Design of Environment for Arrangement of Cultured Muscle Cells

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ABSTRACT

The effect of scaffold and flow on orientation of cultured muscle cells has been studied to make muscle fiber *in vitro*. C2C12 (Mouse myoblast cell line) cells were cultured with High-glucose Dulbecco's Modified Eagle's Medium on a dish to make muscle tubes. The scaffolds were designed with the oriented polypropylene fibers. In the other experiment, the medium was stirred to give flow stimulation on cells. Differentiation was induced with horse serum. Orientation of cells was observed with a phase-contrast microscope. The experimental results show that the direction of cells can be controlled with the direction of the scaffold fiber and the flow.

Keywords: Biomedical Engineering, Muscle Cells, Scaffold, Flow, Differentiation and Orientation

1. INTRODUCTION

A biological muscle generates power even in a small unit, collaborating with environment. The recent progress in cell culture technique has realized muscle cell culture, which has potential to be applied for an engineered actuator [1]. To make an efficient contractile unit with cultured muscle cells, cell culture environment should be designed. The effect of magnetic field on muscle cells [2] and the effect of surface micro morphology on the alignment of skeletal muscle myoblasts [3] have been studied in the previous studies. In the present study, the effect of scaffold and flow on orientation of cultured muscle cells has been studied to make muscle fiber *in vitro*.

2. METHODS

Scaffold

Polypropylene fibers of 0.02 mm diameter were used as the scaffold for muscle cell culture. The fibers were soaked in an ultrasonic washing machine filled with acetone to clean their surface, and dried. Every fiber was arranged parallel each other with the chuck of silicone rubber of 3 mm thick. After the distances between fibers were adjusted between 0.1 mm and 0.2 mm, the fibers were fixed on the stainless frame (Fig. 1). Variations were made about coating on the fibers: Laminin (cell adhesive glycoprotein constituting basement membrane) and collagen. In the former case, the fibers were soaked in phosphate buffer solution including Laminin. In the latter case, the fibers were covered with type 1-A collagen dissolved in concentrated culture medium. The fibers are located at 3 mm above the bottom of the culture dish with the thick of silicone rubber spacer. Cells were seeded on the scaffold and cultured. When cells were seeded, a slide glass coated with cellulose was located under the scaffold frame to avoid cell adhesion to the bottom of the dish (Fig. 2). The slide glass was removed in two days of cultivation. C2C12 (Mouse myoblast cell line) cells were cultured with Dulbecco's Modified Eagle's Medium including ten percent of fetal bovine serum (FBS) and atibiotics. The cells were seeded with density of 3000000 cells per dish.

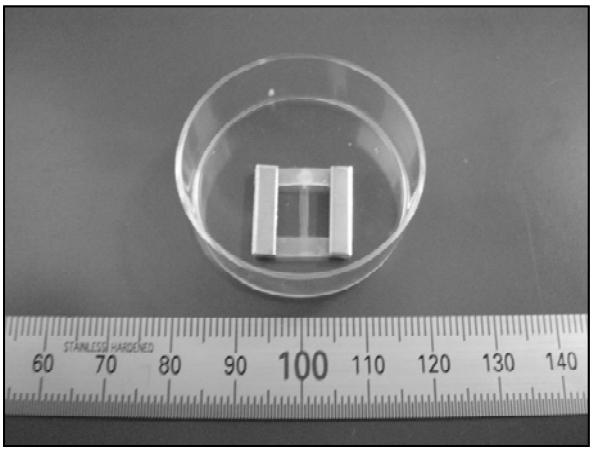

Fig. 1: Scaffold frame with polypropylene fiber.

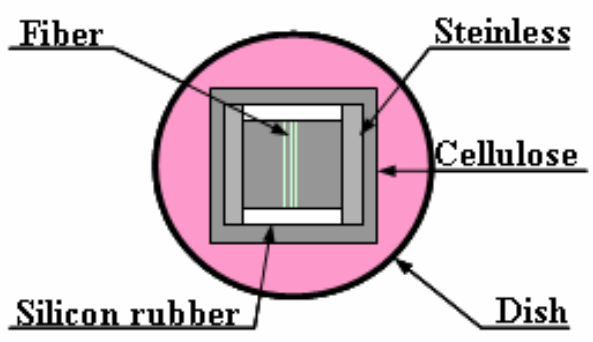

Fig. 2: Arrangement of scaffold in culture dish.

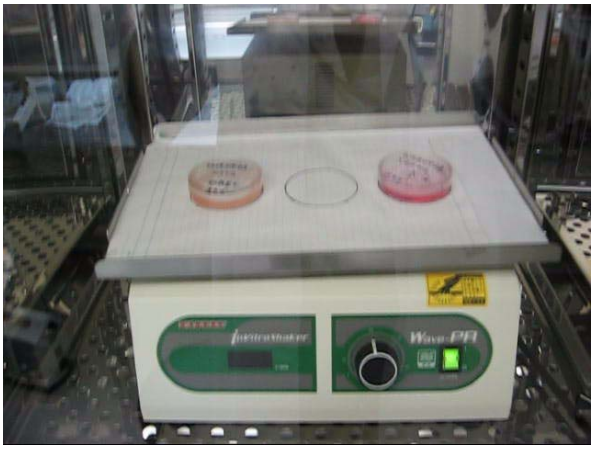

Fig. 3: Tilted rotating disk. Culture dish with (left) and without (right) silicone rubber sheet disk.

The medium was replaced every two days. The cells were cultivated for 16 days, and observed with a phase-contrast microscope.

Reciprocating flow

A culture dish was placed on a plate, which generates seesaw movement. A sheet of silicone rubber was sandwiched between the dish and the disk for a nonslip strip. The tilted angle was set to seven degrees, and the reciprocating speed was set to thirty times per minutes in the test. A dish of 60 mm diameter and a pentagon dish of 75 mm length were placed on the disk. The reciprocating flow was generated in the medium in the culture dish. The seesaw movement was started at the next day from cells seeding, and kept for one week. C2C12 (Mouse myoblast cell line) cells were cultured with Dulbecco's Modified Eagle's Medium including ten volume percent of fetal bovine serum (FBS) and atibiotics. The cells were seeded with density of 10000 cells per square centimeter. After four days cultivation, ten volume percent of FBS was replaced with seven volume percent of horse serum to differentiate cells into myotubes. The medium was replaced every two days. The cells were observed with a phase-contrast microscope.

Rotational flow

A dish of 60 mm diameter was placed on the tilted rotating plate, which generates rotational seesaw wave movement (Fig. 3). The tilted angle was set to six degrees, and the rotating speed was set to fifty revolutions per minutes in the test. The seesaw movement was applied for ten minutes at room temperature when the medium was changed every two days. Circumferential streamline was generated in the medium. C2C12 (Mouse myoblast cell line) cells were cultured with Dulbecco's Modified Eagle's Medium including ten volume percent of fetal bovine serum (FBS) and atibiotics. The cells were seeded with density of 3000 cells per square centimeter. After four days cultivation, ten volume percent of FBS was replaced with seven volume percent of horse serum to differentiate cells into myotubes. The medium was replaced every two days. The cells were observed with a phase-contrast microscope.

Narrowed rotational flow

A silicone rubber sheet disk of 3 mm thickness was placed in the middle on the bottom of culture dish of 60 mm diameter (52 mm inner diameter) to restrict the space for the flow of the medium (Fig. 4). Variation was made in the disk's diameter between 10

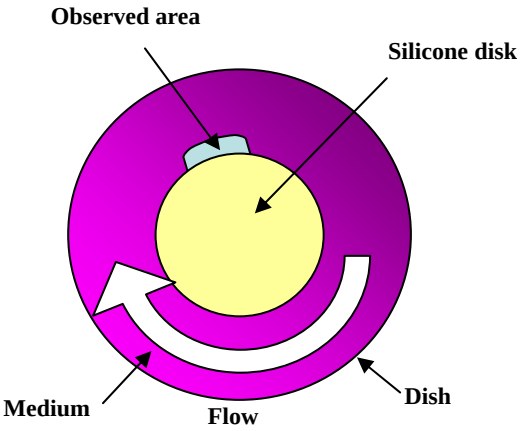

Fig. 4: Tilted rotating disk. Culture dish with (left) and without (right) silicone rubber sheet disk.

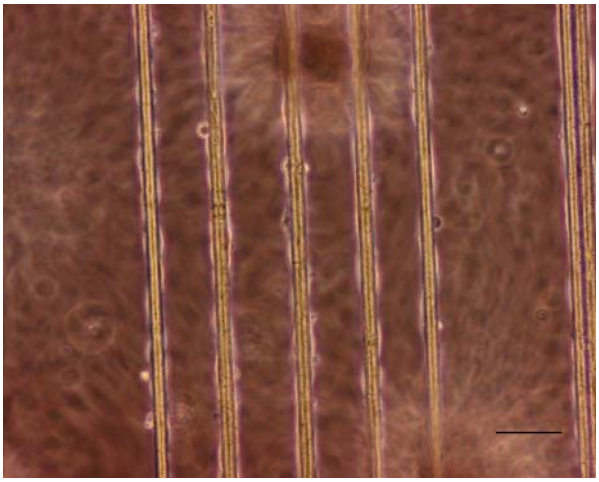

Fig. 5: Cells cultivated for 2 days around Laminin coated fiber. Bar indicates 0.1 mm.

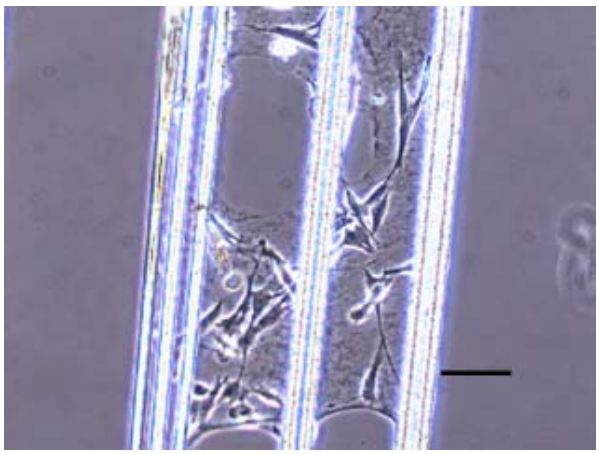

Fig. 6: Cells cultivated for 2 days around collagen coated fiber. Bar indicates 0.1 mm.

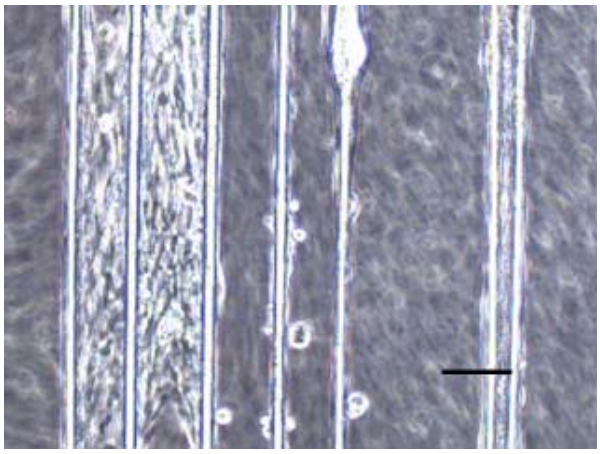

Fig. 7: Cells cultivated for 16 days around Laminin coated fiber. Bar indicates 0.1 mm.

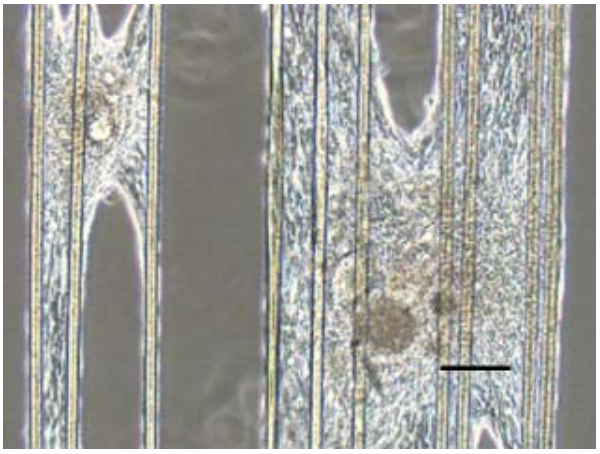

Fig. 8: Cells cultivated for 16 days around collagen coated fiber. Bar indicates 0.1 mm.

mm and 40 mm. The volume of the medium was adjusted not to spread over the superior surface of the disk. One-way rotational flow in the dish was generated with the plate movement of twenty revolutions per minute with six degrees tilted angle. C2C12 cells were cultured with the same method as the preceding section, while the plate was continuously rotating at 37 degrees Celsius in an incubator. The cells were seeded with density of 3000 cells per square centimeter. After six days cultivation, ten volume percent of FBS was replaced with seven volume percent of horse serum to differentiate cells into myotubes. For comparison, cells were cultured in the dish without a silicone rubber sheet at the counter position on the desk (Fig. 3).

3. RESULTS

Scaffold

Figs. 5-8 show the cells around the scaffolds. The experimental results show that cells adhere to the scaffolds, and bridge fibers with sheet of cells in a week. Cells, although, show no orientation between scaffolds in Figs. 4-7. The numbers of adhered cells were larger around fibers coated with

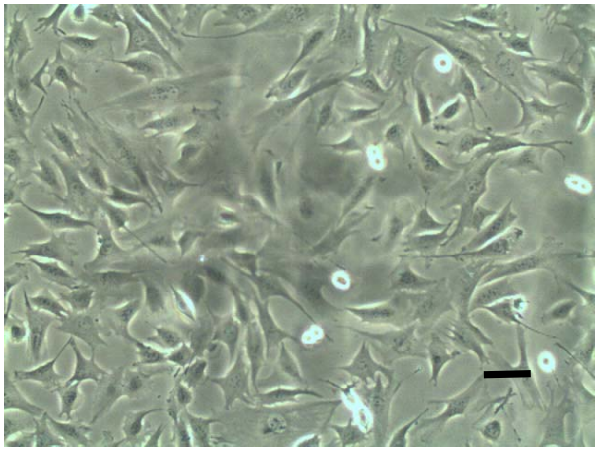

Fig. 9: Cells cultivated for 1 day with reciprocating flow. Bar indicates 0.1 mm.

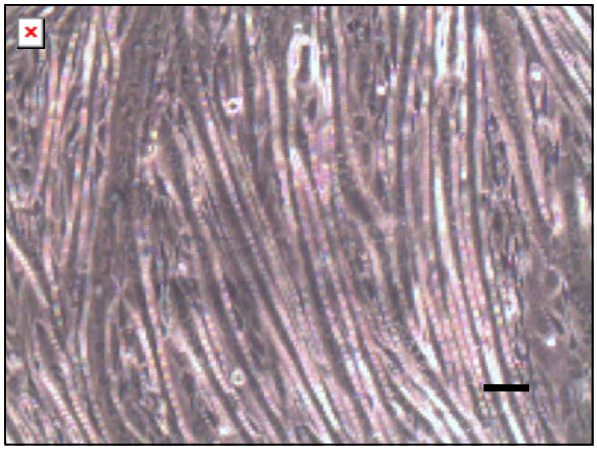

Fig. 10: Cells cultivated for 8 days with reciprocating flow. Bar indicates 0.1 mm.

collagen than those coated with Laminin.

Flow

The experimental results with reciprocating flow show that muscle cells are proliferated, fused and differentiated to myotubes, but their directions are random both in the middle zone and in the peripheral zone of the dish regardless of the shape of the culture dish (Figs. 9 & 10). After flow stimulation for ten minutes, cells were scattered at random, regardless of culture days and of location in the dish (Figs. 11-14). The result of one-way rotational flow shows regular array of myotubes (Figs. 15-18), while that without silicone rubber sheet shows random direction of myotubes (Fig. 19). The cells were attached on the bottom of the dish in two days (Fig. 15) and arranged along the circumferential streamline around the silicone rubber disk in six days (Fig. 16). The array of myotubes grew around the silicone disk day by day, and the alignment curved to the radial direction of the disk as leaving the disk (Figs. 17-18).

4. DISCUSSION

The present study shows following potentials. The collagen

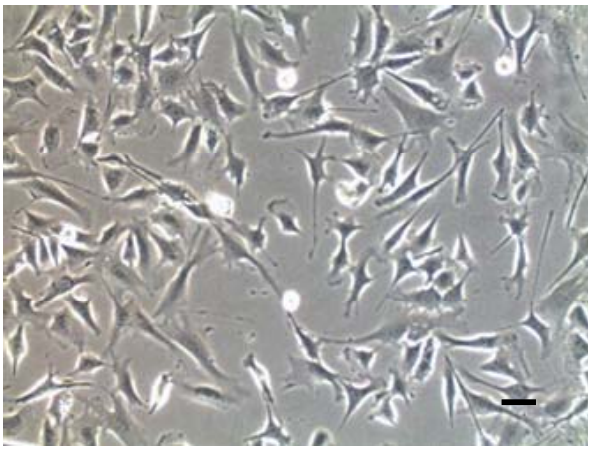

Fig. 11: Cells in the middle zone cultivated for 2 days, before stimulation with flow for ten minutes. Bar indicates 0.1 mm.

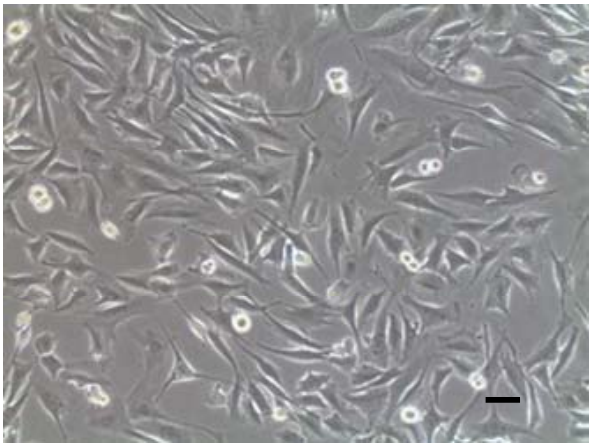

Fig. 12: Cells in the middle zone stimulated by flow for ten minutes after cultivation for 2 days. Bar indicates 0.1 mm.

gel around fibers may supply environment where cells adherence is accelerated. The silicone rubber spacer works to distinguish the scaffold from the bottom of the culture dish. A muscle cell colony can bridge between scaffold fibers, of which distance is shorter than 100 micrometer. In the narrower space between scaffold fibers, cells might line along the fiber.

Reciprocation of flow does not have significant effect to arrange the direction of myotubes. The intermittent flow stimulation for ten minutes does not have visible effect to uniform the arrangement of cells, either. An obstacle of silicone disk in the middle part of culture dish is, on the other hand, is effective to narrow the flowing space, where the rotational circumferential flow occurs with swing motion of the tilted plate. That may also help to restrict the secondary flow in the fluid. The flow and the slope might affect arrangement of myotubes.

5. CONCLUSION

The effect of scaffold and flow on orientation of cultured muscle cells has been studied to make micro actuator *in vitro*. The experimental results show that cells tend to tilt to the direction of the scaffold fiber and the flow.

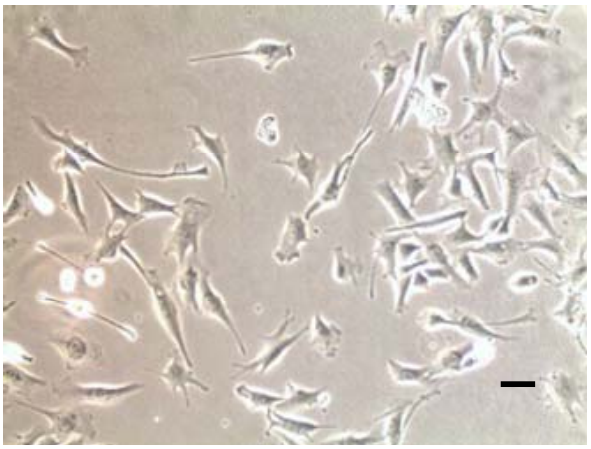

Fig. 13: Cells in the peripheral zone cultivated for 2 days, before stimulation with flow for ten minutes. Bar indicates 0.1 mm.

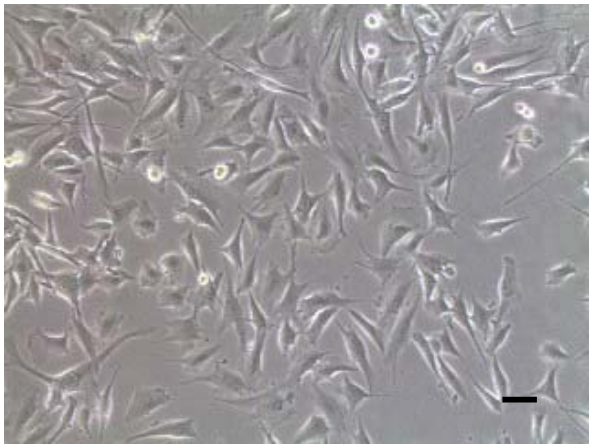

Fig. 14: Cells in the peripheral zone stimulated by flow after cultivation for 2 days. Bar indicates 0.1 mm.

6. ACKNOWLEDGMENT

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Fig. 15: Cells in the peripheral zone stimulated by flow after cultivation for 2 days. 10 mm. Bar indicates 0.1 mm.

Fig. 16: Cells in the peripheral zone stimulated by flow after cultivation for 6 days. 10 mm. Bar indicates 0.1 mm.

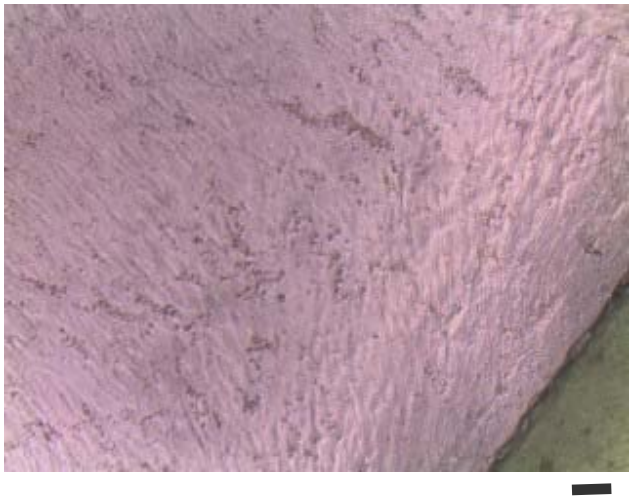

Fig. 17: Cells in the peripheral zone stimulated by flow after cultivation for 11 days. 15 mm. Bar indicates 0.1 mm.

Fig. 18: Cells in the peripheral zone stimulated by flow after cultivation for 11 days. 25 mm. Bar indicates 0.1 mm.

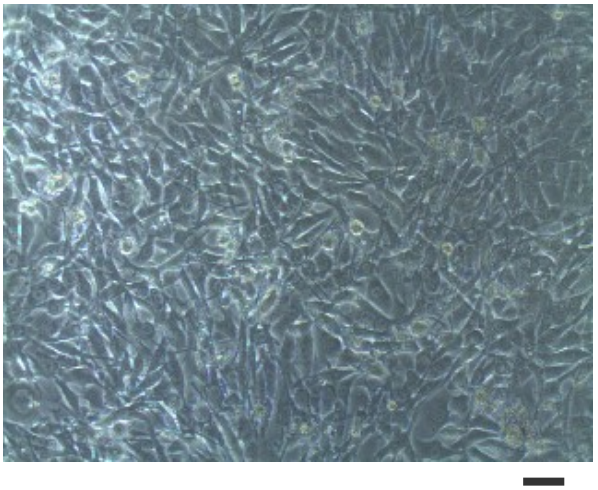

Fig. 19: Cells cultivated in flow for 5 days without silicone rubber sheet. Bar indicates 0.1 mm.