CONTRACTILE SKELETAL MUSCLE MICROTISSUES IN MICROCHANNEL

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ABSTRACT

This paper reports a development of the microfluidic device for fabrication and cultivation of contractile skeletal muscle tissue in microchannels. Microfluidic processes were only used to develop contractile skeletal muscle microtissues in microchannels. Thus, the volume of the collagen gel used to fabricate the microtissues can be defined precisely. The fabricated microtissues can be cultured under a more *in vivo*-like environment and used for higher throughput applications. Tunnel-like microchannels connecting 2 microchannels realized to pattern the collagen gel with cells and supply the medium to the cells (Fig.1). The developed microtissues in microchannels may be powerful tools for drug development and biological research.

KEYWORDS

Cells, 3D-Cell culture, Skeletal muscle, Muscle on a chip, Electrical stimulation, Force

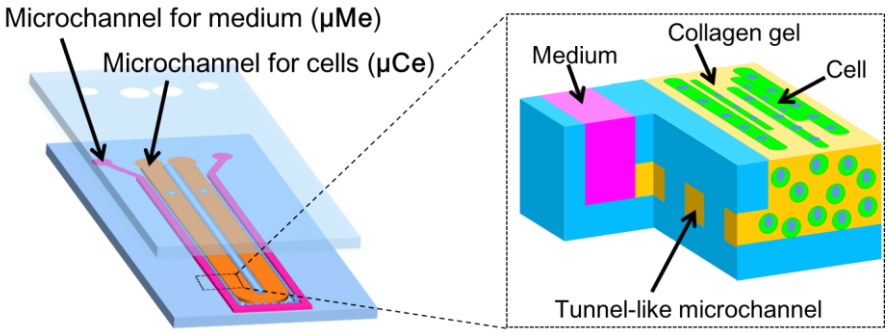

Figure 1: Conceptual illustrations of the device.

INTRODUCTION

Skeletal muscle possesses the physiological significance and outstanding characteristics. So far, some groups including our group have fabricated 3-dimensional (3D) cultured skeletal muscle microtissues in vitro and utilized them for drug screening and biological research [1-3]. Although the microfluidic devices for 3D cell culture have been developed for some cell types (endothelial cells, neural cells, etc), the devices for skeletal muscle cells have not been reported to the best of our knowledge. Previously, the skeletal muscle microtissues were fabricated by using microfabrication techniques and cultured in micro-wells or dishes filled with medium [3-6]. In contrast, in the present study, microfluidic technologies were used to both fabricate and culture the skeletal muscle microtissues.

EXPERIMENT

As shown in Fig.1, the device consists of 2 microchannels for cells (μCe) and medium (μMe). They are connected each other by tunnel-like microchannels. Collagen solution mixed with cells are introduced into the μCe and solidified at 37°C. After that, medium is introduced into the μMe and cells in the collagen gel are cultured. In this process, tunnels play 2 roles. One is that they prevent the collagen solution from leaking into the μMe. The other is that they allow the medium diffuse into the gel in the μCe for cell culture. The device was made of 2 layers of PDMS. Micropatterned slabs were bonded to slabs without patterns (Fig.2).

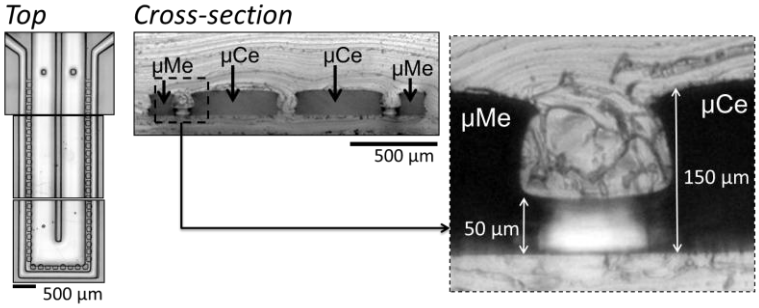

Figure 2. Top and cross-sectional views of the developed microdevice.

The performance of the tunnels was investigated (Figs.3 and 4). When the colored solution was introduced into μCe, no leakage of the solution into μMe was observed (Fig.3). To confirm the diffusion of the medium, FITC-labeled albumin solution was introduced into the μMe after the collagen solution was solidified in the μCe. The albumin diffused throughout the collagen gel within 120 min (Fig.4).

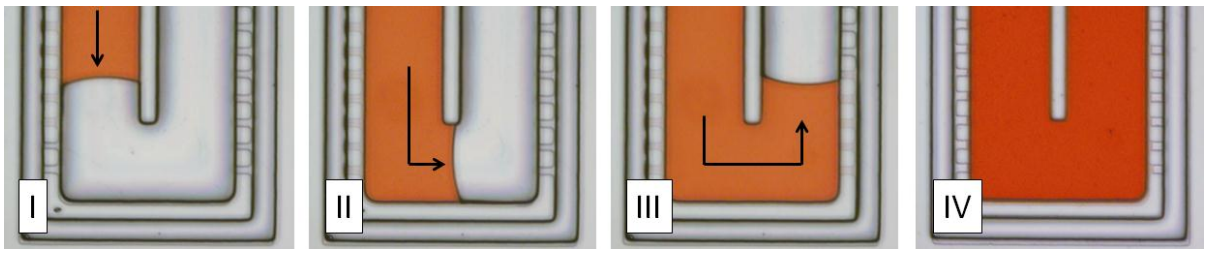

Figure 3. Introduction of the colored solution into the μCe. No leakage was observed to the μMe.

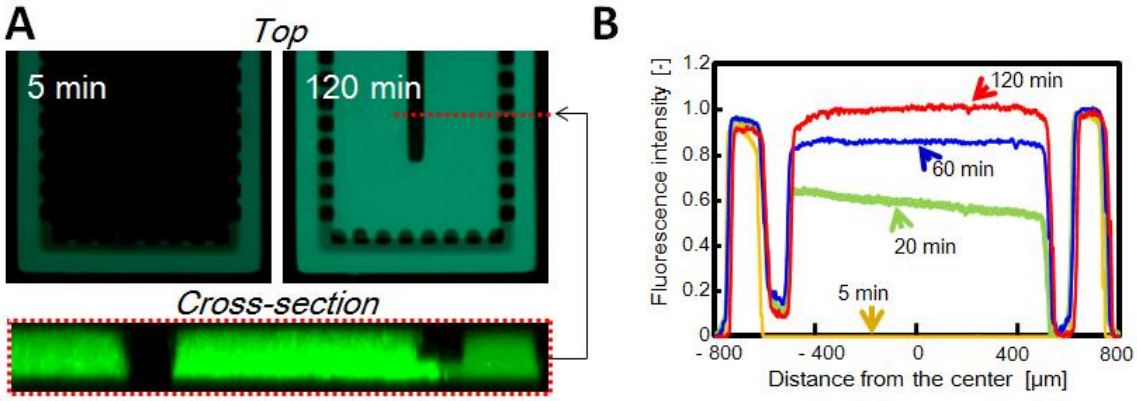

Figure 4. Introduction of the FITC labeled albumin solution into the μMe of the device with collagen gel in the μCe. The albumin was diffused throughout the gel within 120 min (A and B).

Mice skeletal myoblasts (C2C12) was cultured in the device (Fig.5). Myoblasts need to differentiate to myotubes to obtain contractility. The medium containing 2% horse serum was used for inducing differentiation. The collagen solution containing myoblasts (10^7 cells/ml) was introduced and solidified in the μCe. Then, the medium was introduced into the μMe. The gel started to dissociate from the inner surface of the μCe after 4 days and cylindrical 3D micro-tissue was formed after 6 days (Fig.5A). Aligned myotubes (α-actinin positive cells) were observed in the microtissue (Fig.5B) and the cultured tissue in the μCe contracted in response to the applied electric stimulation (Fig.6).

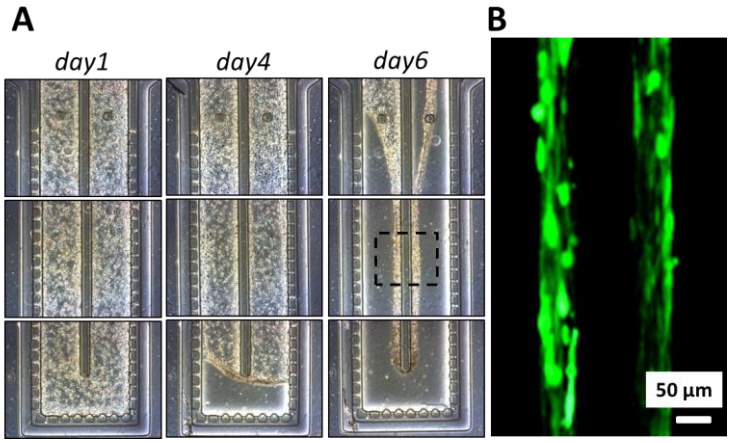

Figure 5. Results of cell culture in the device. A: Process of differentiation. B: Myotubes were visualized (Green: α-Actinin).

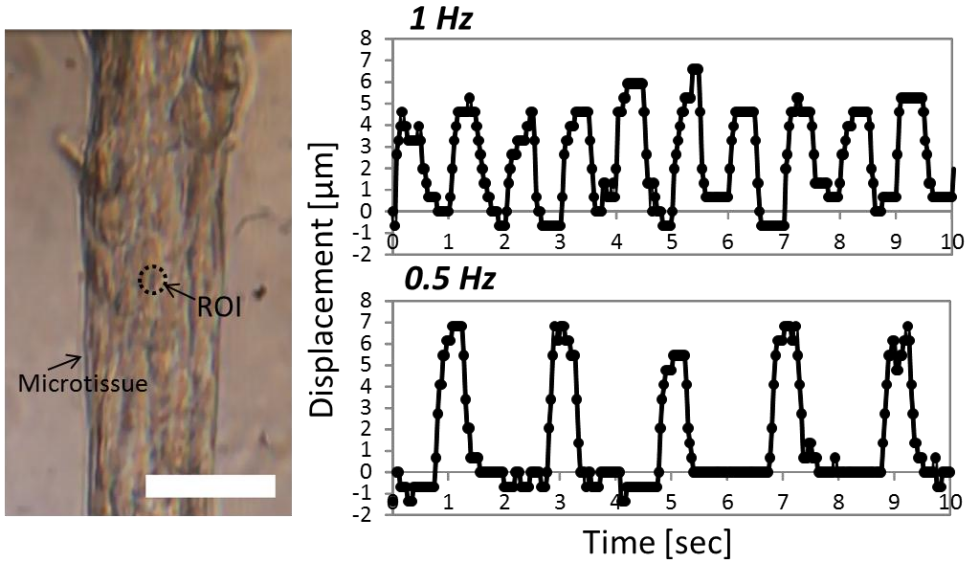

Figure 6. Cultured skeletal muscle microtissues (day6) were contracted in response to the applied electrical stimulation (1 and 0.5 Hz). Scale bar: 50 μm.

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