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Measurement of the signal from a cultured cell using a high-T_c SQUID

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Abstract

Stem cells in developing tissues give rise to the multiple specialized cell types that make up the heart, lung, skin, and other tissues. For scientists, it is important to know the stages of stem cell development. We propose a new method to obtain knowledge of the cell development stages by using a high-T_c SQUID magnetometer. In the first step of the research, we used Wister rat myocardial cells incubated for 12–14 days in a culture dish as a sample, which were not derived from stem cells. With the cells we tried to measure the signal using a SQUID magnetometer. We were able to measure the magnetic signals, which may be generated by ion transport through the cell membrane. A beat signal with a period of 0.6 s was observed. The peak-to-peak value was about 400 pT. This showed good agreement with the result of microscopic observation.

1. Introduction

Research on stem cells is advancing knowledge about how an organism develops from a single cell and how healthy cells replace damaged cells in adult organisms. This promising area of science is also leading scientists to investigate the possibility of cell-based therapies to treat disease, which is often referred to as regenerative or reparative medicine. Stem cells are important for living organisms for many reasons. Stem cells in developing tissues give rise to the multiple specialized cell types that make up the heart, lung, skin, and other tissues. When unspecialized stem cells give rise to specialized cells, the process is called differentiation. Scientists are just beginning to understand the signals inside and outside cells that trigger stem cell differentiation [1].

For scientists, it is important to know the stages of stem cell development. Growing cells in a laboratory is known as cell culture. Stem cells are isolated by transferring the inner cell mass into a plastic laboratory culture dish that contains a nutrient broth known as a culture medium. Scientists are using a patch clump method, which includes a microscope, a microprobe and microvoltmeter to investigate the development stages of the cell. Figure 1 shows the patch clump method. It is possible to discover the growth stage by reading the voltmeter corresponding to the membrane potential of the cell, which voltage is the order of several tens of millivolts. In this method, much skill is required to insert the micropipette. Also, this method is not applicable to the tissue for cell-based regenerative therapies because the stem cell is fragile and easily broken by touching or sting into the surface [2–5]. Therefore, non-invasive methods are desired.

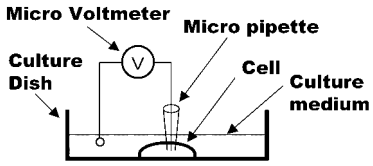

Figure 1. Patch clump method. It is possible to find the growth stage by reading the voltmeter corresponding to the membrane potential of the cell. Using this method, much skill is required to insert the micropipette.

We propose a new method to discover the cell development stages by using a high-T_c SQUID magnetometer. This method is perfectly invasive and does not break the cell. This technology must also be applicable to screening and testing new drugs and toxins. In this paper, we describe the system for the measurement and the results using rat myocardial cells. We note that the cells used here are not derived from stem cells at present.

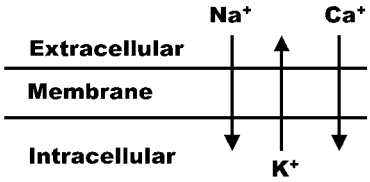

Figure 2. Mechanism for the generation of the membrane potential of the live tissue. There are some pumps such as Na^+/K^+ ATPase and Ca^{2+} ATPase, which are pumping each ion to keep ion balance. As a result, the membrane potential is generated.

2. Principles

Figure 2 shows the mechanism for the generation of the membrane potential of the live tissue. There are some pumps such as Na^+/K^+ ATPase and Ca^{2+} ATPase, which are pumping each ion to keep ion balance. As a result, the membrane potential is generated. For the myocardial cells, the membrane potential temporarily becomes positive by spontaneous excitation. Therefore if a cell sample is placed on a sensitive sensor, it is possible to measure the magnetic field generated by the current.

Suppose there is a live cell tissue in a culture dish, then drugs or chemicals are dropped into the dish and the change of the signal is monitored by the SQUID. As a result, we can find whether the drugs are effective for the tissue. If the cardiac tissue is used as a sample, it is possible to investigate the effectiveness of the drug to the heart, for example.

3. Experimental details

3.1. Cell samples

We used the tissue samples of neonatal Wister rats. All the animals were kept in a temperature-controlled room with a 12 h light–dark cycle, and had free access to water and a standard laboratory diet. All the experiments were carried out in compliance with guidelines on the care and use of laboratory animals from Toyohashi University of Technology. Cardiac muscle tissues were taken from 1–3-day-old Wister rats under CO_2 anaesthesia and were sliced into small pieces. They were cultured in a plastic laboratory culture dish at 32 °C for 12–14 d. The inner surface of the culture dish was coated with collagen and contained 2 mL of HMEM-based culture medium. The size of the cultured cell array was about 500 μm in diameter and 50 μm in height.

The cells were attached to the bottom of the culture dish after three days of culture. After four days, an unstable heartbeat motion in part of the cellular structure, which showed systole and diastole, could be confirmed by microscopic observation. The cells cultured for 12–14 d were used for our experiments. At this stage, it could be seen that the whole cellular structures beat periodically in the culture dishes.

3.2. Measurement system

The dc SQUID magnetometer is made of $Y_1Ba_2Cu_3O_{7-y}$ thin film. The junctions utilized in the SQUID are of the step-edge type. The SQUID type is a dc large washer type, whose

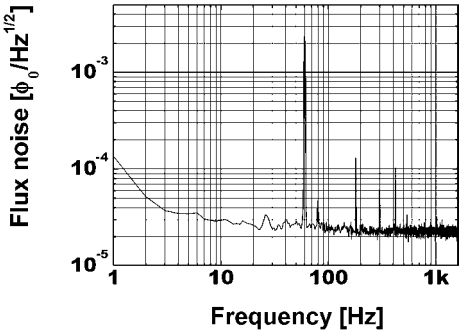

Figure 3. Spectral density of flux noise in the SQUID including the electronics. The white noise is about 20 $\mu\phi_0$ $Hz^{-1/2}$, which corresponds to 0.36 pT $Hz^{-1/2}$.

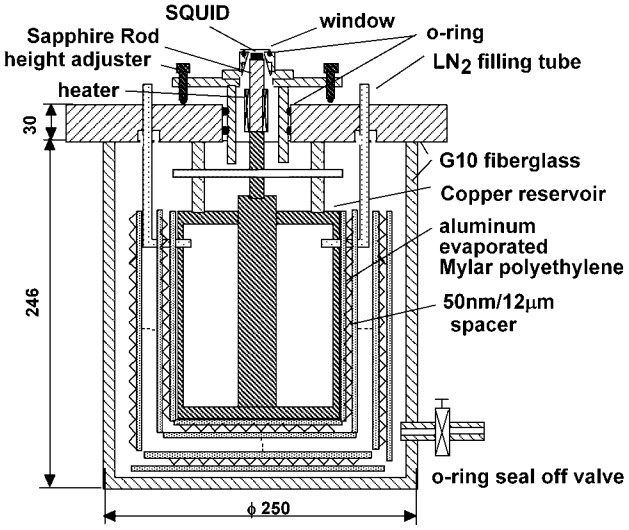

Figure 4. Cross-sectional view of the cryostat. This is specially designed for a SQUID microscope. The minimum separation between the SQUID and the sapphire window is 200 μm.

geometric size is 5.5 × 5.0 mm^2. The effective area of the SQUID is 0.11 mm^2. The spectral density of the flux noise is shown in figure 3. The white noise was about 20 $\mu\phi_0$ $Hz^{-1/2}$. The cryostat was specially designed for a SQUID microscope as shown in figure 4. The SQUID magnetometer was installed in the cryostat. The minimum separation between the SQUID and the sapphire window is 200 μm. A more detailed description can be found elsewhere [6–9].

All of the experiments were performed inside a magnetically shielded room (MSR), with a shielding factor of −50 dB at dc. Figure 5 shows a schematic layout of the system and the data acquisition system. The SQUID was operated in a flux-locked loop with a flux modulation frequency of 256 kHz. The output voltage of the SQUID electronics is passed through a low pass filter at a frequency of 5 kHz and also through a 60 Hz band elimination filter. The signal voltage is coupled to an A/D interface board and stored in a PC.

3.3. Measurement

Figure 6 shows the cell sample in the plastic culture dish. The dish on the left contains a cell sample and that on the right has no samples for comparison. When the sample was

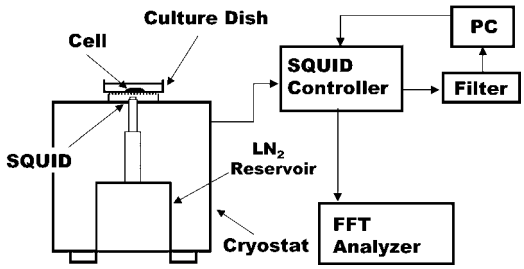

Figure 5. A schematic layout of the system. The SQUID was operated in a flux-locked loop with a flux modulation frequency of 256 kHz. The sample in the plastic culture dish was placed on the top of the vacuum window. The signal output was coupled to an A/D interface board and stored in a PC.

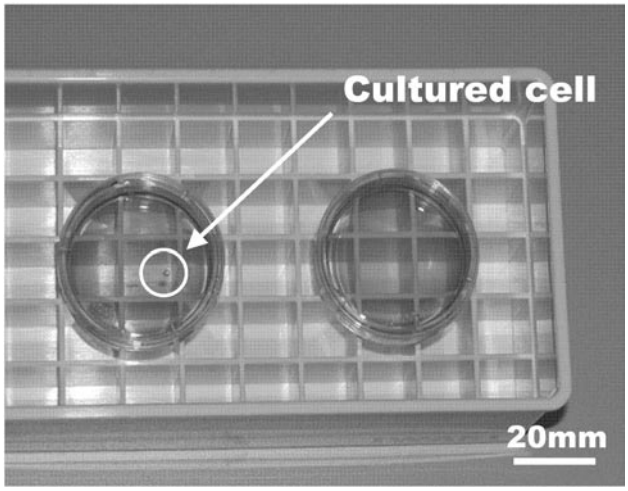

Figure 6. Photograph of the cultured dishes: left, containing a cell sample; right, blank (for control).

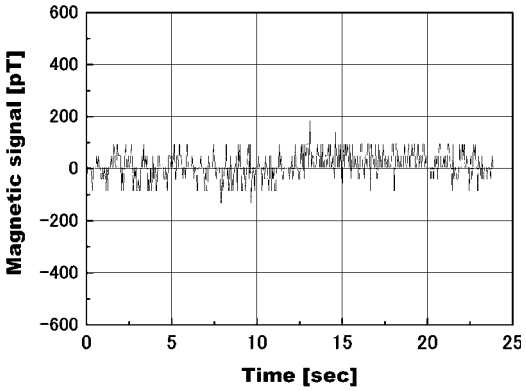

Figure 7. Time domain signal trace of the culture dish without cells (blank). A noise level of 30–40 pT was found in the period.

measured, it was placed on the top of the vacuum window of the cryostat. Then the magnetic signals were recorded. The total spacing between the cell sample and the SQUID magnetometer is approximately 1.8 mm.

4. Results and discussion

First, a culture dish without cells (blank) was measured for the control; the result is shown in figure 7. We found

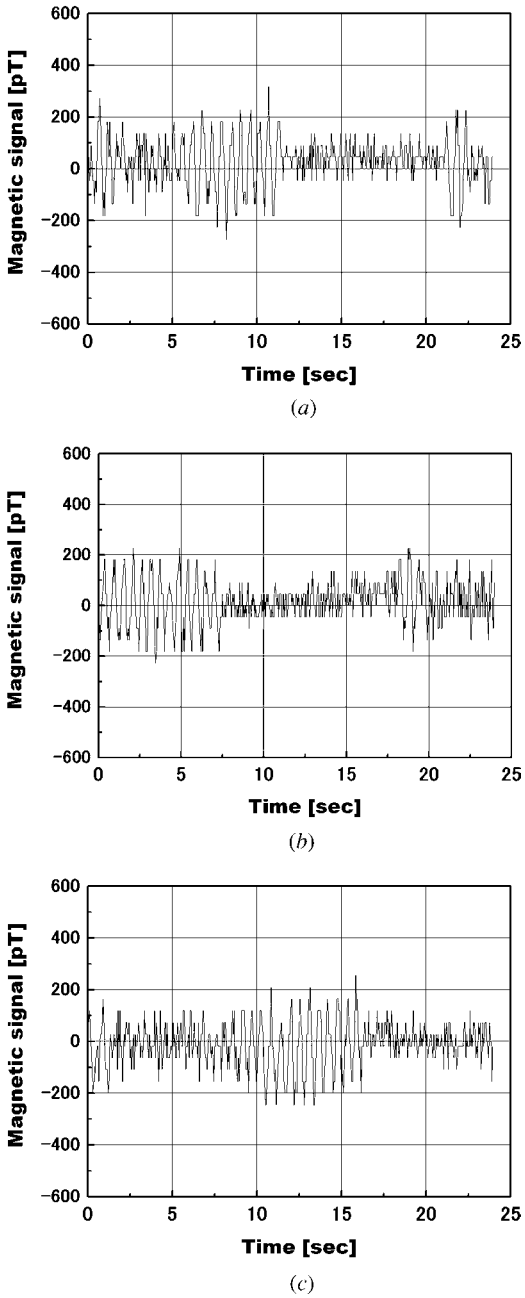

Figure 8. Time domain signal trace of a rat myocardial cell. Three signals were obtained at different times. Periodicities of the three signals are identical. A beat signal with a period of 0.6 s can be seen. The peak-to-peak value is about 400 pT. The beat oscillation is continued for about 8–10 s and stopped for 10 s. This wave set could be periodically observed.

a background noise level of 30–40 pT. The external noise source is microphonic noise due to LN_2 bubbling. Secondly, a cultured cell in the dish was measured. Figure 8 shows three signal traces obtained at different times. A beat signal with a period of 0.6 s can be seen. The peak-to-peak value is about 400 pT. The beat oscillation was continued for 8–10 s and stopped for 10 s even if any stimulation was given. This wave set could be observed. These magnetic signals may be generated by ion transport through the cell membrane. This phenomenon showed good agreement with the result of

microscopic observation. The cells in the culture dish were incubated for 12–14 d and started to form a network in which a coherent excitation took place. This networking may cause cells to give coherency. We believe this result has not been reported to date.

We think some stimuli are needed to perform the measurement for membrane potential of cultured stem cells, which is our goal. However, this technology using a myocardial cell is applicable to screening and testing new drugs and toxins in the near future.

5. Conclusion

We have proposed a new method to discover stem cell development stages by using a high-T_c SQUID magnetometer. As the first step of the experiment, we used rat myocardial cells incubated for 12–14 d in a culture dish, which were not derived from stem cells. For the measurement of the magnetic signals from the cells, a SQUID magnetometer was used. As a result, the magnetic signal from the cellular structure could be detected. The peak-to-peak value was about 400 pT. The beat oscillation was continued for 10 s and stopped for 10 s even if any stimulation was given. These magnetic signals may be generated by ion transport through the cell membrane. This phenomenon showed good agreement with the result of microscopic observation. The cells in the culture dish were incubated for 12–14 d and started to form a network in which a coherent excitation took place. This networking may cause cells to give coherency. This result is a first step to realize a system for measurement of the membrane potential of stem cells. Although some stimuli are needed to perform the measurement for membrane potential of stem cells and any toxins, this technology using a myocardial cell is applicable to screening and testing new drugs in the near future.

Acknowledgment

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