Novel Apparatus for Simultaneous Monitoring of Electrocardiogram in Awake Zebrafish

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***Abstract*—This paper reports polymer-based apparatus with embedded flexible thin-film electrodes to monitor electrocardiogram (ECG) of zebrafish under mild or zero anesthesia. The apparatus were made of polydimethylsiloxane (PDMS) using the molding technique with molds formed by 3D printing. The system is capable of acquiring intrinsic ECG from multiple fish simultaneously, thus introducing a novel method to phenotypically assess the hearts' functionalities, supporting heart regeneration studies, drug screening and other related bio-investigations using the zebrafish model. Smart algorithms were developed to facilitate data processing and analyses. The recorded ECG was compared with that of the same fish under full sedation and a proof-of-concept 4-chamber system was demonstrated. The process does not require a cleanroom, therefore being cost effective and holding promise to accelerate numerous studies in various disciplines using the zebrafish model.**

Keywords—Zebrafish; Electrocardiogram (ECG); Heart Diseases; Phenotype screening; Biological Investigations

I. INTRODUCTION

The zebrafish (*Dario rerio*) model is important for studies in diverse disciplines, including pharmacology, toxicology, neurobiology, behavioral and developmental biology. It has been used to study aspects of gene functions that can be directly related to human genetics and diseases. Unlike mammals, zebrafish possess a remarkable regeneration capacity after cardiac injury, thereby providing a tractable model system to study endogenous heart regeneration [1, 2]. Zebrafish have also proven to be an ideal vertebrate model system for phenotype-based screening owing to their physiological similarity to mammals. For instance, the zebrafish model enables a forward genetic approach to reveal the genetic basis and underlying molecular mechanisms of numerous heart diseases, including arrhythmic diseases which contributed about 350,000 deaths annually in the U.S. only [3]. Last but not least, it is not costly to maintain a vivarium and zebrafish genetically-modified models have been well established during the past decade.

Conventional assessment methods (e.g. immunohistochemical approaches, DNA and protein analyses) of adult zebrafish hearts can elucidate neither the progress of the process (i.e. regeneration and remodeling) of the same samples over time nor the overall myocardium functionalities. In contrast, our team and other groups have been alternatively using electrocardiogram (ECG) lately to monitor zebrafish hearts for phenotype screening and biological studies. Up to date, commercial available ECG acquisition systems, provide only single-channel ECG, are bulky and unable to detect T waves. In the past several years, we have demonstrated the acquisition of ECG in zebrafish using microelectrode array (MEA) membranes, providing favorable signal-to-noise ratio (SNR) with full features of P waves, QRS complexes and T waves [4]. The obtained ECG signals were clearly distinguishable between heart-injured fish and shams; thus enabling a novel tool to investigate zebrafish hearts. However, long-term monitoring required repeated ECG acquisitions with sedated animals, rendering the past work stressful to the fish and inadequate to provide intrinsic ECGs. Further, the determination of aberrant patterns in fish ECG was done manually, making it impossible for long-term screenings of a large quantity of fish.

In this work, we developed a low-cost ECG monitoring system for zebrafish, which can simultaneously record data from multiple awake subjects, thereby providing a novel approach to acquire intrinsic zebrafish ECG with high throughput. The system includes multiple small housings made of polydimethylsiloxane (PDMS) using 3D-printed molds. Flexible electrodes were embedded for acquisition, bringing comfort and thus minimizing motional artifacts. We also developed algorithms to automatically detect the shape and position of the characteristic waves of the recorded ECG with capacity to locate and quantify anomalies, paving the way for precise interpretation of "big ECG data" from multiple fish, otherwise remain impossible with manual processing. **Fig. 1** shows the conceptual design of a single-chamber apparatus.

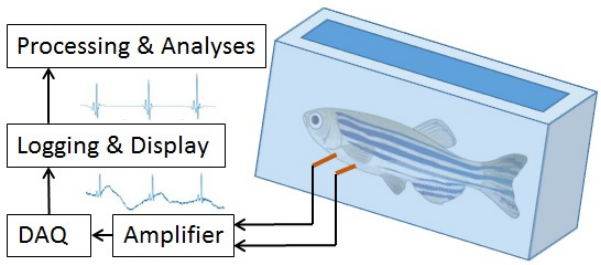

Fig. 1. Conceptual design of the system with PDMS housing.

II. Design, Methods and Implementation

A. PDMS Housing

The molds were designed using the online software TinkerCAD (*Autodesk, Inc., Mill Valley, CA*) and then printed using a 3D printer. The material was polylactic acid (*1.75-mm PLA, JustPLA, San Jose, CA*). There were two parts, a rectangular box with an inner length of 53 mm and width of 25 mm, and an oval-shape object with a length of 44 mm and a maximum width of 14.7 mm. First, PDMS (*Sylgard 184, Dow Corning, Midland, MI*) was poured into the rectangular box and then the oval mold was securely placed into the PDMS partially to form a tapered housing. After the PDMS was cured at room temperature for 24 hours, it was removed from the rectangular box. The oval mold was gently pulled out to make sure the apparatus remained the desired shape.

To integrate ECG readout electrodes, two strips of 125 µm-thick polyimide (*Kapton*, *DuPont, Wilmington, DE)* with sputtered Cu electrodes were inserted from the side of the apparatus through two thin-cut slits. The slits were then sealed by applying PDMS followed by a post-curing process. The strips were placed so that when the fish is loaded into the housing, the two electrodes would securely position at the chest and abdominal areas, acting as recording and reference electrodes, respectively. The process is illustrated in **Fig. 2**.

B. Experimental Setup

All experiments were in compliance with the Institutional Animal Care and Use Committee (IACUC) protocols (#4389-01) approved through the University of Washington to minimize the stress to animals. Adult zebrafish are considered under full anesthesia when they are sedated in a buffer solution with 150-200 mg/l tricaine methane sulfonate (Tricaine). First, an open-chest surgery was conducted to form an incision as described in [4], and then the fish would be ready for recording in several days to a few weeks.

Chambers could be filled with the fish system water containing different concentrations of Tricaine. **Fig. 2** (lower panel) shows a 4-chamber apparatus with 4 different zebrafish ready for simultaneously recording of ECG. The pairs of electrodes were connected to a differential amplifier (*A-M Systems Inc. 1700 Differential Amplifier, Carlsborg, WA*) to amplify the input ECG by 10,000-fold, using the bandpass range 0.1-500 Hz and at a cut-off frequency of 60 Hz (notch). The filtered signals were then digitized at a sampling rate of 1,000 Hz (*National Instruments USB-6251 DAQ device, Austin TX, and LabVIEW 2016*) before being recorded into a computer.

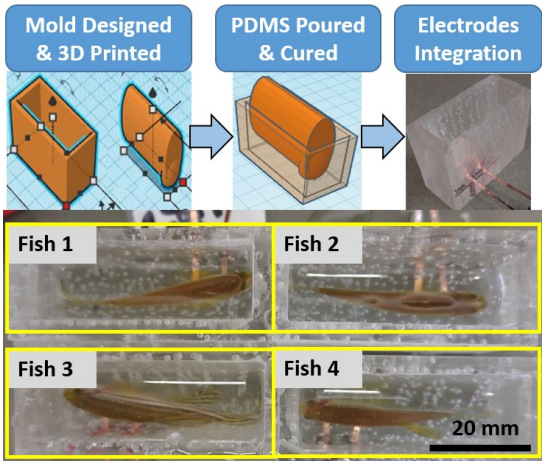

Fig. 2. Processes and the 4-chamber apparatus with fish.

C. Signal Processing and Interpretation

The obtained signals were de-noised using the Wavelet transform and thresholding technique as described in our previous work [4]. Here, we developed additional algorithms to accurately detect all peaks of ECG, namely P, Q, R, S and T; and then important segments, such as ST and QT, would be automatically determined and quantified. In our program, QRS complexes would be detected first, and then P and T waves. All was implemented using MATLAB (*R2016A, MathWorks, Natick, MA*), with the Wavelet and Signal Processing Toolboxes. The algorithm flow can be seen in **Fig. 3.**

First, the signal would be transformed from the time domain to the frequency domain using the Fast Fourier Transform (FFT), and then the peak screening window would be used to find out the temporary-peak positions. The window size was set as the average heart rate value or RR interval

$$Window\ size = \frac{60}{\text{RR interval}} \quad (1)$$

The size of window filter can be adjusted to get more or fewer peaks following

$$New\ window\ size = 2 * \min(\text{real}(\text{RR intervals}) - \text{QR interval} \quad (2)$$

All peaks will be scaled with the rate of the highest peaks as

$$Peak\ scaling = All\ peaks\ /\ (the\ highest\ peak\ /\ 7) \quad (3)$$

Then peak screening would be performed by thresholding. Those peaks that are 4/7 times higher than the highest peak will be determined as R peaks. Next, Q peaks were determined by finding the first lowest points to the left of R peaks. Similarly, S peaks can be found on the right of R peaks. The J intermediate point, which is the termination of the QRS complex and the beginning of the ST segment was determined as the rising point to the right of the S peak.

T waves could be detected after S peaks. In order to distinguish negative and positive ridges, any of which could be T, it's necessary to set a baseline or isoelectric line from the J point to the T peak; and then, we determine the minimum and

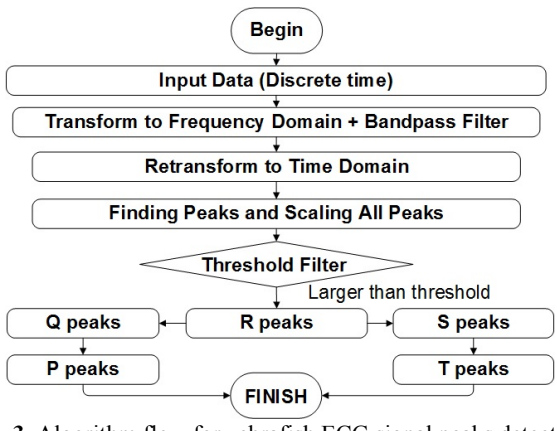

Fig. 3. Algorithm flow for zebrafish ECG signal peaks detection.

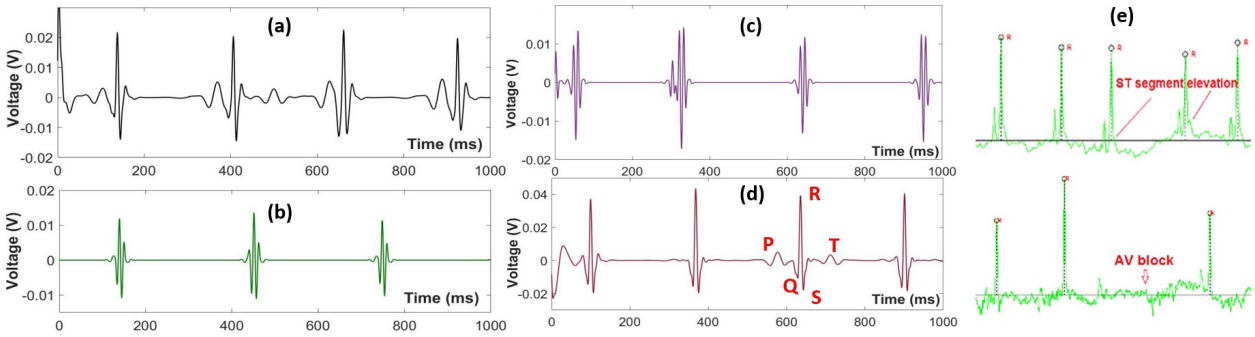

Fig. 5. (a) — (d) Simultaneous recording from 4 different fish. (**e**) The automated program found ST depression and AV block patterns.

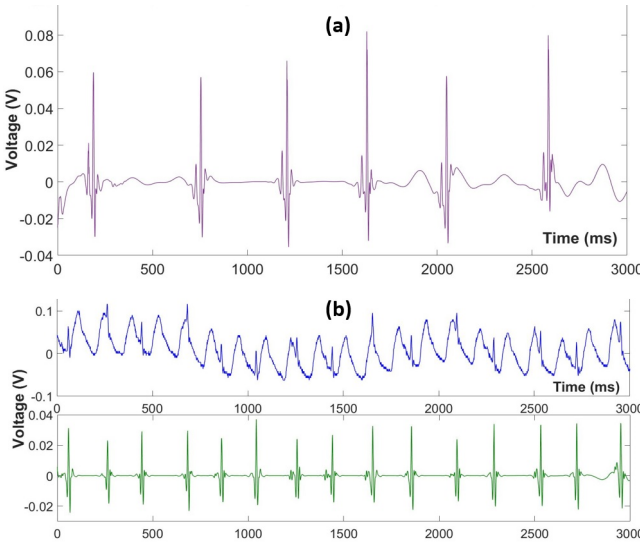

Fig. 4. ECG of fish (**a**) fully sedated and (**b**) under 50% sedation.

maximum points in the interval from the J point to the end of period. Comparing the two values, if the amplitude of maximum point is bigger, then the T wave is at the positive peak and vice versa. With the same approach, we can detect P waves. Unlike those of humans' ECG, the P waves in zebrafish ECG may have higher amplitudes compared to other peaks and they may be closer to the Q peaks, thus we chose P as the nearest temporary highest peak before R.

III. Results and Discussion

A comparison of ECG signals obtained from the same fish, under full anesthesia and under 50% anesthesia, is showed in **Fig. 4**. The upper panel in **Fig. 4b** displays the raw signal while the filtered one is showed in the lower panel. It can be seen that, the sedation drug has affected the cardiac activity of zebrafish, resulting in a much slower heartrate. The ECG recorded while the fish was partially awake, also comprised low-frequency artifacts from gill motions and even the beating heart; however, they were successfully removed by our de-noising technique using the Wavelet transform.

ECGs of 4 zebrafish under 50% anesthesia from simultaneous recording using our 4-chamber apparatus are shown in **Fig. 5 (a-d)**. Since the motional artifacts may be dominant in the obtained signal, we faced difficulties time to time, in order to extract full-feature ECG. For instance, only panel (**d**) provides an ECG with distinguishable P waves, QRS complexes and T waves. The T waves were diminished after signal processing in the rest, especially in (**b**) and (**c**). This calls for the development of better algorithms.

The peak detection and pattern analysis algorithms were then validated with various aberrant ECGs. ST elevation and atrioventricular (AV) block patterns, to name a few, were found and they are showed in **Fig. 5e.** ST elevation indicates acute myocardial infarction, coronary vasospasm, etc. while AV block may be due to ischemia infarction or fibrosis. The success rates for peak detection of P, Q, R, S, T peaks are 85%, 85%, 95%, 90% and 70%, respectively. It is obvious that T waves are the most challenging ones to determine.

IV. Conclusions

With the enormous amount of data from a large quantity of subjects used in bio-studies, automated analyses and interpretation algorithms are essential. The recent advances in big data and cloud computing could help facilitate the process in both studies and patient health monitoring. In this context, our demonstrated system with simultaneous monitoring of zebrafish as well as ECG pattern analyses, holds translational implications to realize integrated systems, for both animal models and humans, supporting healthcare and biological investigations.

Acknowledgment

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