AN APPLICATION OF INTERDIGITATED ARRAY OF Pt ELECTRODES FOR ELECTRICAL STIMULATION OF ENGINEERED MUSCLE TISSUE

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ABSTRACT

Electrical field stimulation provides an efficient way to differentiate muscle cells, which results in an increase in the contractile activity and the formation of muscle myofibers. Here, we introduced the use of an interdigitated array of Pt (IDA-Pt) electrodes as a novel platform for the electrical stimulation (ES) of engineered muscle tissues.

KEYWORDS

C2C12 skeletal muscle cells, muscle tissue engineering, electrical stimulation, interdigitated array of electrodes.

INTRODUCTION

Muscle tissue engineering has been proposed as an alternative therapy to regenerate or recover damaged muscle tissues in body [1]. Engineered muscle tissues also have *in vitro* applications, such as for drug screening [2] or as bio-actuators [3]. Electrical field stimulation stands an efficient way to differentiate muscle cells, which results in an increase in the contractile activity and the formation of muscle myofibers. Here, we suggest the use of an interdigitated array of Pt electrodes (Figure 1) as a novel device to electrically stimulate engineered muscle tissues with the following advantages: (1) because the electrodes were permanently fixed on the glass substrate they easily provided a highly accurate, reproducible and well-defined electric field; (2) this technology made it possible to make high resolution and different electrode designs mimicking physiological feature sizes and topographies; (3) the ES was equally distributed over the whole tissue; (4) lower energy was needed to create a specified electric field compared to the conventional setups for the ES. We should mention that traditional ES setups use a pair of conductive materials inside the tissue culture medium, in close proximity to the muscle tissue. In such a configuration, the electric current should pass through the surrounding medium of the tissue. Due to the inherent resistance of the medium, there is a loss in the electric field, and the resulting electric field sensed by the muscle tissue may not be effective in the tissue stimulation.

EXPERIMENT

The experimental procedure used to obtain the functional muscle tissues is illustrated in Figure 1. Briefly, micromolded gelatin methacrylate (GelMA) fabricated by the PDMS stamp (12 mm×8 mm) was created on the IDA-Pt electrodes. GelMA hydrogel is a photopolymerizable hydrogel comprised of gelatin with the methacrylic anhydride groups. The topography created by the GelMA hydrogel influenced the cell morphology and alignment. Most C2C12 myoblasts that loaded onto the GelMA micropattern changed their morphology, oriented, and elongated along the ridge-groove direction after 1 day of culture (Figure 2). This phenomenon is the so-called *contact guidance*. Note that muscle cells were stained using phalloidin (AlexaFluor® 594, Invitrogen) and 4,6-diamidino-2-phenylindole (DAPI) (Vector Laboratories Inc.) to reveal F-actin (red) and cell nuclei (blue), respectively. After 2 days of culture, the muscle cell differentiation was induced by which the C2C12 myoblasts started to fuse together making multinucleated myotubes. On day 8 of culture, the engineered muscle tissue was electrically stimulated through the IDA-Pt electrodes as depicted in Figure 1. As control system, the muscle tissue was stimulated with long platinum wires with 0.6 mm diameter placed 1.5 cm apart. Electrical pulses were applied to the muscle tissue using a waveform generator under two different ES regimes, namely, regime 1 (voltage 0.5 V, frequency 1 Hz, and duration 10 ms) and regime 2 (voltage 6 V, frequency 1 Hz, and duration 10 ms) for 3 days or 1 day, respectively.

C2C12 myotubes stimulated through the IDA-Pt electrodes showed higher amount of alignment (~80%) compared to those stimulated using the Pt wires (~60%). High degree of alignment and organization of myotubes are basic requirements of an engineered muscle tissue [4]. Note that the close elasticity of the 20% GelMA hydrogel (~40 kPa) to that of the native muscle tissue (~12 kPa) [5] is an essential factor enabling such a myotube arrangement because the myotubes could move and alter their directions. High maturation and alignment of stained myotubes due to applying the electric field through IDA-Pt electrodes under regime 2 are shown in Figure 3. The cultures that were electrically stimulated using IDA-Pt electrodes were further compared to their corresponding controls and those stimulated using the conventional ES setup of Pt wires in terms of gene expression of muscle transcription factors (*i.e.*, MyoD, Myf-5, myogenin, MRF4, Mef2c, and MLP) and proteins biomarkers (*i.e.*, sarcomeric actin, α-actinin, perinatal myosin heavy chain (MHC-pn), MHC-IId/x, MHC-IIa, and MHC-IIb). As demonstrated in Figure 4, the gene expression levels were generally higher for the cultures stimulated by the IDA-Pt electrodes under regime 2 compared with the controls and those stimulated through Pt wires. However, in general, there was no statistically significant difference in gene

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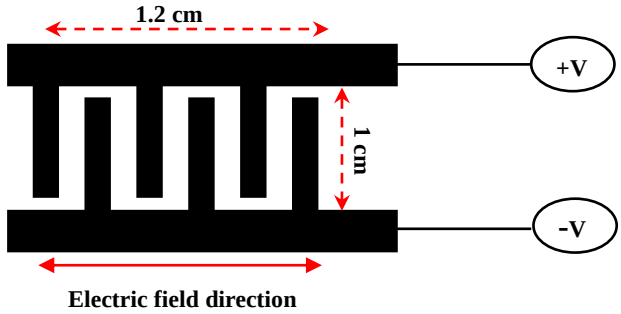
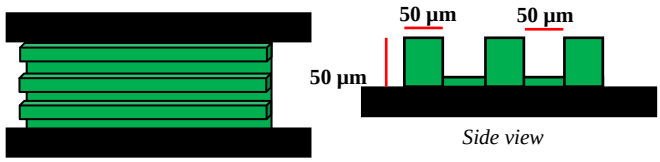
expression levels between the muscle tissues stimulated under paradigm 1 through IDA-Pt electrodes and Pt wires, indicating that 0.5 V is probably less than the threshold voltage required for the ES of a functional muscle tissue [6]. We also observed the contraction of myotubes under regime 2. In contrast, no such phenomenon was observed for the myotubes exposed to ES under regime 1.

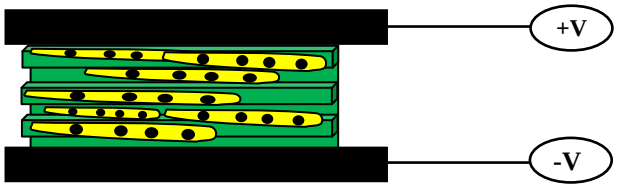
Taken together, we showed that the IDA-Pt electrodes were more efficient in the stimulation of muscle tissues compared to conventional setups. Due to the wide array of potential applications of ES to two- and three-dimensional (2D and 3D) engineered tissues, the suggested platform could be employed for a variety of cell and tissue structures to more efficiently investigate their responses to electrical fields.



(1) Fabrication of IDA-Pt electrodes on the glass slide.



(2) Micromolding of 20% GelMA hydrogel.



(3)Applying AC electric field through IDA-Pt electrodes to C2C12 myotubes at day 8 of culture.

Figure 1. Schematic procedure of IDA-Pt electrodes and their use to stimulate skeletal muscle tissues.

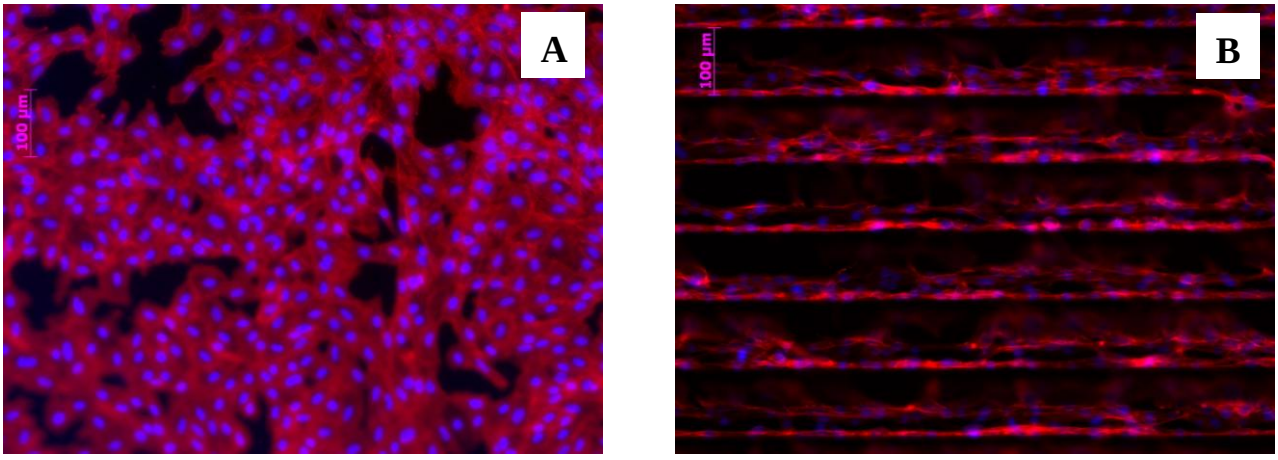


Figure 2. C2C12 myoblasts alignment and elongation within the unpatterned (A) and micropatterned GelMA hydrogels after 1 day of culture. The cell nuclei and filamentous F-actin were stained by DAPI (blue) and phalloidin (red), respectively.

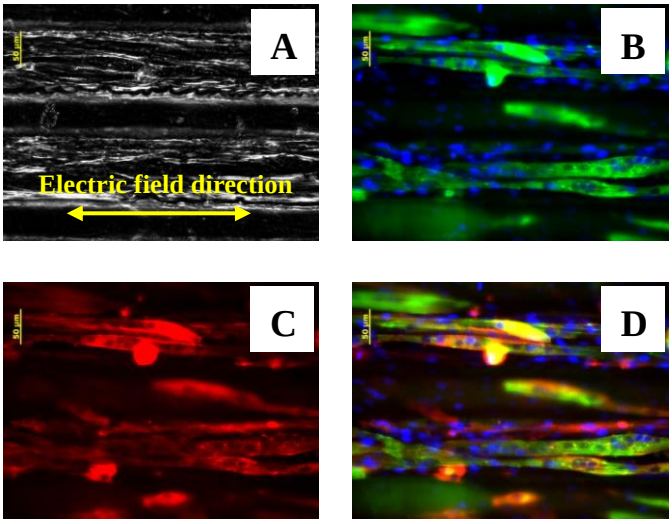


Figure 3. C2C12 myotubes micrographs after applying ES through IDA-Pt electrodes under regime 2 (voltage 6 V, frequency 1 Hz, and duration 10 ms) as shown by phase contrast microscope (A) and as stained to reveal the myosin heavy chain (green), cell nuclei (blue), F-actin (red) (B-D).

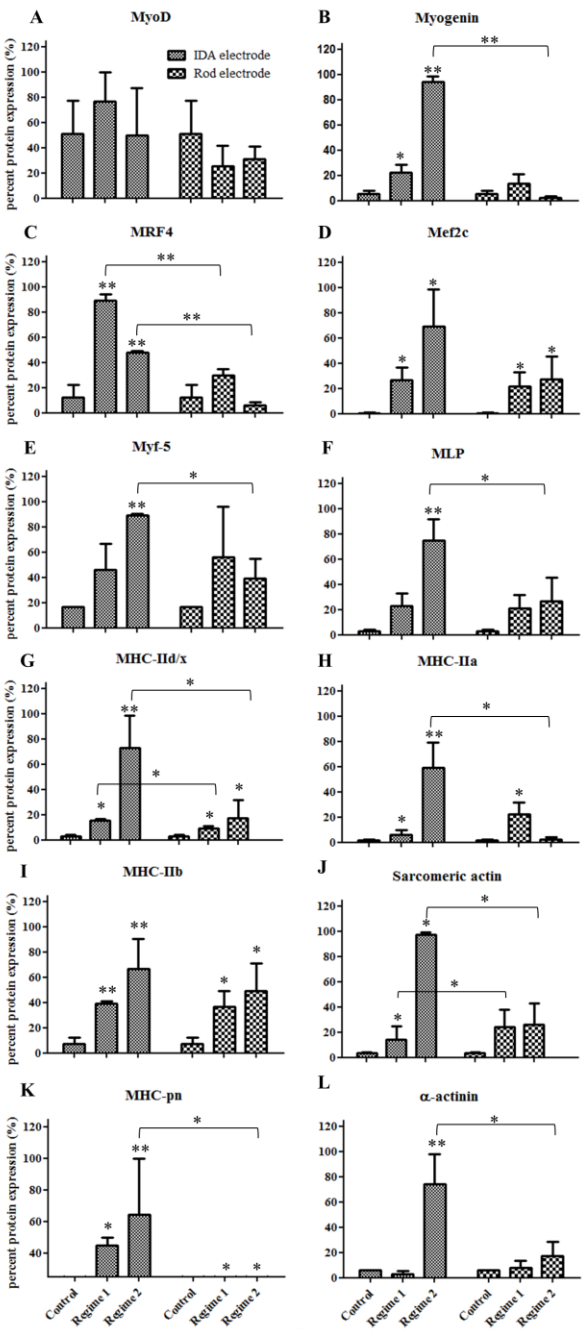


*Figure 4. Changes in the expression levels of genes as a result of the different ES paradigms of regime 1 (voltage 0.5 V, frequency 1 Hz, and duration 10 ms), regime 2 (voltage 6 V, frequency 1 Hz, and duration 10 ms), and control (without ES). Expression levels were normalized with respect to the internal reference gene GAPDH (*p <0.05 and **p <0.001).*

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