SEQUENTIAL ASSEMBLY OF THE FUNCTIONAL MATERIAL MICROPATTERNS ON THE HYDROGEL SHEET FOR CONSTRUCTING SKELETAL MUSCLE CELL-BASED ASSAY SYSTEM

Kuniaki Nagamine[1,2], Shingo Otani[1], Syuntaro Ito[1], Hirokazu Kaji[1,2], Makoto Kanzaki[1,2], Matsuhiko Nishizawa[1,2]

[1]Tohoku University, Japan, [2]JST-CREST, Japan

ABSTRACT

The functional material micropatterns-assembled hydrogel sheet has been prepared by means of sequential transfer technique to construct the skeletal muscle cell-based assay system. We demonstrated to construct micropatterned synaptic connection between motor neurons-skeletal muscle cells using this technique. Besides, assembly of the non-biological functional materials has also advantage to confer additional functions to a hydrogel assay system. The fluorescent microbeads were assembled onto a hydrogel as sensing elements of contractile force and metabolic activity of skeletal muscle cells. These flexible sensor sheets would be useful to study the relationship between exercise and metabolic activity of muscle in type 2 diabetes.

KEYWORDS

Skeletal muscle cell, Hydrogel, Assembly, Bioassay

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, for example, 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 have established the micropatterning technique of functional materials on a wet hydrogel sheet by means of the sequential transfer to construct the skeletal muscle cell-based assay system. This technique utilized conventional 2-dimensional micropatterning technique on the substrate followed by transferring the patterned materials onto a hydrogel surface via chemical interaction between them. We demonstrated to construct micropatterned synaptic connection between motor neuron-skeletal muscle cells using this technique. Besides, functional microbeads were assembled onto the hydrogel as sensing elements of contractile force and metabolic activity of skeletal muscle cells.

EXPERIMENT

Preparation of a synapse sheet

Figure 1(A) shows the process of sequential transfer of cellular micropatterns from a culture dish onto a fibrin gel. A fibrinogen and thrombin mixture solution was poured over C2C12 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 substrate, the gel sheet with myotube line patterns (line width: 250 μm) were obtained. The resulting gel sheet was then attached onto NG108-15 motoneuron-like cellular line patterned on the other culture dish and was left for 4 h to allow additional attachment of the cells onto myotube-patterned fibrin gel. Fig. 1(B) shows the photograph of the fibrin gel with cellular patterns and (C) shows the micrograph of the grid micropattern formed by myotubes in the vertical line and NG108-15 cells in the horizontal line. As can be seen, the neurite extension from NG108-15 micropatterns toward the myotube line patterns was clearly identified. In conventional co-culture method, as both cells were randomly mixed in a culture dish, it has been difficult to identify synaptic connections between the cells in microscopic observation. The present

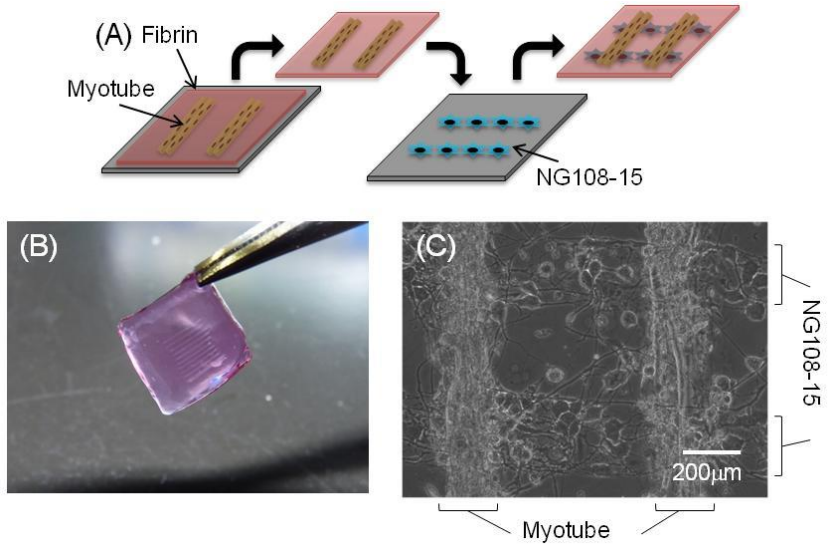

Figure 1. Process of sequential transfer of the cellular micropatterns from a culture dish onto a fibrin gel. (B)Photograph of the fibrin gel with cellular micropatterns. (C)Micrograph of the grid micropattern formed by myotubes in the vertical line and motor neurons in the horizontal line.

co-culture micropattern enabled easily focusing on the specific structure on the cells such as neuromuscular junction.

Contractile force sensor sheet

Figure 2 shows another process of sequential transfer of microbeads and myotubes onto a same fibrin gel for muscle contractile force measurement. A microbeads-patterned fibrin was prepared in an electrode chamber by using positive dielectrophoresis (Fig, 2(1)), followed by encapsulation of the patterned beads into a fibrin gel (Fig, 2(2)). Then, the prepared microbeads/fibrin gel was attached onto myotube line patterns on a culture dish to additionally transfer the cells onto the gel (Fig, 2(3)). Fig. 3 shows a micrograph of a grid micropattern formed by myotubes and microbeads. When 1 Hz of electrical pulses was applied to the myotubes, the bright fluorescent microbeads contracted synchronously with the cellular motion. This result suggested that the beads were useful as a marker of myotube contraction, leading to calculation of the contractile force from the beads motion as shown in Fig. 4(A). Now, we are examining influence of an obesity-related proinflammatory cytoine, TNF-α, on muscle contractile force using the gel sheet (Fig. 4(B)).

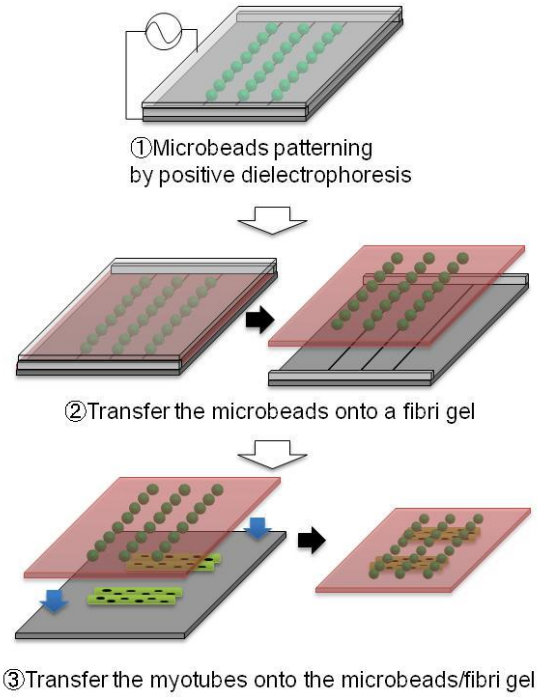

Figure 2. Process of sequential transfer of microbeads and myoyubes onto a fibrin gel.

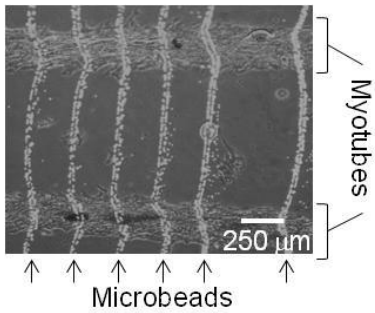

Figure 3. Micrograph of grid micropatterns on a fibrin gel formed by myotubes and microbeads line patterns.

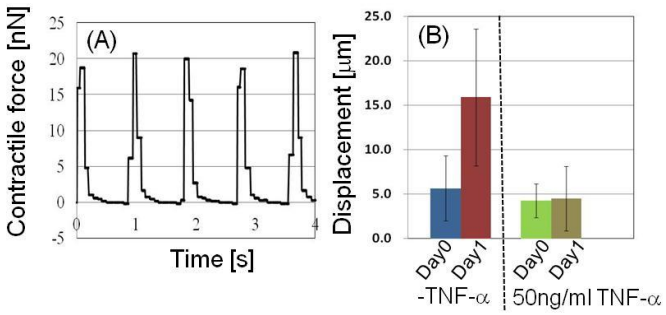

Figure 4. (A) Time-course of contractile force when the myotubes were stimulated with 1Hz of electrical pulses. (B) Change of twitch contractile displacement of myotubes treated with or without 50 ng/ml TNF-α upon application of continuous electrical pulses (1Hz).

Oxygen responsive microbeads-patterned hydrogel sheet

Figure 5(A) shows the schematic view of the oxygen responsive microbeads-assembled hydrogel sheet for sensing cellular metabolic activity [3]. Oxygen responsive microbeads were prepared as follows. A 3.5 mL of aqueous polystyrene microparticle suspension (1.0 μm diameter) was mixed with 1.5 mL of tetrahydrofuran (THF) under ultrasonication for 10 min to swell the particles. A 0.5 mL solution of oxygen responsive phosphorescent dye, platinum octaethylporphyrin (PtOEP), dissolved in dimethyl sulfoxide was added to the particle suspension followed by another 20 min ultrasonication to incorporate the PtOEP into the particles. Then, THF in the mixture was evaporated for 12 h at room temperature. The particles were separated by centrifugation, and washed two times with ethanol. The resulting oxygen responsive microbeads were immobilized on a poly(acrylamide) gel surface by using same process as Fig. 2(1), (2).

The sensor sheet can be directly attached onto target cells to image cellular oxygen consuming activity. In this study, we demonstrated oxygen concentration imaging around glucose oxidase (GOX) pattern as a cellular model, which consumes oxygen during glucose oxidation reaction. GOX pattern was prepared by patterning GOX-modified microbeads onto a glass substrate via cross form stenciled sheet. Oxygen-responsive dye, PtOEP, encapsulated into the beads is based on the ability of molecular oxygen to quench the dye selectively. Therefore, the phosphorescent intensity increases as the oxygen concentration decreases. As can be seen in Fig. 5B, the

cross-formed phosphorescence was displayed corresponds to the GOX pattern, suggesting oxygen consuming activity of GOX was successfully imaged using the sheet.

In this study, functional material patterned hydrogel film was prepared by means of sequential transfer of 2-dimensional patterns on the solid substrate onto a gel surface. Skeletal muscle-motor neuron co-culture was successfully patterned on a gel film allowing to easily focusing on a specific cellular constructs and functions such as neuromuscular junction. Assemblies of contractile force sensor beads and oxygen sensor beads were also beneficial to study the relationship between exercise and glucose metabolism of skeletal muscle cells in type 2 diabetes. Bright fluorescent beads acted as remarkable marker of contractile displacement of myotubes on a gel to quantitatively assess contractile force of the cells. Oxygen-responsible beads enabled successful imaging of oxygen-consumption activity of GOD pattern as a cellular model, suggesting applicability of the imaging sheet to cellular metabolic assay.

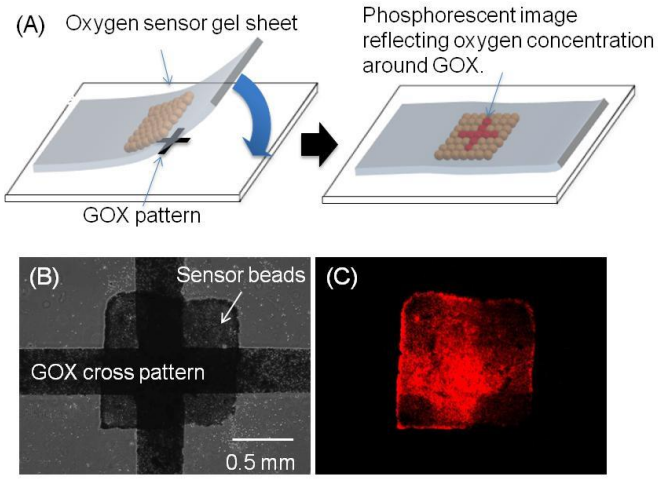

Figure 5. (A) Schematic view of oxygen imaging around the GOX pattern using the oxygen sensor gel sheet. (B,C) Phase-contrast (B) and phosphorescent micrograph (C) of the oxygen imaging sheet attached onto the cross-formed GOX pattern.

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CONTACT

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