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Hydrogel-Based Contractile Electrodes for Stimulation of Cells and Tissues

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We developed techniques for preparing a muscular cells-arrayed hydrogel sheet and a microelectrodes-printed hydrogel sheet. The combination of these two hydrogel sheets enables the metabolic assay for muscular tissue under a controlled contractile motion.

I. Introduction

In-vitro bioassay using cultured cells becomes increasingly important as the alternative to animal experiments in biological basic research and drug discovery. For the assays with neuronal and muscular cells require smart interface between the cells and the microelectrodes for stimulation. We have developed a system named "Electrochemical Biolithography" [1-4] that can versatilely prepare a variety of biochips including (1) cellular arrays-embedded hydrogel sheet and (2) conducting polymer microelectrode-printed hydrogel sheet.

Here, we will present the in-vitro bioassay chip incorporating skeletal muscle cells that is required to reveal the complex mechanisms of type-2 diabetes closely associating with defection of glucose uptake in skeletal muscle cells. Since the muscle cells are sensitive to the stiffness of culture substrate [5], we developed first the technique to culture micropatterned C2C12 myotubes on soft, moist fibrin gel sheet [6,7]. The muscular culture maintained stable contractile activity for a longer period of time (more than a week) than the myotubes on conventional dish system (less than a few days). On the other hand, we developed also a novel process for micropatterning conducting polymer electrode on hydrogels to provide a fully-organic, permeable, flexible electrode [8,9]. The microelectrodes of poly (3,4-ethylenedioxythiophene) (PEDOT) are prepared on hydrogels through two electrochemical processes: the electropolymerization of PEDOT into the hydrogel and the electrochemical actuation-assisted peeling. For the PEDOT printed on the agarose sheet, the surface resistance was measured in a wet condition using a 4-point probe method as ca. 50 Ω/sq, this value being in agreement with previous reports for PEDOT film prepared by dry printing processes. The PEDOT microelectrodes can be prepared on a variety of hydrogels, including glucomannan (konjac), collagen and polyvinyl alcohol.

By laminating the myotubes-patterned gel and the PEDOT-patterned gel, the resulting contractile cellular chip enables detail and site-specific investigation of electrical stimulation-mediated alteration in myotube metabolism, which could mimic control of skeletal muscle metabolism via locally-formed neuromuscular junction in vivo. 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 [7]. On these experiments, the electrical stimulation supplied through a PEDOT electrode can induce contraction of muscle cells. And importantly, the PEDOT electrode also contracted synchronously with the motion of the cells.

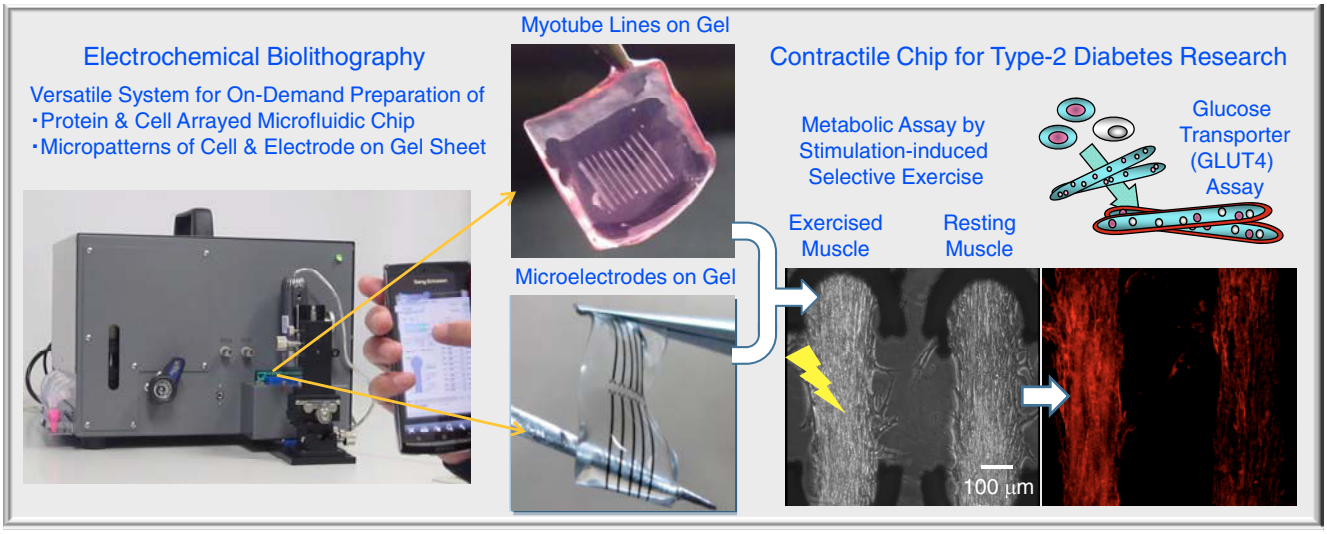

Figure 1 Overview of this research: metabolic assay chip prepared by the electrochemical biolithography.

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II. Electrochemical Biolithography

The interfacing between the biomolecules and device materials is one of the most important subjects for both in-vitro and in-vivo medical devices. We have been studying the potential use of microelectrode techniques as the tool for controlling bionic interfaces; special control of protein adsorption and cell adhesion / growth. The technique is simple enough to be integrated to the small and closed systems such as AFM system and microfluidic devices. Recently, we have succeeded to transfer cellular micropatterns to a biocompatible gel sheet towards advanced bioassay using contractile muscle cells.

We have developed electrochemical-based biolithography (ECBL) [1,2] that realizes in-situ, spatiotemporal control of cellular shape, motility, and functions. We modified an atomic force microscope (AFM) to accommodate ECBL by converting the tip of a commercially available AFM cantilever probe into an electrode and achieved in-situ patterning of fibronectin in a few um scale. As demonstrated in Fig. 2, the sub-cellular resolution of this system enabled the lithographic manipulation of the environment surrounding a single cultured cell (NIH-3T3 fibroblasts) [3]. The navigated cellular outgrowth retained the shape typically around 2 days in the cell cultivation condition. An interesting experiment using the ECBL-AFM system would be the construction of a neuronal/muscle cell network that could be used to study the

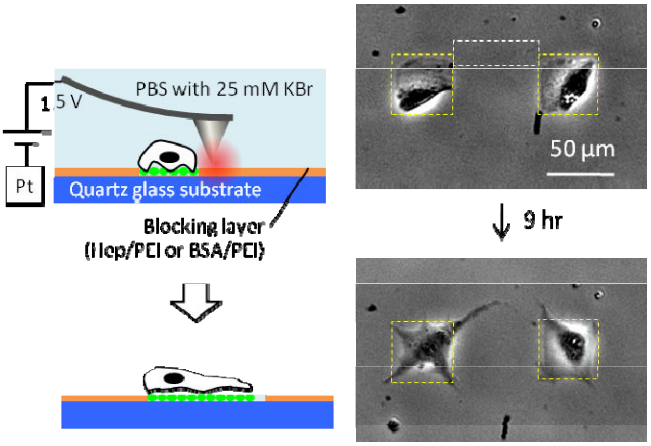

Figure 2 A schematic diagramming single cell in-situ manipulation and resulting micrographs.

interactions of cell junctions.

The cell-based assay and diagnosis require alignment of cells within a microchanel. We have recently made a prototype system that can quickly prepare a microfluidic chip containing array of multiple proteins and cells by applying the techniques of ECBL. As shown in Fig. 3, Pt microelectrode array was prepared at the upper wall of the channel for ECBL and negative DEP for collecting the dispersing cells to the area for adhesion. We studied continuous collection of cells in a fluid flow. In the symposium, the success in selective capture of neutrophils and eosinophils from a mixture of leucocytes will be presented [4]

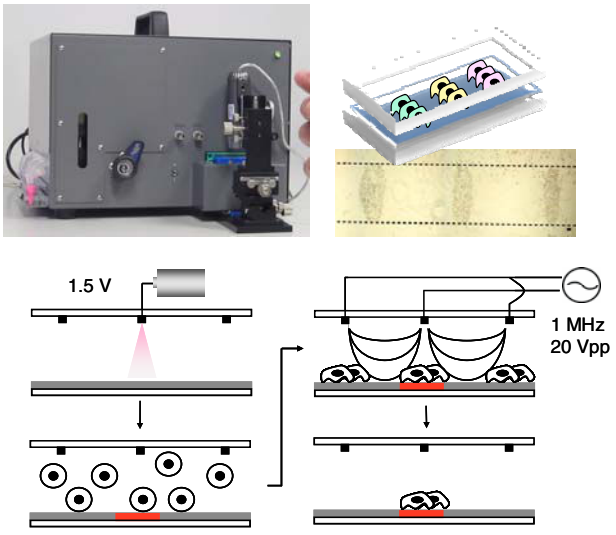

Figure 3 The electrochemical biolithography system, and the schematic representation of the cellular patterning within the microchannel; combination of the electrochemical biolithography and negative DEP.

We found that micropatterned cell cultures can be transferred into or on a fibrin gel sheet from a glass substrate with keeping their patterns during the transfer process. For example, we prepared contractile C2C12 myotube line patterns embedded in a fibrin gel (Fig. 4). The myotubes supported by the elastic gel showed larger contractile displacement than when they were attached on a conventional culture dish and retained their contractile activity for a week. Such assay system incorporating skeletal muscle cells is required to characterize the molecular mechanisms involved in glucose homeostasis and insulin- and contraction-mediated glucose uptake toward elucidating complex mechanisms of type2 diabetes.

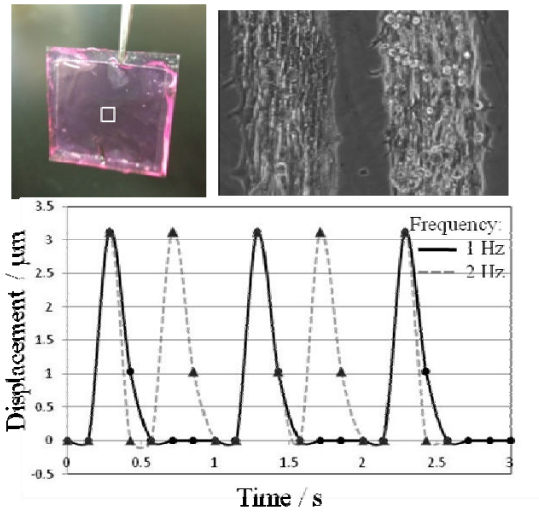

Figure 4 The phase-contrast micrograph of patterned lines of myotubes transferred from a glass substrate to a fibrin gel surface. Contractile displacements with time as functions of electrical pulse frequency (Hz). The amplitude was 0.7 V mm^{-1}.

III. Conducting Polymer / Hydrogel Electrode for Contractile Assay

This paper reports novel process for micropatterning conducting polymer electrode on hydrogels to provide a fully-organic, permeable, flexible electrode. All of the existing printing methods using screens, ink-jet systems or microstamps, require the drying of fluid inks, and thus cannot be used for printing on a moist gel substrate. In contrast, our new process is based on the electrochemical deposition of electrodes into gel films [8,9].

The microelectrodes of poly (3,4-ethylenedioxythiophene) (PEDOT) are prepared on hydrogels through two electrochemical processes as schemed in Figure 5: the electropolymerization of PEDOT into the hydrogel and the electrochemical actuation-assisted peeling. A hydrogel film was placed over a Pt microelectrode fabricated on glass plate, followed by electropolymerization of the monomer on the gel-covered electrode at 1.0 V vs. Ag/AgCl. The electropolymerized PEDOT film is adhering to the Pt master electrode, and therefore mechanically aggressive peeling collapses the soft hydrogel. We found that electrochemical elastic actuation of PEDOT (±0.5 V vs. Ag/AgCl) was effective for nondestructively peeling off the soft gel from the master electrodes. The volume change of PEDOT may induce stress at the polymer/electrode interface, and cause detachment of the film. The print resolution depends on the polymerization time (polymerization charge). In the case of 60 min polymerization for the agarose, it can be seen that the PEDOT pattern becomes ca. 10 μm thicker than the Pt master electrode, indicating that the PEDOT grew ca. 5 μm in the vertical direction into the gel film during the polymerization. For the PEDOT printed on the agarose sheet by 60 min polymerization, the surface resistance was measured in a wet condition using a 4-point probe method as ca. 50 kΩ/sq, this value being in agreement with previous reports for PEDOT.

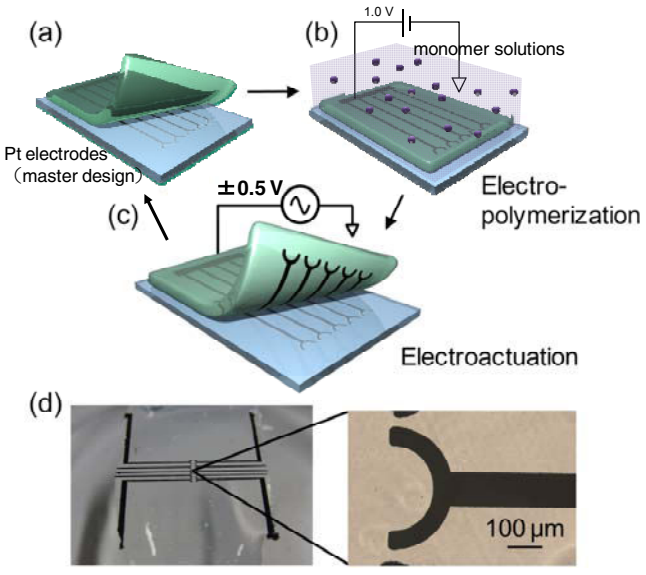

Figure 5 Schematic illustrations of the fabrication process for a conducting polymer/hydrogel electrode. (a-c) (d) Photograph of the PEDOT microelectrode array printed on an agarose gel sheet.

The present technique is versatile; PEDOT microelectrodes can be prepared on a variety of hydrogels, including agarose, glucomannan (konjac), collagen and polyvinyl alcohol or on poly (hydroxyethyl methacrylate) (HEMA) (commercial soft contact lens), as shown in Figure 6.

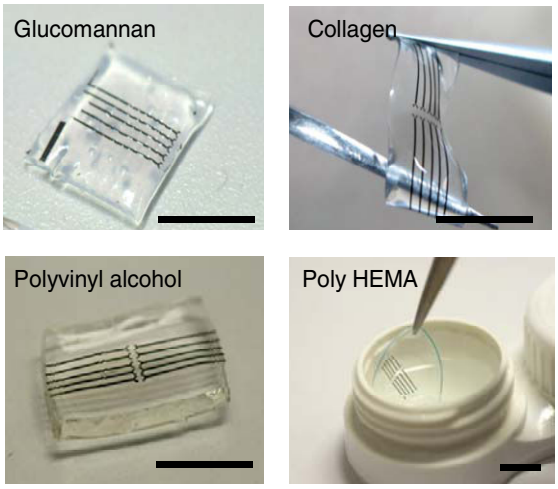

Figure 6 Photographs of the printed microelectrodes on various hydrogels including glucomannan (konjac), collagen and polyvinyl alcohol or on poly-HEMA) (commercial soft contact lens). Scale bar, 5 mm.

Such a moist, permeable and flexible electrode should have many unique applications such as an in-vivo lapping electrode and in-vitro cell cultivation. As an example, we demonstrate herein the advantage of the present electrode for the electrical stimulation of C2C12 myotubes that is required for research on type2 diabetes [6,7]. Figure 7a illustrates how, in the present study, the cell-embedded fibrin sheet was set on the PEDOT/agarose electrode, which was connected to an electric stimulator. A periodic voltage pulse (6V, 0.6 s) was applied at 1 Hz to induce the cellular contraction, and the motion of the myotubes and PEDOT electrodes were analyzed. As is seen in Figure 7b, the electrical stimulation supplied

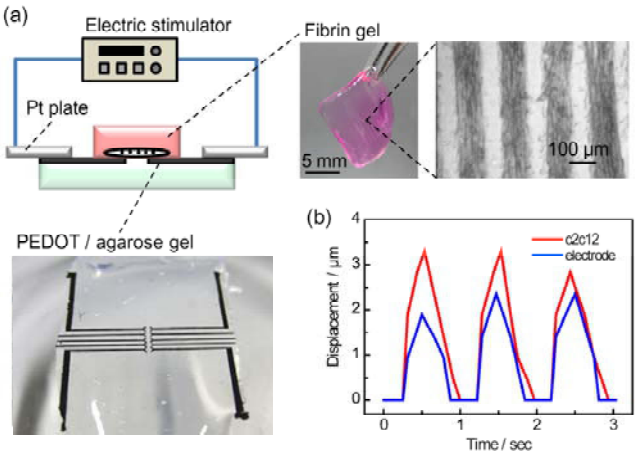

Figure 7. (a) Experimental setup for stimulation of myotubes. Fibrin gel sheet containing the micropatterned C2C12 myotubes was laid on the PEDOT/agarose electrode. (b) Time course of contractile displacements of C2C12 myotubes (red) and PEDOT hydrogel electrode (blue).

through a PEDOT electrode can induce contraction of muscle cells. And importantly, the PEDOT electrode also contracted synchronously with the motion of the cells.

ACKNOWLEDGMENT

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