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Development of Engineered Skeletal Muscle Tissue Under Geometric Constraint

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Abstract: In recent years, robots driven by skeletal muscle tissue consisting of muscle cells has been developed. One of the major challenges for these biohybrid robots is to increase the contractile force of the engineered skeletal muscle tissue. Some studies have used the stimulus response of muscle cells to improve engineered skeletal muscle tissue. However, these methods require various equipments and complicated processes. In this study, we proposed a culture method of engineered skeletal muscle tissue under geometric constraint by pins and it simplified stimulation process. We observed the orientation of the muscle fibers in the engineered skeletal muscle tissue cultured with geometric constraint condition. This result suggests that our method may improve the contractile force of the engineered skeletal muscle tissue.

Keywords: bio-robot, muscle cell, tissue engineering

1. INTRODUCTION

In recent years, engineered skeletal muscle tissue consisting of extracellular matrices (e.g., collagen) and skeletal muscle cells have been developed [1, 2]. Furthermore, as an engineering application of engineered skeletal muscle tissue, many researchers have proposed robots implemented the engineered skeletal muscle tissue [3–5]. These robots have the flexibility and adaptability of living things. When applying engineered skeletal muscle tissue to robots, it is important to maximize the contraction force of the muscles. Some studies have used the stimulus response of muscle cells to improve engineered skeletal muscle tissue [6–9]. These studies showed that culturing skeletal muscle cells under mechanical stimulation promoted cell maturation [6, 7, 9] and oriented muscle fibers, which are mature muscle cells [8, 9]. As muscle cells mature, their contractile force improves and skeletal muscle cells generate uniaxial force [9]. Following these findings, the culturing under mechanical stimulation contributes to the improvement of the contractile force of engineered skeletal muscle tissue. However, the conventional methods have used various devices such as extension devices, and which complicates the stimulation process.

In this study, we aim to establish a simple method that can improve contractile force by giving mechanical stimulation to engineered skeletal muscle tissue without various devices. We proposed a culture method that fixes the endpoints by pins (geometric constriant). Engineered skeletal muscle tissue have the property of spontaneously shrinking during the process of culturing. This property gives mechanical stimulation to cells with geometric constraint. In this report, we demonstrated whether the culture method of engineered skeletal muscle tissue under geometric constraint induced the orientation of muscle fibers.

2. RESULTS

The following shows the state of engineered skeletal muscle tissue cultured under the conditions with and without pins (with/without geometric constraint). The study of Heher *et al.* showed the orientation of muscle fibers of the engineered skeletal muscle tissue was only observed when culturing with mechanical stimulation [9]. Therefore, we considered the orientation of muscle fibers as an indicator of cell maturity. We performed immunostaining of engineered skeletal muscle tissue with anti-myosin heavy chain(MHC) antibody. anti-MHC antibody conventionally have been used to stain matured muscle fibers [5]. Figure.1 shows immunostaining of engineered skeletal muscle tissue cultured with/without geometric constraint. As Fig.1 shows, the orientation of muscle fibers was observed in a certain direction only with geometric constraint but it wasn't observed without geometric constraint. These results suggest that our method may improve the contractile force of the engineered skeletal muscle tissue. In the future, we plan to design a robot setup that incorporates this culture method and conduct drive experiments for robot applications.

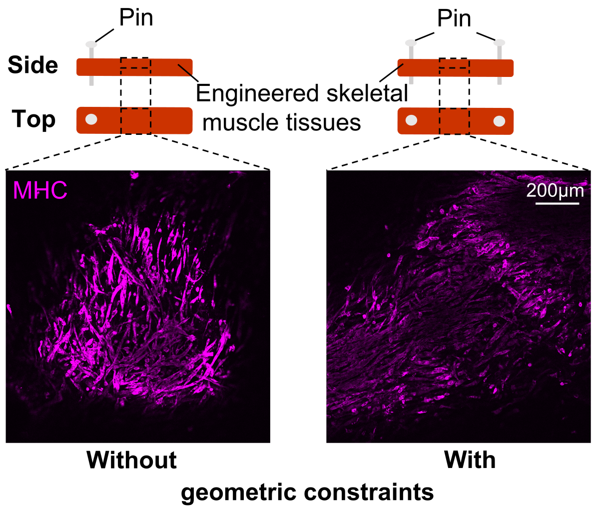

Fig. 1. Muscle fiber with/without geometric constraint

† Ryo Teramae is the presenter of this paper.

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