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Research Article

Spike Sorting Tool for Analysis of Cardiac Extracellular Signals Recorded by 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, extracellular potentials of cardiac muscle cells were measured on an array of microelectrodes using Thin-Film-Transistor (TFT) technology, and electrophysiological data was analyzed using a spike sorting technique. This study shows the possibility of extracting useful bioelectrical information from the extracellular signals recorded by TFT arrays.

1. INTRODUCTION

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

However, signal processing and data analysis of extracellular recordings remain intensive, and new tools are needed to make electrophysiological data handling and analysis easier.

In light of this problem, this paper proposes a spike sorting algorithm for the analysis of cardiac extracellular potentials measured on an array of microelectrodes using a new Thin-Film-Transistor (TFT) sensor array developed by Sharp Corporation. Measurement of neuronal potentials was 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 biosignal processing. The recorded signals were filtered for the detection of spikes, which were then grouped into clusters according to their similar features. Through this analysis, the experiments demonstrated the possibility of obtaining accurate spike sorting of extracellular recordings on TFT arrays. Future implementation of the program in Field Programmable Gate Array (FPGA) will allow real-time analysis for automatic cell stimulation with a closed-loop system.

2. MEASUREMENT METHOD

2.1. Thin-Film-Transistor Arrays

Thin-film-transistor arrays were used for *in vitro* recording of extracellular signals from 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 150×150 transparent microelectrodes and is mounted on a printed circuit board. Microelectrodes are composed of indium tin oxide with a size of 100×100 μm². 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

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source/drain lines. The columns of the array control the gates of the TFTs, while the rows control the sources. When a 12 V DC voltage is applied to one gate line, all the microelectrodes connected to that line are activated. One or more source lines are then connected to a measurement system for sensing.

Extracellular signals of cardiomyocytes were measured using a Multi-Channel Measurement System and optical observations were performed simultaneously with an inverted microscope. Figure 1 describes the working principle of TFT arrays and the experimental setup.

2.2. Culture of Cardiomyocytes

Cardiomyocytes were dissociated into single-cell suspension from neonatal mice hearts 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 the neonatal heart dissociation kit for mouse from Miltenyi Biotec (GmbH, Bergisch Gladbach, Germany) and gentleMACS™ 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. Finally, red blood cells were lysed and cardiomyocytes were resuspended with appropriate cell culture medium.

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 electrophysiological data analysis. This data processing technique consists in identifying the spiking activities of cells that contribute to the signal recorded by each microelectrode [4]. The identified basic functions are: (1) bandwidth reduction for selective band amplification and noise reduction; (2) discrimination threshold computation; (3) detection and alignment of biological spike signals; (4) extraction of spike shape features; and finally (5) spike clustering. Those functions are depicted in Figure 2.

3. RESULTS

3.1. Bioelectrical Signal Recording

In this study, extracellular potentials of cardiomyocytes were recorded on an array of 28 × 28 microelectrodes. The measured noise level was approximately ±50 μV. Extracellular recordings of cardiomyocyte cultures was confirmed by optical visualization of cell contraction using an inverted microscope (Figure 3). Here, a line of four microelectrodes was selected and data was extracted for the ensuing processing of the bioelectrical signals.

3.2. Bioelectrical Signal Processing

Bioelectrical signal processing of extracellular signals acquired by TFT arrays was performed. This technique provided valuable information about the characteristics of spontaneous bioelectrical signals in cardiomyocyte networks. In this paper, electrophysiological data of a decrease in temperature was analyzed.

3.2.1. Filtered data

Filtering of raw data was performed for noise reduction by removing undesired signals according to their frequency. To detect cardiomyocyte electrical activity, signals were band-pass filtered with
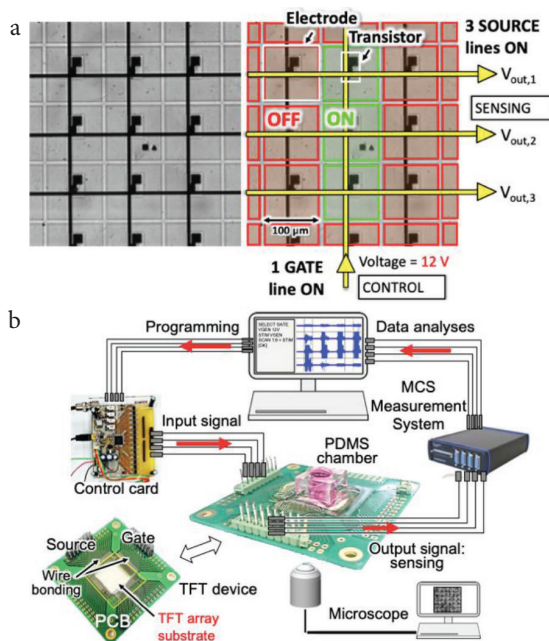

Figure 1 | (a) Working principle of the transparent TFT substrate, with a close-up view of the array of 100 μm² microelectrodes. (b) Experimental setup.
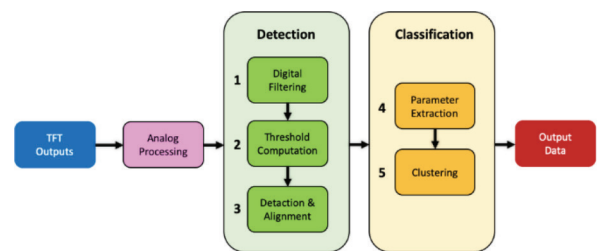

Figure 2 | Workflow of the functions performed by the spike sorting algorithm.
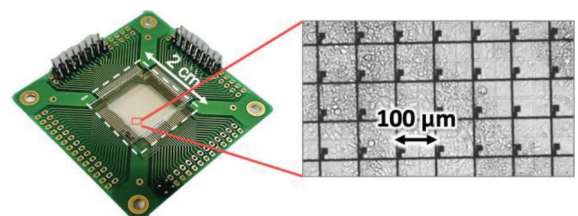

Figure 3 | Culture of cardiomyocytes on TFT array device, 3 days after cell seeding.

cut-off frequencies at 200 and 3000 Hz. The sampling rate of the recorded data was 10 kHz. Figure 4 shows an example of raw and filtered data.

3.2.2. Spike detection and alignment

A predefined threshold was used for distinguishing extracellular potentials of cardiomyocytes from background noise. For each microelectrode, spikes were then detected and aligned with respect to the maximum of their absolute value, as shown in Figure 5.

3.2.3. Spike intervals

Spike intervals were then classified. Figure 6 shows the histograms that display the number of spikes according to their time intervals on one electrode at two temperatures.

3.2.4. Clustering

Finally, Principal Component Analysis (PCA) was used for parameters extraction, such as the amplitude or spike width, and data dimension reduction. In this analysis, each spike became a point
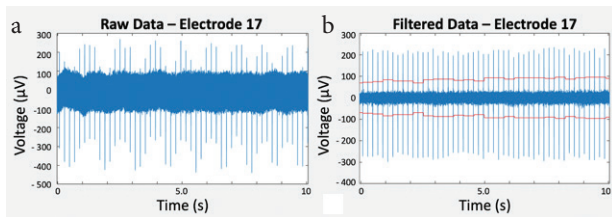

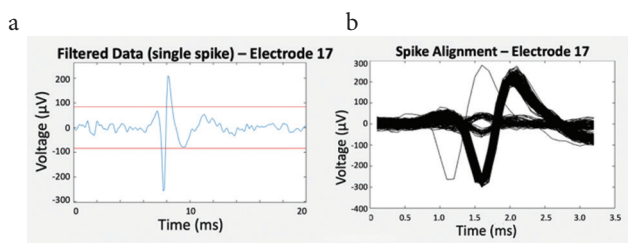**Figure 4** | (a) Raw data and (b) filtered data within 10 s of measurement.

Figure 5 | (a) Filtered data (blue) and threshold lines (red) for spike detection, and (b) spike alignment.
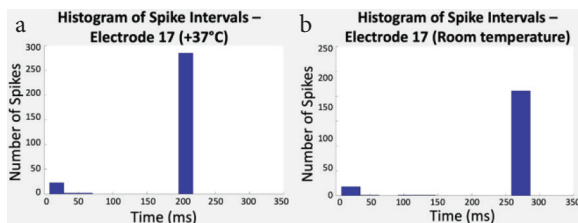

Figure 6 | (a) Histogram of spike intervals at +37°C and (b) room temperature (RT). In this study, spike intervals went from 200 ms (5.0 Hz) to 270 ms (3.7 Hz).
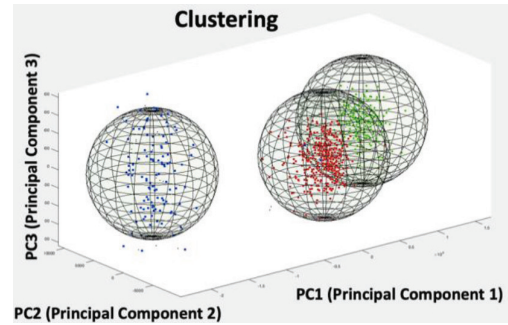

Figure 7 | Three clusters of spikes detected on one microelectrode.

in a three-dimension space. As a result, spikes were divided into clusters in such a way that the spikes in the same cluster are more similar to each other than to those in other clusters (Figure 7).

4. DISCUSSION

Analysis of electrophysiological data with a spike sorting program allowed the extraction of useful information about spontaneous bioelectrical signals in cardiomyocyte networks recorded by TFT sensor arrays. The shape of the extracellular potentials is similar to what we can obtain with other Micro Electrode Arrays (MEA). Decrease of the beating rate with temperature (from +37°C to room temperature) was observed optically during the data acquisition with a system from Multi Channel Systems (MCS) (GmbH, Reutlingen, Germany). This observation was confirmed by the program with a decrease rate of around −6.5 beats per minute/°C. 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. A modification of the signal peak-to-peak voltage amplitude was also observed as a result of decrease in temperature, with a shift from ±300 to ±200 µV. The program could also successfully distinguish spikes and divide them into different clusters. A spike raster plot available in the program (not shown here) also revealed the spike synchronicity of extracellular signals recorded on each microelectrode. This observation confirmed the synchronicity of the bioelectrical conduction among the network of cardiomyocytes, which was also observed experimentally under microscope.

5. CONCLUSION

In this paper, a spike sorting algorithm for electrophysiological data processing was successfully used for analysis of spontaneous electrical signals generated by a network of cardiomyocytes measured by TFT sensor arrays. As this program was written for future operation with a FPGA, a closed-loop for automatic cell stimulation with real-time analysis could be implemented. Combining this technique with deep-learning algorithms [5] could conjointly allow *in vitro* identification of abnormal functional behaviors of cardiac biosignaling networks and provide a powerful tool for drug screening.

CONFLICTS OF INTEREST

The authors declare they have no conflicts of interest.

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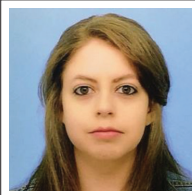
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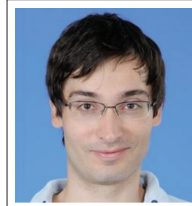
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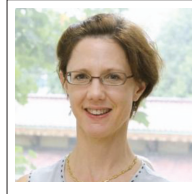
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