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Contraction of Cultured Muscles Induced by Extra Cellular Current

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1. INTRODUCTION

In the field of regeneration medicine, some organs can be produced from a stem cell. The technology of the regeneration medicine attracts attention from not only medical field but also engineering field. Especially, many researches which try to apply cultured muscles to actuators have been reported.[1, 2]

Cells have an ionic permselective membrane which produces potential difference between intracellular and extracellular. The potential difference is called membrane potential. The muscle cells also have the membrane potential and they can generate action potentials as well as a nerve. The action potential is a wave of the membrane potential change which carries signals in nerves and muscles.

The cultured muscle cell can be contracted by the electrical stimulation and its membrane potential can be measured by a microelectrode. In this study, the relation between the membrane potential change and the contraction motion was investigated.

2. EXPERIMENTAL

2.1 Cultured muscles

The mouse myoblast cell line (C2C12) was used for the experiment. Because the muscle cells can't proliferate any more, the myoblasts which are a kind of the stem cell are incubated. The myoblasts are gathered from a mouse and can be proliferated in a dish easily. When the culture medium in the dish is changed to a special one for differentiation, the myoblasts start to differentiate to the myotubes. The myotubes have contraction ability. The myoblasts become the myotubes in three days and a self contraction can be observed as well as the field induced contraction.

2.2 Electrodes for measurements

Two electrodes were installed in the dish in which the myotubes were cultured as shown in Fig.1. The one for a common electrode was a platinum plate of 30mm × 15mm, and the other was a platinum wire of 1 mm in diameter. For the electrical stimulation, the pulse voltage for 20ms was applied to the electrodes.

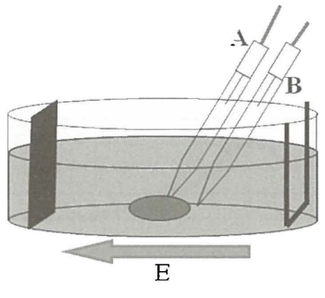

Fig.1 A setup for the measurement.

To measure the membrane potential, a micro-glass electrode was stung into the cell as a probe. The electrode was filled by 3M KCl-aqueous solution. Because the supplied

current gives the potential gradient in the dish, the external potential near the measuring position was also measured by another electrode.

2.3 Measurement of the contraction

In order to measure the contraction quantitatively, phase-contrast microscopic images of the cell during the contraction were taken by a high speed camera. The picture taken before the contraction and the picture taken during the contraction were compared, and the difference of the brightness of each pixel was mapped as shown in Fig.2. The amount of contraction is expressed by standard deviation of the brightness. Figure 3 shows a change of the standard deviation during the contraction and relaxation of the myotube. The standard deviation represents the statistic value of all myotubes in the image.

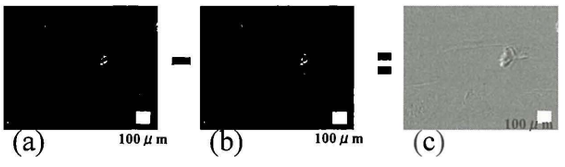

Fig.2 Image processing for the measurement of the contraction: (a) during the contraction, (b) before the contraction and (c) difference.

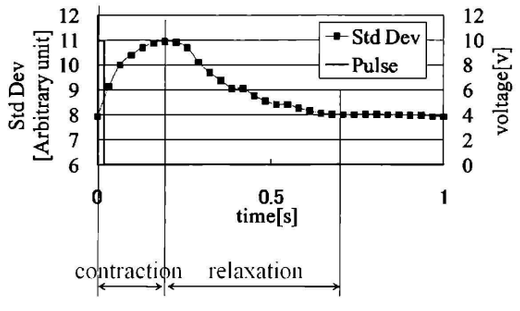

Fig.3 The standard deviation during the contraction and relaxation.

3. RESULTS AND DISCUSSION

The contractions induced by the pulse voltage (Fig.4) were measured.

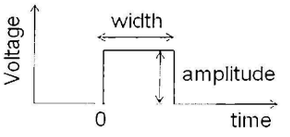

Fig.4 Applied voltage pulse.

Figure 5 shows a pulse width dependence of the contraction. The contractions were measured for various pulse widths with constant amplitude and frequency. Both the speed and magnitude of the contraction depend on the width.

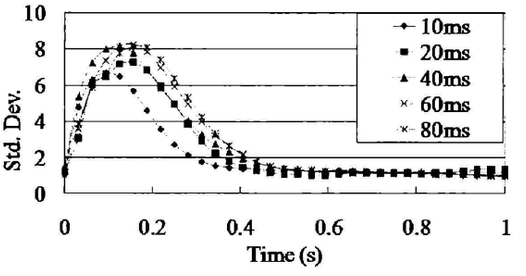

Fig.5 Pulse width dependence of the contraction.

Figure 6 shows an amplitude dependence of the contraction. The contractions were measured for various amplitudes with constant width and frequency. The magnitude of the contraction depends on the amplitude, although the speed of the contraction is independent on the amplitude.

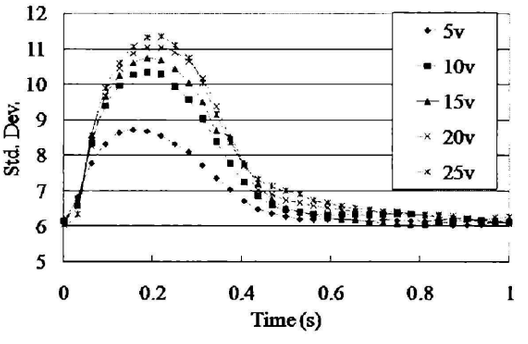

Fig.6 Pulse amplitude dependence of the contraction.

The membrane potentials were measured at three positions of the myotube (a, b and c) as shown in Fig.7. The membrane potential at the position "a" is higher than the resting potential and the membrane potential at "c" is lower than the resting potential as shown in Fig.8. The external current makes gradient in the membrane potential.

This result can be explained by a simple equivalent circuit model as shown in Fig.9. According to the model, the local membrane potentials in the myotube are described as

$$\begin{cases} V_1 - U_1 = V_m - \dfrac{V_L}{2} \\ V_2 - U_2 = V_m + RI - \dfrac{V_L}{2} \\ V_3 - U_3 = V_m + 2RI - \dfrac{V_L}{2} \\ \quad\vdots \\ V_n - U_n = V_m + (n-1)RI - \dfrac{V_L}{2} \\ \quad = V_m + \dfrac{V_L}{2} \end{cases} \tag{1}$$

These equations represent the gradient of the membrane potential.

4. SUMMARY

Magnitude of the electrically induced contraction of cultured muscles was quantitatively measured by standard deviation of brightness change in microscopic images. Local membrane potentials were measured and can be explained by the equivalent model.

References

[1] Yuki Ohnishi, Yusuke Kawakita, Kenichi Yamasaki, Toshia Fujisato and Sadahito Uto, Journal of the Society of Electrical Materials Engineering, Vol.17, No1, pp.38-43, 2008. (in Japanese).

[2] Kawahara Y, *et al*, Pathobiology 73, pp.288-294 (2006).

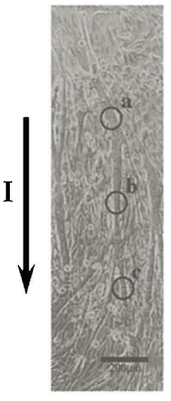

Fig.7 Current direction and measurement positions.

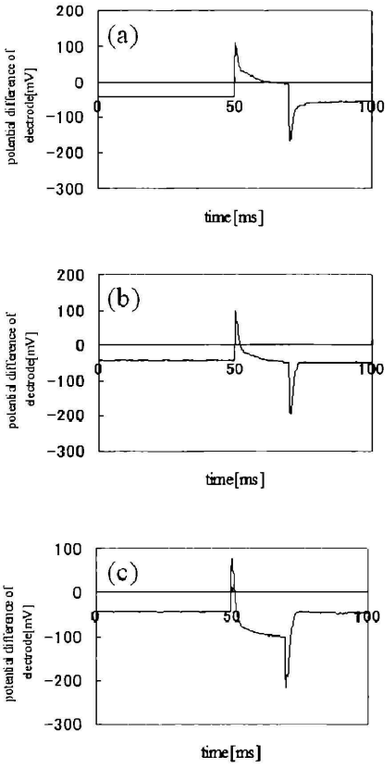

Fig.8 Membrane potentials measured at a, b and c in Fig.7.

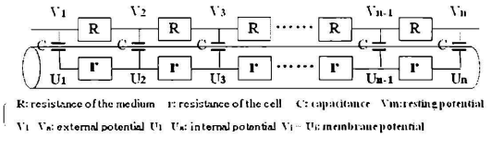

Fig.9 Equivalent model of the myotube.