figure 2 that i) a rhythm of fast and slow movement was present within the extracted time histories, and ii) a phase shift among waveforms extracted from atrium, ventricle and bulb was present, as expected from the physiology on the heart (e.g., peaks in the ventricular waveforms, which were expected to represent the peak ventricular systole, were delayed with respect to the atrial corresponding peaks). A typical example of waveform of static pixels for a no treated 96 hpf zebrafish embryo is shown in Figure 3. The waveform exhibited a rapid upstroke (a_{max}) of the amount of static pixels followed by a drop (a_{min}), showing the rhythmic change of blood cell velocity. This rhythmic fluctuation of dynamic pixels was consistent with the direct observation of pulsatile flow of blood cells observed under confocal microscope. Figure 4 shows the PSD of waveforms extracted from bulb image analysis on a treated and a no treated embryo: the PSD clearly shows that the two embryos exhibited peaks in the spectrum at distinct frequencies (the sedated embryo at 2.923 Hz, the no-treated one at 4.733 Hz), in consequence of the anaesthetic administration (tricaine) to one of them. Hence, the lowered heart rhythm of tricaine-treated embryos indirectly validated the herein proposed method. In conclusion, our preliminary results demonstrated that an evaluation of cardiac rhythm in zebrafish embryos directly from images is feasible. The main advantage of using this method was that it was rapid and fully automated, thus avoiding genetists and biologists of measuring zebrafish embryos cardiac rhythm by visual inspection.

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