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Hydrogel-supported skeletal muscle cell-based assay device

Kuniaki Nagamine[1,2], Shingo Otani[1], Shuntaro Ito[1], Hirokazu Kaji[1,2], and Matsuhiko Nishizawa[1,2]

[1]Department of Bioengineering and Robotics, Graduate School of Engineering, Tohoku University, 6-6-01 Aramaki, Aoba-ku, Sendai, 980-8579, Japan
Phone: +81-22-795-3586 E-mail: nagamine@biomems.mech.tohoku.ac.jp
[2] JST-CREST, Sanbancho, Chiyoda-ku, Tokyo 102-0075, Japan

1. Introduction

In vitro bioassay system incorporating skeletal muscle cells could be required to reveal the complex mechanisms involved in the development and maintenance of type2 diabetes because type2 diabetes is closely associated with defection of glucose uptake in skeletal muscle cells. An assay system, currently available, that is used to monitor skeletal muscle cell contraction and activity consists of myotube monolayer cultured on substrates with a pair of electrodes for stimulation. By applying electric pulse stimulation, sarcomere assembly is accelerated and allows for cell contraction. However, the contracting myotubes were difficult to maintain their structure for a long period of time because they readily detached from the substrate within a few days.

In this study, we have developed the contractile C_2C_{12} myotube line patterns transferred onto a fibrin gel and succeeded in maintenance of stable contractile activity for a longer period of time (one week) than the myotubes on the conventional dish system (less than a few days). Besides, combining the myotube/fibriin gel sheet with the microelectrode array device made it possible to apply localized and arbitral site-selective electrical stimulation against the patterned cells. We successfully demonstrated fluorescent imaging of the contraction-induced translocation of the glucose transporter, GLUT4, from intracellular vesicles to the plasma membrane of the myotubes. Defect of GLUT4 translocation in a skeletal muscle cell is closely associated with the development of type 2 diabetes.

2. Experiments

Preparation of myotube line patterns on a fibrin gel

Fig. 1 (A) shows the process of preparing the hydrogel-supported C_2C_{12} myotube culture. C_2C_{12} myoblasts were grown and differentiated into myotubes on a glass substrate with a line-patterned cell-resistant polymer, 2-methacryloyloxyethyl phosphorylcholine (MPC). Then, the myotubes were transferred onto a fibrin gel. A fibrinogen mixture solution was poured over the cells, and the substrate was left undisturbed for 2 h at 37 ^{o}C to facilitate the mixture gelation and to allow the cells to adhere to the gel. Fig. 1(B) shows a photograph of the fibrin gel sheet (size, 1 cm x 1 cm; thickness, 2 mm) with myotube line patterns after detaching the gel from the substrate. The white lines seen at the center of the gel are myotube line patterns. Fig. 1(C) shows a phase-contrast micrograph of myotube line patterns on the gel. The width of each line and the gap between the lines were set at 250 μm. The myotube/finbrin gel was finally placed in a carbon electrode chamber and periodic electrical pulse (amplitude, 0.7 V mm^{-1}; frequency, 1 Hz; duration, 2 ms) was applied to endow the cells with contractile activity.

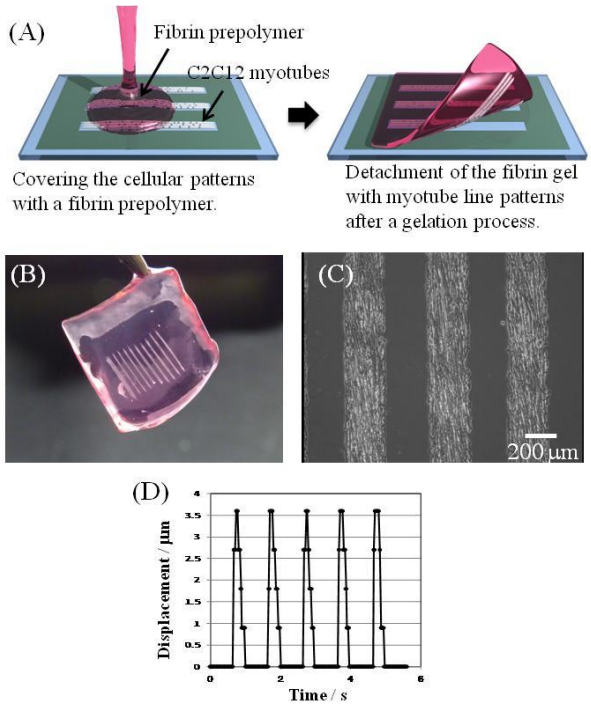

Fig. 1 (A) Process of cellular patterns transfer onto a fibrin gel. (B) Photograph of a fibrin gel with myotube line patterns. (C) Phase-contrast micrograph of myotube line patterns on the gel. (D) Time-course of contractile displacement of cells on a fibrin gel stimulated with continuous electrical pulse at 1.0 Hz.

3. Results and discussion

The C_2C_{12} myotube/fibrin gel sheet was prepared by transferring line-patterned myotubes monolayer from a glass substrate to a fibrin gel while retaining their original patterns of myotubes (Fig.1(A)-(C)). The myotube line patterns on the gel exhibited vigorous contraction depending on electrical pulse stimulation applied using a pair of carbon electrode positioned at either side of the gel (Fig. 1(D)). We found that the myotubes supported by an elastic fibrin gel maintained their contractile activity for a longer period of time (one week) than myotubes on a con-

ventional culture dish, suggesting that the myotube/fibrin gel system would be better suited, than a dish-based system, to stable skeletal muscle cell-based bioassay.

The C_2C_{12} myotube/fibrin gel sheet was easy to handle, allowing to be attached and be aligned onto the microelectrode arrays chip (Fig. 2). Fig. 3 depicts the expression assay of GLUT4 by the selective stimulation of the myotube line patterns. Myotube line pattern on the left side was electrically stimulated and the right side pattern was rested (Fig. 3(A)). Then, the cells were then fixed with 1% paraformaldehyde, followed by immunostaining with Alexa594-conjugated antibody. Fluorescent images were observed using a confocal microscope. As can be seen in Fig. 3(B, C), electrically stimulated myotubes displayed an increase in fluorescent intensity above that of unstimulated cells by about 4-fold. Arbitral control of micropatterned myotubes contraction with localized electrical stimulation enabled high-contrast imaging of contraction-induced GLUT4 translocation phenomena in myotubes.

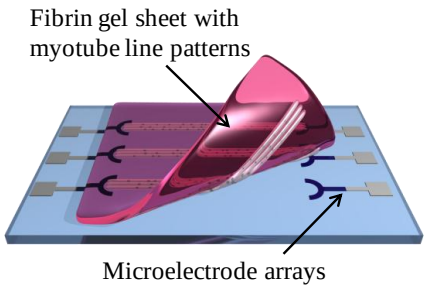

Fig. 2 Overview of the myotube/fibrin gel sheet combined with the microelectrode array chip.

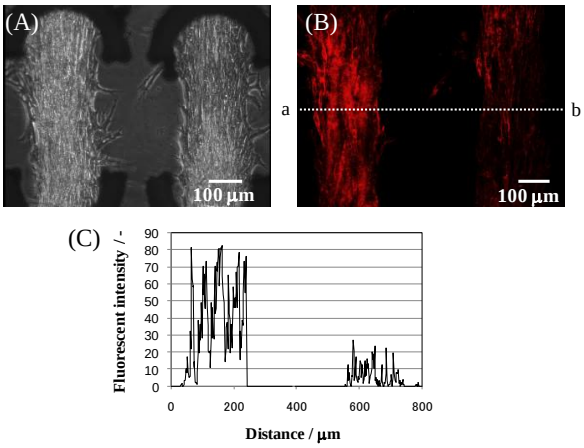

Figure 3 (A) Phase-contrast micrograph of the myotube line patterns locally stimulated with the microelectrode arrays. (B) Fluorescent image of the myotube line patterns immunostained with an Alexa Fluor 594-conjugated antibody. (C) Fluorescent intensity of each immunostained myotube line pattern along the cross section a-b in (B).

3. Conclusions

We have developed a micropatterned C_2C_{12} myotubes/fibrin gel culture system integrated with a microelectrode array chip for skeletal muscle cell-based bioassay. Arbitrary control of micropatterned myotubes contraction with localized electrical stimulation enabled high-contrast imaging of contraction-induced GLUT4 translocation phenomena in myotubes. This device would easily permit focusing the stimulation site on a desired specific tissue construct, such as a neuromuscular junction formed in a neuron-skeletal muscle cell co-culture. Such applications would be reported in future studies.

Acknowledgements

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