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Non-invasive measurement of glucose uptake of skeletal muscle tissue models using a glucose nanobiosensor

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ABSTRACT

Skeletal muscle tissues play a significant role to maintain the glucose level of whole body and any dysfunction of this tissue leads to the diabetes disease. A culture medium was created in which the muscle cells could survive for a long time and meanwhile it did not interfere with the glucose sensing. We fabricated a model of skeletal muscle tissues *in vitro* to monitor its glucose uptake. A nanoporous gold as a high sensitive nanobiosensor was then successfully developed and employed to detect the glucose uptake of the tissue models in this medium upon applying the electrical stimulation in a rapid, and non-invasive approach. The response of the glucose sensor was linear in a wide concentration range of 1–50 mM, with a detection limit of 3 μM at a signal-to-noise ratio of 3.0. The skeletal muscle tissue was electrically stimulated during 24 h and glucose uptake was monitored during this period. During the first 3 h of stimulation, electrically stimulated muscle tissue consumed almost twice the amount of glucose than counterpart non-stimulated sample. In total, the glucose consumption of muscle tissues was higher for the electrically stimulated tissues compared to those without applying the electrical field.

1. Introduction

Tight control of glucose level in body is crucial to decrease the disease complications. Therefore, lots of research groups have been explored robust and reliable methods for monitoring of glucose concentration. Electrochemical-based methods to measure the glucose have gained unique attention because of low cost, simplicity, and high selectivity and sensitivity (Wang, 2007). In general, electrochemical glucose biosensors can be divided into two main categories, enzyme-based biosensors and non-enzyme based ones (Heller and Feldman, 2008). Enzyme-based glucose biosensors were the first generation of electrochemical glucose biosensors. They employ glucose oxidase, glucose dehydrogenase, or other enzymes to detect the glucose. In our recent work, we proposed an enzyme-modified nanoporous gold to detect the glucose in physiological conditions with commonly available interferents (Chen et al., 2012). Even though the enzyme-based glucose biosensors have already been commercialized, their applications have been restricted mainly due to chemical and thermal instabilities of used enzymes. Non-enzyme based glucose biosensors have been emerged to tackle these problems and to enhance the selectivity and sensitivity of electrochemical biosensors. In addition, rapidly growing nanotechnologies and nanomaterials have provided more opportunity to improve and optimize design and fabrication of the non-enzyme based glucose biosensors (Hu et al., 2012). In our previous works, we reported the applications of free-standing nanoporous gold (NPG) films and gold covered nanoporous copper composites for the non-enzyme electrochemical sensing of glucose (Chen et al., 2010, 2011). We showed rapid response, high sensitivity and selectivity of these nanomaterials for the glucose detection. However, most non-enzyme-based biosensors using nanomaterials have been shown to efficiently work in glucose-contained buffer solutions, which are far from physiological samples such as serum or blood (Cash and Clark, 2010). The reason could be adverse effects of components in these samples on the glucose detection as to occupy the active sites of biosensors and prevent effective adsorption of glucose. Therefore, it is required to extend the application of glucose nanobiosensors to measure the glucose uptake in physiological conditions for biological samples, such as muscle tissues in a real-time, accurate, and non-invasive manner.

In vitro cell culture (or tissue) models having physiological functions can be used to assess biological phenomena in an easy,

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accurate, and controllable manner. They provide an invaluable tool to replace corresponding animal experiments, which are very costly, laborious, and time-consuming. Skeletal muscle tissue has major responsibility for the maintenance of glucose in the whole body (Corcoran et al., 2007; Nedachi et al., 2008). Problems in glucose uptake of skeletal muscle tissues lead to the type 2 diabetes, the most common type of diabetes (Hayashi et al., 1997; Oshikawa et al., 2004). Therefore, development of an *in vitro* model of muscle tissues is of great interest. It can be used to analyze drugs against type 2 diabetes and mechanisms of exercise- and insulin-induced glucose uptake by the skeletal muscle tissue, which have usually been performed by longtime and expensive animal experiments because current *in vitro* muscle tissue models are not able to contract. The contractile ability of a muscle tissue is needed to study mechanical properties and physiologically relevant energy phenomena occurred in a muscle tissue (Kaji et al., 2010).

In this investigation, an *in vitro* contractile muscle tissue model was fabricated. A nanoporous gold biosensor was then employed to measure the glucose consumption of the contractile muscle tissue. This is the first report to successfully use a nanomaterial as a high sensitive glucose detector for skeletal muscle tissue. This system opens new avenues for the research in this field helping to understand biological phenomena related to the diabetes and to screen novel drugs and treatments for this worldwide disease.

2. Materials and methods

2.1. Materials

Following materials were purchased and used without further purification: gelatin Type A from porcine skin, methacrylate anhydrate, trimethoxysilyl propyl methacrylate (TMSPMA), penicillin/streptomycin (P/S), and trichloro(1H, 2H, 2H-perfluorooctyl) silane (all from Sigma-Aldrich, USA), polydimethylsiloxane (PDMS) (SILPOT 184, Dow Corning Toray, Japan), Dulbecco's modified Eagle's medium (DMEM), trypsin/EDTA, MEM essential amino acid, Dulbecco's phosphate buffer saline (DPBS), MEM nonessential amino acid, horse serum, and insulin (all from Invitrogen, USA), fetal bovine serum (FBS) (Bioserum, Japan), indium tin oxide (ITO) glass slide (Sanyo Vacuum Industries, Japan), positive g-line photoresist (*i.e.*, S1818) and developer (*i.e.*, MF CD-26) (Shipley Far East, Japan), 2-Hydroxy-1-(4-(hydroxyethoxy) phenyl)-2-methyl-1-propanone (*i.e.*, Irgacure 2959) (Ciba Chemicals, Japan), and hexamethyldisilazane (Tokyo Ohka Kogyo, Japan), anhydrous $MgSO_4$, $Na_2HPO_4.2H_2O$, KH_2PO_4, $NaHCO_3$, and glucose (all from Wako Pure Chemical Industries, Japan).

2.2. Cell culture

Murine C2C12 myoblast cells (from the American Type Culture Collection (ATCC)) were cultured under a 5% CO_2 atmosphere at 37 °C in DMEM, 10% (v/v) FBS, 1% (v/v) P/S. When ~70% confluency was reached, the cells were detached by using 0.25% (w/v) trypsin/0.1% (w/v) EDTA and then subcultured or used in the experiment.

2.3. Preparation of gelatin methacrylate (GelMA) prepolymer

GelMA was synthesized as delineated in our previous work (Nichol et al., 2010). In brief, highly (~80%) methacrylated gelatin was produced by mixing 8 mL methacrylic anhydride with 10 g of type-A porcine-skin gelatin in 100 mL DPBS. The mixture was stirred at 50 °C for 3 h. The reaction was stopped by diluting the mixture fourfold with warm (40 °C) DBPS. The mixture, in a 12–14 kDa cutoff dialysis tube, was dialyzed against distilled water for 1 week at 40 °C and then lyophilized. GelMA prepolymer was prepared by dissolving 20% (w/v) GelMA and 1% (w/v) photoinitiator (Irgacure 2959) in DPBS at 70 °C.

2.4. PDMS stamp preparation

A silicon wafer patterned with a SU-8 photoresist pattern was made using the conventional photolithography method (Ostrovidov et al., 2004). This template was then used to make PDMS stamps as follows. PDMS prepolymer and its curing agent were mixed at 10:1 ratio (w/w) and poured onto the silicon master mold and the mold was held under vacuum for 15 min to remove air bubbles. After the system was cured at 70 °C for 2 h, PDMS stamps were peeled off the master mold and then silanized with trichloro(1H, 2H, 2H-perfluorooctyl)silane to prevent adhesion of the GelMA hydrogel to the stamp.

2.5. Design and fabrication of interdigitated array of Pt (IDA-Pt) electrodes

IDA-Pt electrodes were fabricated such that the effective electrode dimensions were $8 \times 12\ mm^2$ as shown in Fig. 1. The band electrode was 50 μm wide and had a 50 μm gap. The electrodes were patterned on the glass slide (thickness 1 mm; Matsunami, Japan) by the conventional photolithography procedure. Briefly, hexamethyldisilazane and S1818 were poured onto a glass slide, and the slide was baked at 90 °C for 10 min. The slide was then irradiated with UV light through a mask aligner (MA-20; Mikasa, Japan) and developed at MF CD-26. A Ti adhesive layer was then seeded onto the glass slide followed by a Pt film (100 nm thickness). The electrode design was revealed by the lift-off technique. Eventually, a passivation layer of SU-8 photoresist was patterned on the device such that only the electrode bands remained intact. The photoresist layer was then hard baked to create an inert polymer resin.

2.6. Microcontact molding of GelMA hydrogels and cell seeding

GelMA hydrogels were molded on the IDA-Pt electrodes. Briefly, 40 μL of the GelMA prepolymer was poured on the IDA-Pt electrodes and the PDMS stamp was placed into the prepolymer. The stamp was softly rubbed so that its microgrooves were filled with the prepolymer and then the stamp and prepolymer were exposed to $7\ mW/cm^2$ UV light (Hayashi UL-410UV-1, Hayashi Electronic Shenzen, Japan) for 150 s. The stamp was then gently removed to obtain a micropatterned GelMA hydrogel. Feature sizes of the GelMA micropattern were 50 μm groove, 50 μm ridge, and 50 μm height. To culture the C2C12 cells on the microgrooved GelMA hydrogel (Fig. 1), the cells were first trypsinized, counted, and then resuspended in DMEM at a density of 1.5×10^7 cells/mL. 100 μL of the cell suspension was placed onto the micropatterned hydrogel and then the system was left undisturbed for 20 min at 37 °C to allow the C2C12 myoblasts to attach to the insides of the grooves. Culture medium was then added to the dish and immediately aspirated to remove non-adherent cells. Fresh medium was then added and the cells were cultured for 2 days. The medium was then replaced with differentiation medium (DMEM, 2% (v/v) horse serum, 1 nM insulin, and 1% (v/v) P/S). The differentiation medium was normally changed every 2 days.

2.7. Electrical stimulation of the engineered muscle tissue

On day 9 of culture, the engineered muscle tissue was electrically stimulated through the IDA-Pt electrodes as depicted in Fig. 1. For the electrical stimulation of muscle tissue, the