EXERCISE- AND DRUG DOSE-DEPENDENT METABOLIC ASSAY DEVICE USING THE HYDROGEL-SUPPORTED SKELETAL MUSCLE CELLS

Kuniaki Nagamine[1,2], Hirokazu Kaji[1,2], Makoto Kanzaki[1,2], Matsuhiko Nishizawa[1,2]
[1]Tohoku University, Japan, [2]JST-CREST, Japan

ABSTRACT

We have prepared contractile skeletal muscle cellular micropatterns supported by the hydrogel to investigate the relationship between exercise or drug administration and development of type 2 diabetes under controlled culture conditions. The muscle cell-patterned gel sheet can be handled easily while sustaining the cellular structure and activity, allowing for the first time to be combined with the various analytical devices on demand. In this study, two types of bioassay devices were constructed by combining the gel sheet with our original microelectrode-printed flexible gel sheet for electrically inducible exercise-dependent metabolic assay, and with a microfluidics for drug screening.

KEYWORDS

Skeletal muscle cell, Hydrogel, Microfluidics

INTRODUCTION

In vitro bioassay systems incorporating cells with physiological activity have been developed as an alternative to whole animal experiments. Systems using skeletal muscle cells are one of the promising devices to reveal the complex mechanisms of type 2 diabetes because the disease is closely associated with a disorder of insulin- or contraction-induced glucose metabolism in a skeletal muscle cell in vivo. Such a bioassay system could be also useful for screening candidate drugs against type 2 diabetes

In this study, we prepared aligned contractile skeletal muscle cellular micropatterns supported by the hydrogel to investigate the relationship between exercise or drug administration and type 2 diabetes development under controlled culture conditions. The muscle cellular gel sheet can be handled easily while sustaining the cellular structure and activity, allowing for the first time to be combined with the various analytical devices on demand. The gel sheet combined with our original microelectrode-printed flexible gel sheet, which is soft enough to contract synchronously with the myotube motion, were applied to spatiotemporally control of each micropatterned muscle contraction with localized electrical stimulation. This device enabled high-contrast imaging of contraction-induced glucose transporter, GLUT4, translocation from intracellular vesicles to the plasma membrane of the cells. GLUT4 is a principal mediator of glucose uptake in the skeletal muscle cell in response to insulin and exercise and its defection is closely associated with the development of type 2 diabetes. Furthermore, combination with the microfluidics enabled localized drug administration to the cellular micropatterns on the gel avoiding direct subjection of the cells to the fluidic shear stress. This device would allow drug dose-dependent metabolic assay such as insulin-induced control of glucose metabolism in the skeletal muscle cells.

EXPERIMENT

Preparation of a fibrin gel with contractile myotube micropatterns combined with a conducting polymer electrodes-printed hydrogel

Figure 1(A) shows a photograph of a fibrin gel with C2C12 myotube line patterns and (B) shows conducting polymer electrodes printed on a hydrogel. The gel sheet with cellular pattens was prepared by transferring cellular micropatterns from a culture dish onto a fibrin gel[1,2)]. A fibrinogen and thrombin mixture solution was poured over the myotubes patterned on a culture dish, and the dish was left undisturbed for 4 h at 37 ^{o}C under a 5% CO_2 atmosphere to facilitate the mixture gelation and to allow the cells to adhere to the gel. By gently detaching the gel from the dish, the gel sheet with myotube line patterns (line width: 250 μm) were prepared (Fig. 1(A).

Poly(3,4-ethylenedioxythiophene) (PEDOT) micropatterns were prepared on a hydrogel as follows[3,4)]. Poly(acrylamide) hydrogel film was laminated on Pt microelectrodes prepared on a glass substrate using a conventional photolithography and lift-off technique. Electropolymerization was

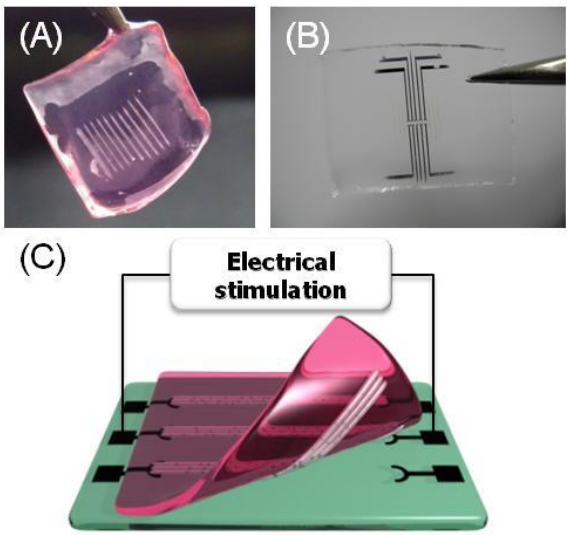

Figure 1. (A, B)Photograph of a fibrin gel with myotube line patterns (A) and a conducting polymer PEDOT-printed hydrogel (B). (C) Myotube/fibrin gel combined with the PEDOT microelectrode-printed hydrogel sheet.

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conducted on the gel-covered electrode in a aqueous monomer solution. Finally, the hydrogel film with PEDOT micropatterns was peeled from the Pt electrode substrate by taking advantage of the electrochemical elastic actuation of PEDOT.

The myotube gel sheet was patched onto the microelectrode array sheet as shown in Fig.1(C) to construct a skeletal muscle cell-based bioassay device. Contractile behavior of each myotube line pattern on the gel was individually controlled by localized electrical stimulation using the microelectrode arrays. Similar to natural skeletal muscle tissues, the myotubes showed twitch contractions upon application of 1 Hz electrical pulses, while tetanic contraction was observed at 40 Hz (Figure 2).

GLUT4 translocation assay

Figure 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)). GLUT4 on the plasma membrane was stained with Alexa594-conjugated antibody after fixation of the cells using paraformaldehyde. As can be seen in Fig. 3(B), electrically stimulated myotubes displayed an increase in fluorescent intensity above that of unstimulated cells by about 4-fold. In a conventional random-cultured myotube monolayer, it was difficult to match the target myotubes with their GLUT4 translocation activity after fixation process because it is hard to seek the ‘‘contracted target myotubes’’ on the culture dish during the electrical stimulation. Therefore, the averaged GLUT4 translocation activity of all cultured cells was detected by the western blot analysis, which would underestimate the true activity of the contractile myotubes. Our device made it possible to easily identify the GLUT4 translocation activity of the contracting myotubes for the first time, by artificially patterning and locally targeted stimulation of the myotubes on the gel sheet. Furthermore, by patterning myotube lines subjected to different stimulation conditions next to each other, high-contrast imaging of the contraction effect on GLUT4 translocation in myotubes was achieved. This device would be applicable for quantitative bioassays of various contraction-induced metabolic alterations in myotubes.

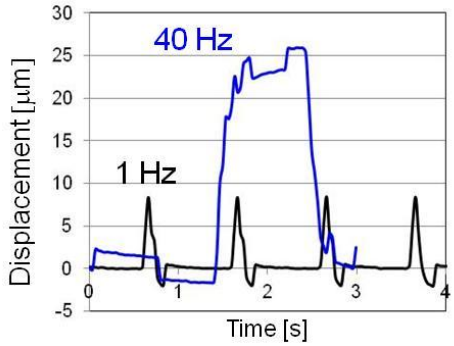

Figure 2. Contractile displacement of myotube line pattern when stimulated with 1 Hz or 40 Hz of electrical pulses.

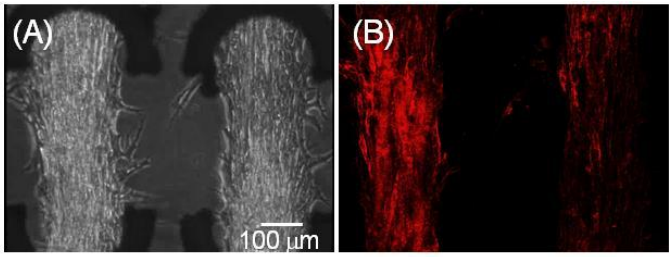

Figure 3. Phase-contrast (A) and fluorescent micrograph (B) of myotube line patterns locally stimulated using the aligned microelectrodes. GLUT 4 was stained with Alexa-594 conjugated anctibody.

Localized chemical dose into a fibrin gel using a microfluidic device

The myotube/microelectrode gel sheet can be combined with a microfluidic channel by simply stacking a microchannel layer on the gel as shown in Figure 4(A). The chip consists of four layers: a PDMS top layer, a silicone rubber middle layer with stenciled microchannel pattern, a myotube/fibrin gel layer set in a silicone frame, and Microelectrode bottom layer. Fig. 4(B) shows the micrograph of myotube line patterns in the microchannel and Figure 5(A) shows the illustration of the device cross-section. The microchannel was attached on

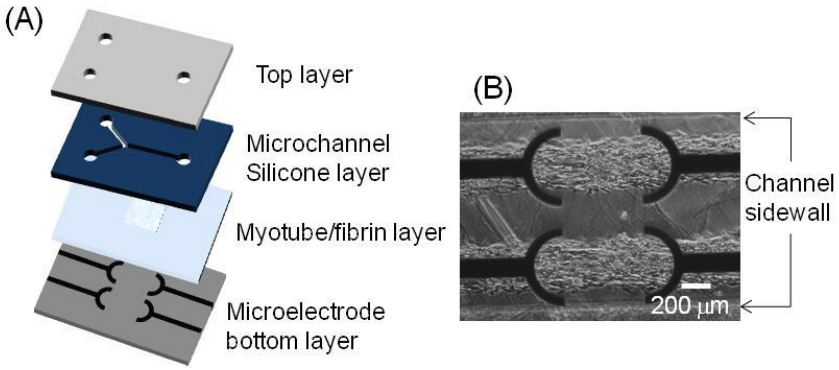

Figure 4. (A)Structure of the myotubes/microelectrode gel sheet combined with the microfluidic channel. (B) Phase-contrast micrograph of the myotube line patterns in the microchannel.

the surface of the fibrin gel and the myotubes were located on the bottom surface of the gel contacting with the microelectrodes. This structure has an advantage for avoiding direct subjection of the cells to the fluidic shear stress.

Fig. 5(B) demonstrated localized chemical dose into the fibrin gel using the device. Two-phase laminar flow of fluorescein solution (left half of the channel, the source flow) and water (right half of the channel, the sink flow) was formed in the microchannel. Fluorescein diffused from the source flow into the fibrin gel while the dye diffusing into the gel over the laminar flow interface was flushed out by the sink flow, resulting in successful localized delivery of the dye into the gel as shown in Fig. 5(B).

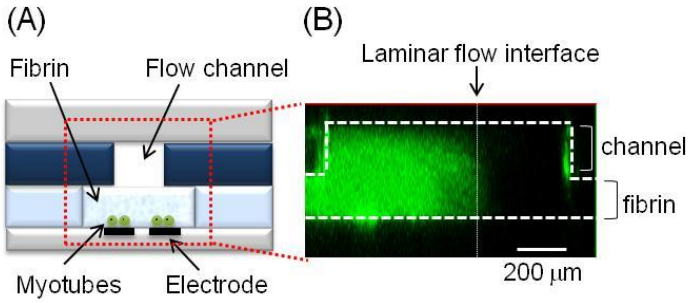

Figure 5. (A) Illustration of the device cross-section. (B) Fluorescent micrograph of the device cross-section when two-phase laminar flow of fluorescein solution and water was formed in the microchannel.

In this study, we have developed a micropatterned myotubes/fibrin gel-based bioassay system combined with analytical microdevices. Combination with a PEDOT microelectrode array chip enabled arbitrary control of micropatterned myotubes contraction with localized electrical stimulation, resulted in high-contrast imaging of contraction-induced GLUT4 translocation phenomena in myotubes. The microfluidic chip made it possible to administer chemicals in 3-dimentionaly localized area avoiding direct subjection of the cells to the fluidic shear stress. These devices 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 in the future study.

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CONTACT

Kuniaki Nagamine nagamine@biomems.mech.tohoku.ac.jp