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. For this reason there is 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 Thin-Film-Transistor (TFT) technology, and electrophysiological data were analyzed. This study shows the possibility of 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* research of the general behavior of cardiomyocytes can help to understand arrhythmia, long Q-T syndrome, and cardiotoxicity. As a result, *in vitro* study of cardiomyocytes represents a valuable tool for drug

discovery and cardiac research. However, the mechanism of the cardiac biosignaling 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*

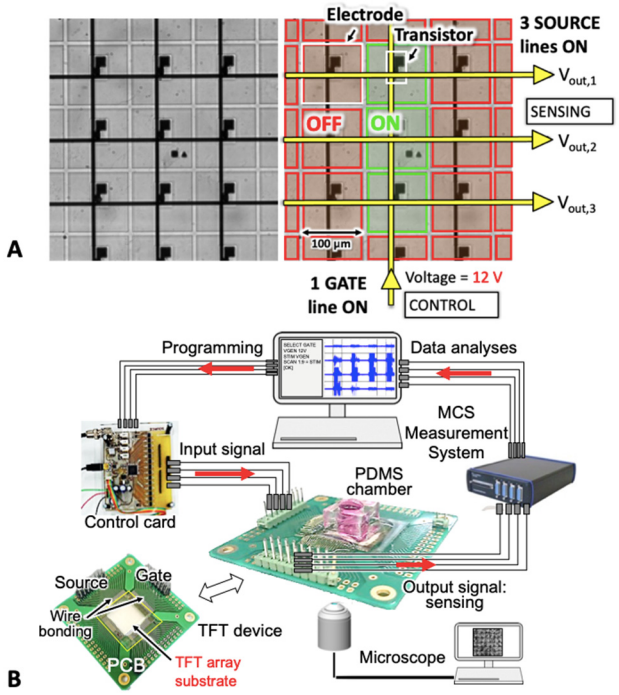
The TFT arrays were used for extracellular recordings *in vitro* of cardiomyocytes. TFT technology is well-known for Liquid Crystal Display in appliances including television sets, computer monitors, or mobile phones. Here, TFT technology is used for biological applications.[3]

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 the 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 are connected 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 simultaneously 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 red blood cell lysis 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, a spike sorting algorithm was used for data analysis. 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.

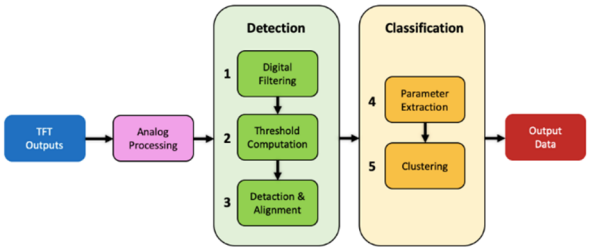


Fig. 2. Workflow of the functions performed for spike sorting.

3. Results

3.1. *Bioelectrical Signal Recording*

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

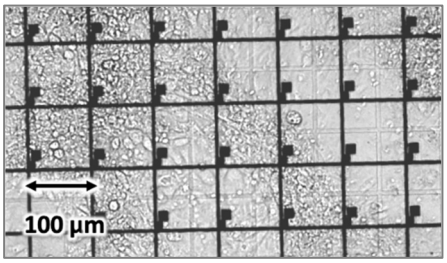


Fig. 3. Culture of cardiomyocytes on TFT array device, 3 days after cell seeding.

3.2. *Bioelectrical Signal Processing*

Bioelectrical signal processing of the data acquired from the measurements was performed. Each analysis provided valuable information about the bioelectrical signals of the cardiomyocytes. In this paper, the measurement data of 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.

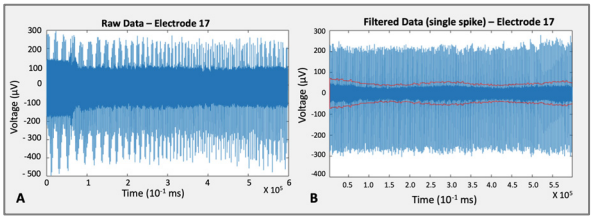


Fig. 4. (A) Raw data of the total measurement time (7 min) and (B) close-up view of a single spike on filtered data.

3.2.2. Spike Detection and Alignment

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

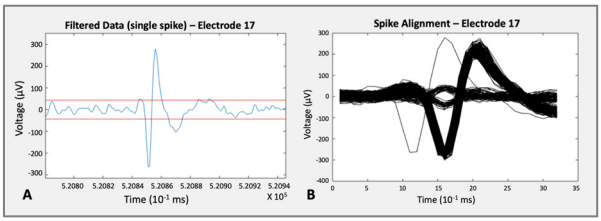


Fig. 5. (A) Filtered data and threshold lines in red for spike detection, and (B) spike alignment for 1 microelectrode.

3.2.3. Spike Intervals

For each microelectrode, the spike intervals were then classified. Fig. 6 shows the histograms that display the number of spikes according to time interval.

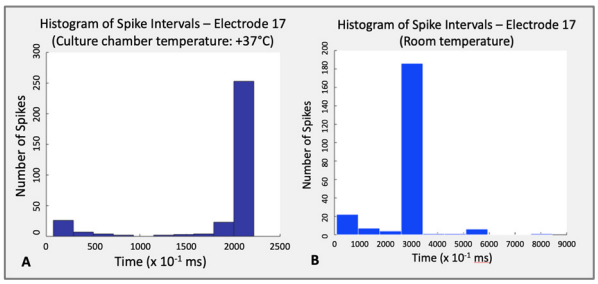


Fig. 6. (A) Histogram of the spike intervals detected on 1 microelectrode when the temperature of the culture chamber is at +37°C and (B) at room temperature (RT). In this study, the spike intervals went from 200 ms (5.0 Hz) at +37°C to 270 ms (3.7 Hz) at RT.

3.2.4. Clustering

Spikes were finally divided into clusters. 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. Fig.7 shows the mean spikes for each cluster.

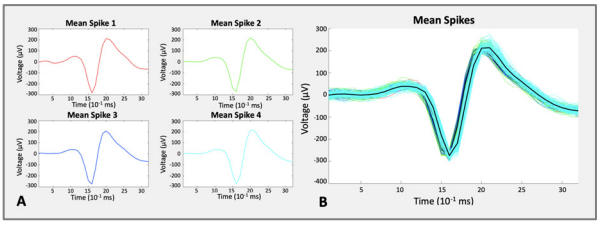


Fig. 7. (A) Mean spikes of each 4 clusters identified after analysis of 4 microelectrodes, and (B) alignment of the mean spikes of each cluster.

4. Discussion

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 the extraction of useful information about the bioelectric signals generated by cardiomyocytes. Decrease of the beating rate with temperature (from +37°C to room temperature) was observed during the data acquisition with MCS measurement system. This observation is confirmed by the program with a decrease rate of around – 6.5 beats per minute/°C for each microelectrode. 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 of temperature. Although one cluster of spikes is identified for each microelectrode, they share an 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 spike synchronicity between 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 a spike sorting technique. This analysis confirms that TFT arrays can efficiently detect the bioelectrical signals generated by cardiomyocytes. Combining this technique with deep-learning algorithms[5] could allow the *in vitro* identification of abnormal cardiac cell conduction and aid for drug screening.

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