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Bioelectrical Signal Analysis of Mouse Cardiomyocyte Culture recorded on Thin-Film-Transistor Sensor Arrays

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Abstract

The dynamical property of the heart bioelectrical system is closely associated with cardiac diseases. There is thus a growing interest in the development of system analysis for studying the cardiac signaling network. In this article, the electrical potentials of cardiac muscle cells have been measured on an array of microelectrodes using the Thin-Film-Transistor (TFT) technology, and electrophysiological data were analyzed. This study shows the possibility of obtaining and accurately analyzing extracellular signals measured on TFT arrays.

Keywords: Thin-Film-Transistor Arrays, Bioelectrical Signal Processing, Electrophysiology, Cardiomyocytes

1. Introduction

Cardiomyocytes are primary muscle cells derived from heart tissue that can generate and conduct bioelectrical signals for cardiac contraction and blood flow. A

problem occurring in the cellular bioelectrical network can range from minor to fatal inconvenience.¹ The cells can retain their physiological functions and thus provide a useful *in vitro* model to look at the beating rate, the duration and the shape of the field potential. *In vitro*

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research of the general behavior of cardiomyocytes can thus help understanding arrhythmias, long Q-T syndrome and cardiotoxicity, As a result, *in vitro* study of cardiomyocytes represent a valuable tool for drug discovery and cardiac research. However, the mechanism of the cardiac bio-signaling network is still poorly understood.

In light of this problem, this paper proposes the analysis of bioelectrical signals of cardiomyocytes measured on an array of microelectrodes using a new Thin-Film-Transistor (TFT) sensor array. Measurements of the bioelectrical potentials of neurons were already demonstrated with TFT arrays.² The data flow generated by large arrays must be compressed to envision compact data acquisition systems. Hence, the electrical signals have been analyzed using a MATLAB program developed for bioelectrical processing of electrogenic cells. The recorded signals were filtered for the detection of spikes, and grouped into clusters according to their similar features. Through this analysis, the experiments demonstrated the possibility of obtaining accurate spike sorting and analysis from extracellular recordings on TFT arrays.

2. Measurement Method

2.1. Thin-Film-Transistor Arrays

The TFT arrays were used for extracellular recordings *in vitro* of cardiomyocytes. Here, the TFT technology well-known for Liquid Crystal Display is used for biological applications.³ The standard type of TFT array comes in a pattern of 150 x 150 transparent microelectrodes and is mounted on a printed circuit board (PCB). Microelectrodes are composed of indium tin oxide (ITO) with a size of 100 x 100 μm^2 . The array of microelectrodes is controlled by an array of TFTs, which are used for switching ON/OFF their respective microelectrodes. The TFTs are controlled by means of gate and source/drain lines. The columns of the array control the gates of the TFTs, while the rows control the sources. When a 12V DC voltage is applied to one gate line, all the microelectrodes connected to that line are activated. Then one or more source lines were reconnected to a measurement system for sensing.

The bioelectrical signals of cardiomyocytes were measured using a Multi-Channel Measurement System (MCS USB-ME32-FAI-System) and optical

observations were performed with an inverted microscope. Fig. 1 describes the working principle of TFT arrays and the experimental setup.

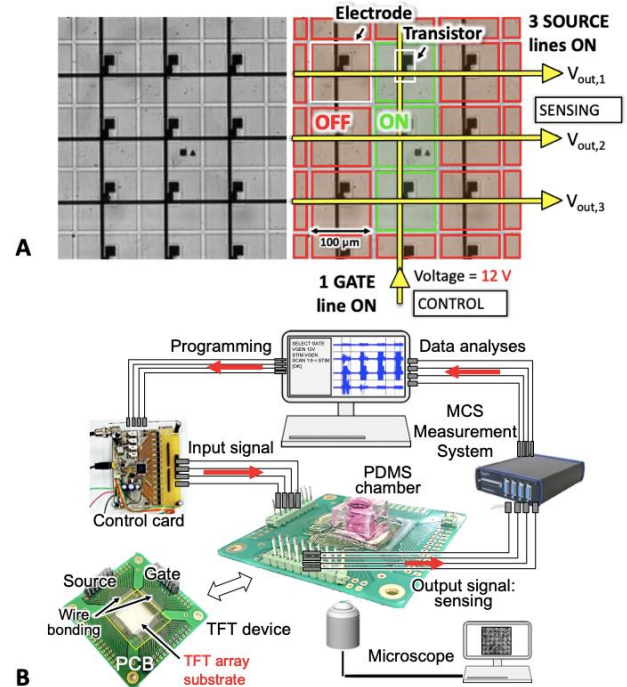


Fig. 1. (A) Working principle of the transparent TFT substrate, with a close-up view of the array of 100 μm square microelectrodes; (B) Experimental setup

2.2. Culture of Cardiomyocytes

Cardiomyocytes were dissociated into single-cell suspension from neonatal mice by combining mechanical dissociation with enzymatic degradation of the extracellular matrix, which maintains the structural integrity of tissues.

The neonatal hearts were enzymatically digested using a neonatal heart dissociation kit for mouse from Miltenyi Biotec and a dissociator was used for the mechanical dissociation steps. After dissociation, the sample was filtered to remove any remaining larger particles from the single-cell suspension, and the lysis of red blood cells was performed.

Cardiomyocytes were finally cultured for 3 days on the TFT array devices without surface treatment.

2.3. Bioelectrical Analysis

Embedded signal processing is an essential step in the development of recording instrumentation. Here, spike sorting algorithm was used. This data processing technique consists in identifying the cells that contribute to the signal recorded by each microelectrode, their number and their spiking activities.⁴ The identified basic functions are (1) bandwidth reduction for selective band amplification and noise reduction, (2) discrimination threshold computation, (3) extraction and alignment of biological spike signals, (4) data dimension reduction using principal component analysis (PCA) or spike shape features and finally (5) online spike clustering. Those functions are depicted in Fig. 2.

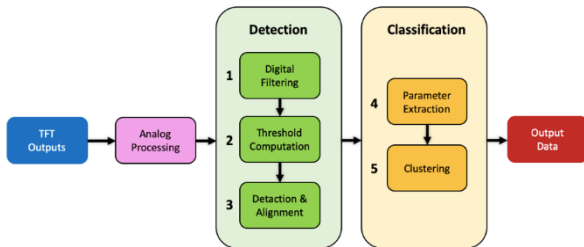


Fig. 2. Workflow of the functions performed for spike sorting

3. Results

3.1. Bioelectrical Signal Recording

In this study, the extracellular electrical potentials of cardiomyocytes were first recorded on 28x28 microelectrodes. The measured noise level was approximately 50 μ V. The bioelectrical activity of the culture of cardiomyocytes was confirmed by optical visualization of the cell contraction using the inverted microscope (Fig.3.). Here, a line of 4 microelectrodes was selected and data were extracted for the ensuing processing of the bioelectrical signals.

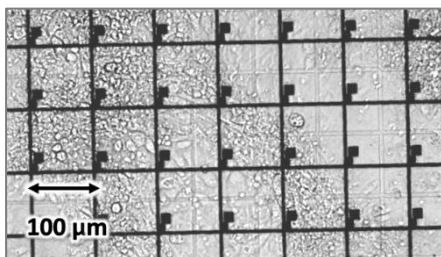


Fig. 3. Culture of cardiomyocytes on TFT array device, 3 days after cell seeding.

3.2. Bioelectrical Signal Processing

Bioelectrical signal processing of the data from several measurements was performed. Each analysis provided valuable information about the bioelectrical signals of the cardiomyocytes. In this study, the data from a decrease in temperature has been analyzed.

3.2.1. Filtered Data

Raw data were first filtered to remove undesired signals according to their frequency. This low-pass filter passes signals with a frequency lower than the selected cutoff frequency of 200 Hz. The sampling rate of the recorded data was 10 kHz. Fig. 4. shows an example of data obtained before and after filtering.

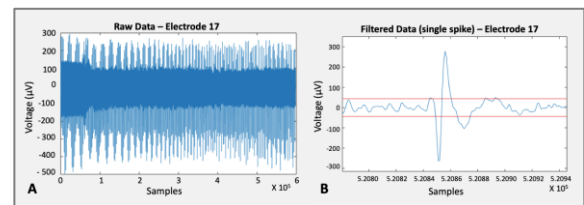


Fig. 4. (A) Raw data of the total measurement time (7 min) and (B) close-up view of a single spike on filtered data.

3.2.2. Spike Detection and Alignment

The action potentials of cardiomyocytes are then distinguished from the noise according to a predefined threshold. The spikes are then aligned with respect to the maximum absolute value of the detected signal as shown in Fig. 5.

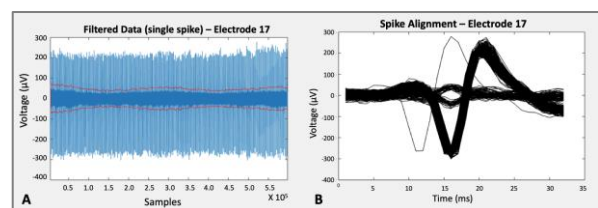


Fig. 5. (A) Filtered data and threshold lines in red for spike detection, and (B) spike alignment one 1 microelectrode.

3.2.3. Spike Intervals

For each microelectrode, the spike intervals are classified. Histograms display the number of spikes according to their period (Fig.6.).

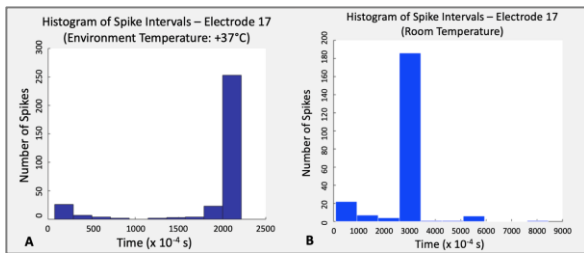


Fig. 6. (A) Histogram of the spike intervals detected on 1 microelectrode when the temperature of the culture chamber is at +37°C and (B) at room temperature (RT). In this study, the spike intervals increased with decrease of temperature: from 190 ms (5.3 Hz) at +37°C to 270 ms (3.7 Hz) at RT.

3.2.4. Clustering

Spikes were finally divided into a number of clusters such that spikes of the same group are more similar than those in other groups. To reduce sample space dimension, Principal Component Analysis (PCA) was used. This statistical technique provides features that are directions in the high-dimensional space. In this study, each spike became a point in a 3-dimension space.

4. Discussion

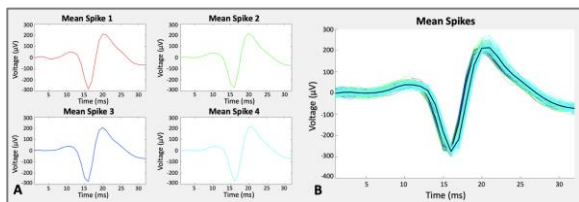


Fig. 7. (A) Mean spikes of each 4 clusters identified after analysis of 4 microelectrodes, and (B) alignment of the mean spikes from each cluster.

The transparent TFT array device can provide a high spatial resolution of cell culture activity by surpassing currently available MEAs in terms of density of microelectrodes, and its cm-sized measurement surface area. With regard to the bioelectrical analysis, the spike sorting program allowed to extract useful information about the bioelectric signals generated by cardiomyocytes. A decrease of the beating rate with the temperature (from +37°C environment to room temperature) at around -6.4 beats per minute/°C on each microelectrode is confirmed by the program. A possible explanation would be that the temperature drop depresses

the speed of ion exchange, as it increases the permeability of the membrane to ions. The program also identified a strong irregularity of the peak-to-peak voltage amplitude with the decrease in temperature. However, one main cluster of spikes is identified for each microelectrodes and share equivalent waveform of the mean spikes. This waveform is highly similar to the typical cardiac action potential in electrocardiogram with the typical T wave associated with ventricular repolarization. A spike raster plot also revealed the coincidence of spike occurrence for each microelectrode. This confirms the synchronicity of the bioelectrical conduction among the cell culture, which was also observed in parallel with the microscope.

5. Conclusion

In this paper, the bioelectrical activity of cardiomyocytes was successfully measured on TFT sensor arrays and analyzed by using spike sorting technique.

This analysis confirms that TFT arrays can efficiently detect the bioelectrical signals generated by cardiomyocytes. Combining this technique with deep-learning algorithms⁵ could allow the *in vitro* identification of abnormal cardiac cell conduction and aid for drug screening.

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