RESEARCH ARTICLE



# Are Quantitatively Micro-machined Scaffolds Effective for Cell Technology?

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**Biological cells adsorb on the scaffold, and show activities: migration, deformation, proliferation, and differentiation. The micro morphology (close to the cell size) on the surface of the scaffold (made by the photolithography technique) is effective for several applications: the marker to trace each cell, and the tool to control the activity of each cell. C2C12 (mouse myoblast) is used in the present study. The typical diameter of the cell is 20 μm, when it is suspended in the medium. The cell aligns along the micro step of the height (> 0.7 μm). The micro-striped groove can control the cell orientation in the flow channel. The aspect ratio of the checkered convexo-concave pattern can control the orientation of cells. When cells are cultured on the thin film (thickness 6 μm, polydimethylsiloxane) with the micro markers at the counter surface, the local contraction movement of myotubes by the electrical-pulse stimulation can be microscopically measured through the transparent scaffold.**

## Introduction

Cell culture technique has been progressed recently. Most of biological cells adsorb on the scaffold, and show activities: migration, deformation, proliferation, and differentiation. These activities depend on properties of the scaffold. Many kinds of scaffolds were designed to control activities of cells for tissue engineering *in vitro*: fibers, porous membrane-based substrates [**1**], or three-dimensional structure made by printer. The property of the surface of the scaffold was also modified by chemical methodology [**2**]. The biological scaffold is degradable and time-dependent for making tissue dynamically. Bio-mimicking three-dimensional (3D) porous scaffold has been designed in many studies [**3**]. Cells are sensitive to topography as well as stiffness of the scaffold substrate [**4**]. As biological materials, decellularized organ was also used for the extracellular matrix [**5**]. Scaffolds technology is helpful to understand for cellular systems: tissue engineering for drug delivery system, complex 3D structure, and functions associated with cellular transport characteristics.

By the cell culture technique, myoblasts can be differentiated into myotubes *in vitro*. The differentiation can be easily confirmed by their cyclic contraction synchronous to cyclic electric pulse stimulation to the culture medium. For the effective contraction movement, the myotube should make orientation, which depends on the orientation of myoblasts. In the conventional way, cells were cultured between walls of the narrow channel to make orientation of cells.

Micro-machine technology has also progressed recently. The cell has sensitivity to the micro morphology of the surface of the scaffold [**6**]. The optimum micro morphology to control the behavior of cell has not been analyzed quantitatively.

In the present study, the optimum scaffold micro-topography to make orientation of cells has been investigated. As the convenient substrate for the surface micro-machining, polydimethylsiloxane (PDMS) has been selected. Behavior of cell is quantitatively analyzed with several parameters *in vitro*: the height of the micro step, the alignment of the microgroove on the bottom wall of the flow channel, and the aspect ratio of the micro square ridge. The micro-machine technique has also been applied to fabricate the scaffold of transparent film with micro markers.

## Experimental

### *Cells*

As one of the myoblasts from cell-line, which is convenient for cell culture, C2C12 (mouse myoblast cell line originated with cross-striated muscle of C3H mouse) was selected in the test. D-MEM (Dulbecco's Modified Eagle Medium) containing 10% FBS (Fetal Bovine Serum) and 1% penicillin/streptomycin was used for the medium. Each culture plate was exposed to the oxygen gas in a reactive ion etching system to be characterized as hydrophilic (oxygen plasma ashing) before seeding of cells.

### *Height of micro step*

The stripe pattern with micro-ridges (width of 3 μm) was made on the surface of polydimethylsiloxane (PDMS) by photolithography technique [**7**]. Variation was made on the height ($H$) of the ridges between 0.3 μm and 3.5 μm (**Fig. 1**). The angle between the longitudinal axis of the cell

adjacent to the ridge and the longitudinal direction of the ridge was measured.

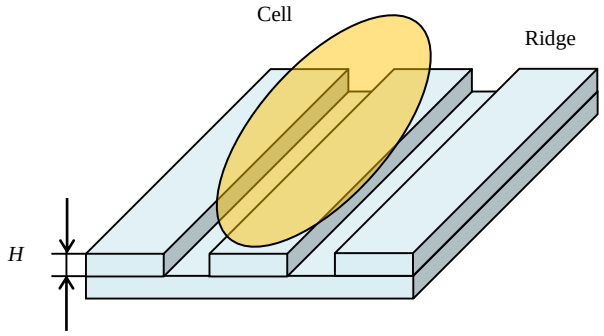


**Fig. 1**. Cell on micro-ridge with height (*H*).

### *Micro groove alignment on bottom of flow channel*

In the flow channel, micro-patterns were fabricated by the photolithography technique in the central part of the upper surface of the lower disk of borosilicate glass for the scaffold of the cell culture [**8**]. Several parallel lines of micro grooves have been made on the lower disk by etching with a reactive ion etching system. The depth, the width, and the interval of the rectangular grooves are 1 μm, 3 μm, and 3 μm, respectively. Variation has been made on the angle ($\theta$) between the longitudinal direction of the groove and the flow direction: 0 degree (parallel), 45 degree and 90 degree (perpendicular) (**Fig. 2**). Each pattern is drawn in the rectangle area of 1.6 mm × 0.4 mm. After several cells adhered on the micro-pattern in the flow channel in two hours, the wall shear stress (< 3 Pa) was applied on the cells by the flow. Both the migration and the deformation of each cell on the micro grooves under the shear flow were observed.

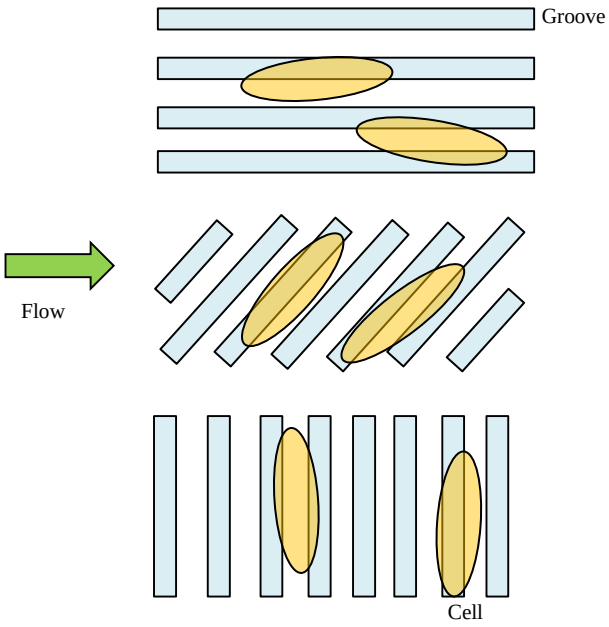


**Fig. 2**. Micro grooves alignment against flow for pre-orientation of cell.

### *Aspect ratio of micro ridge*

The checkered convex-concave patterns have been designed with micro quadrangular prisms at the square area (1 mm × 1 mm) on the disk of glass for the scaffold by the lithography technique [**9**]. Each prism has the following dimension. The height of the prism is 0.7 μm. The length of the top rectangular surface is 10 μm length. Variation has been made on the width of the top square of each prism: 5 μm, 8 μm, and 10 μm. The variation of the width makes the variation on the aspect ratio *R* of the top rectangular surface of each prism: $R$ = 1, $R$ = 1.25, and $R$ = 2. The arctangent of each ratio *R* is 45 degree, 51 degree, and 63 degree, respectively. After cell culture for several days, the mean value of $\varphi$ (between the longitudinal direction of the cell and the direction of the side of the square of each prism) is calculated on the microscopic image (**Fig. 3**).

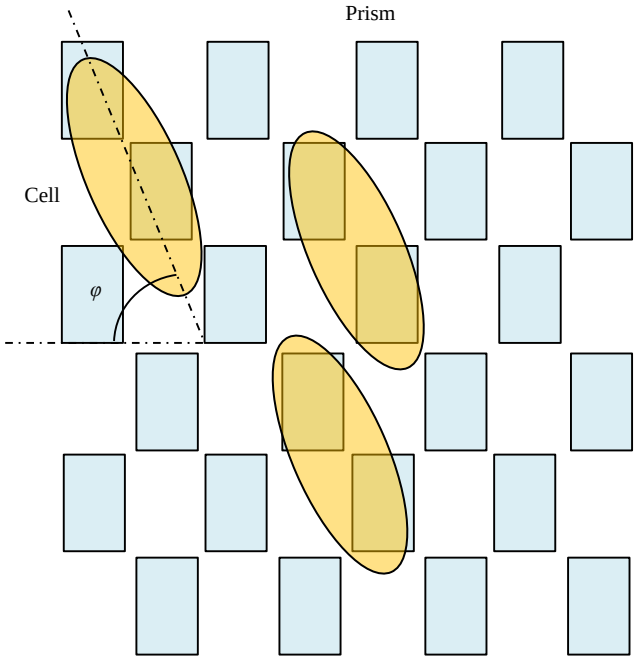


**Fig. 3**. Cells on checkered convexo-concave pattern.

### *Micro markers*

PDMS was mixed with the curing agent. The volume ratio of PDMS to curing agent is ten to one. The protrusions on PDMS film were made by the photolithography technique [**10**]. After many trials, the dimension has been selected as follows. Each protrusion has the hemisphere shape (4 μm diameter, 2 μm height). The pitch between adjacent protrusions is 0.03 mm (**Fig. 4**). The thickness of the base film of PDMS is 6 μm. The side without protrusions is used for the scaffold of the cell culture. The protrusions play the role of the position marker. The array of protrusions is made in the square area of 3 mm × 3 mm at the centre of the film. PDMS ring (inner diameter of 15 mm) was placed on the thin film of PDMS. After the film was baked with the ring, PDMS film with PDMS ring was carefully peeled off from the mould.

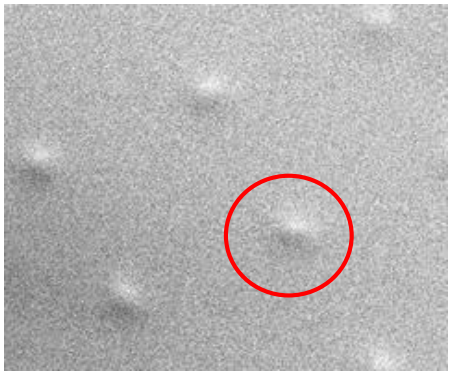


**Fig. 4.** SEM image of micro protrusions (circle) array on back side of scaffold film [**7**].

## Results and discussion

### *Height of micro step*

The typical diameter of C2C12 was 20 μm, when it was floating in the medium. Conventional methods to make orientation of cells were the cell culture in the channel between walls with the distance larger than diameter of the cell to restrict the cell migration. In the present study, cells are cultured near the micro step with dimension smaller than cell size. The experiments show the following results. Cells fall in the groove between ridges higher than 3.5 μm. Each cell tends to align along the micro step higher than 0.7 μm. Cells do not align to the direction of the longitudinal step lower than 0.3 μm.

### *Micro groove for pre-orientation*

The behaviour of cell depends on direction of the flow stimulation. The experimental results show that each cell makes orientation along the micro grooves (**Fig. 5**) on the bottom wall of the flow channel before exposure to the flow. The micro-striped groove can control the cell pre-orientation in the flow channel.

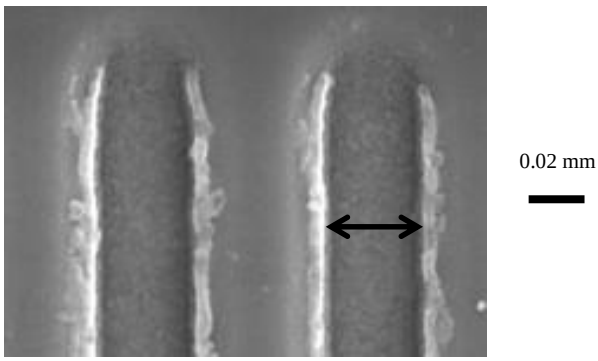


**Fig. 5**. SEM image of micro grooves (arrow section) [**8**].

### *Aspect ratio of micro ridge*

The mean value of $\varphi$ is 45 degree at the random distribution between 0 degree and 90 degree. The angle shows random distribution in the area of $R$ = 1 and control (without micro pattern). The mean value of $\varphi$ approaches to the value of the arctangent of $R$, in the area of $R$ = 2 and $R$ = 1.25. The experimental results show that the major axis of myoblast tends to tilt to the diagonal direction of the square face on the checkered convex-concave patterns. The aspect ratio of the checkered convex-concave patterns can control the orientation of cells.

### *Micro markers*

The following results have been shown in the present experiment. Myoblasts adhere on the counter surface against the micro markers and proliferate to the confluent manner. When the cells are cultured on the side with protrusions on the film, the alignment of the cell depends on the morphology of the surface of the scaffold. When the cells were cultured on the counter side of the scaffold film, alignment of the cell did not depend on the position of the back markers. Durotactic migration effect [**10**], for example, was not observed on the scaffold. Myoblasts differentiate into myotubes on the scaffold surface (**Fig. 6**). When the cyclic electric pulses were applied to the medium of the cell culture, the local cyclic contraction of myotubes was microscopically observed through the transparent film of the scaffold. The film of the scaffold with micro markers has successfully fabricated with optimised properties: transparency for observation, balanced elasticity against the force of contraction of the cultured myotube, and affinity with the myotube. Both the size and the interval of micro markers on the scaffold film are optimised to measure the local movement of cultured myotubes *in vitro* (**Fig. 7**).

C2C12 is convenient to confirm the viability during the cell culture test by the differentiation to the myotube, which shows the repetitive contractive movement by cyclic electric pulse stimulation to the medium. The stain techniques on protein are popular, but they stop the cell culture test intermediately.

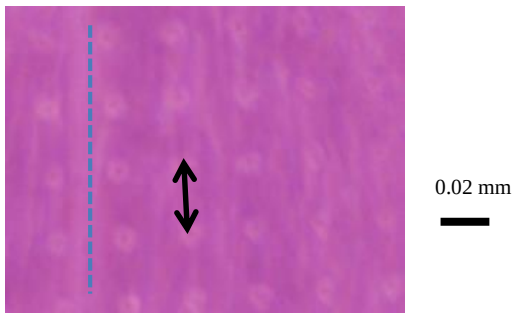


**Fig. 6**. Contraction of myotubes (vertical direction: parallel to dotted line) is measured by tracing of distance between protrusions (arrow) on scaffold film [**11**].

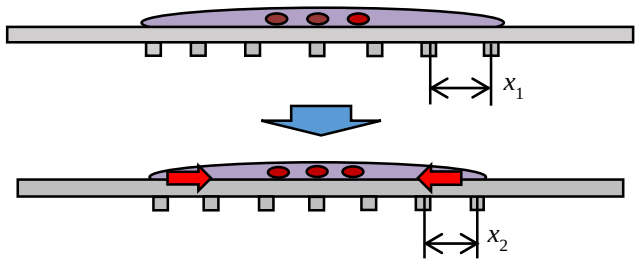


**Fig. 7**. Contraction of myotube is measured by tracing of distance between protrusions (from $x_1$ to $x_2$) [**10**].

## Conclusion

The following aspects of the micro-machined scaffold have been analyzed in the present study: the minimum height of the micro step to make align of the cell, effectiveness of the micro grooves for pre-orientation of the cells in the flow channel, the aspect ratio of the checkered convexo-concave pattern to make orientation of the cell, thickness of the scaffold film of PDMS to observe contraction of the myotube, and the dimension of the micro markers to trace the movement of the local contraction of myotubes. The quantitatively micro-machined scaffold is effective to control cell activities: migration, deformation, and orientation. The results will contribute to several applications: cell sorting, medical diagnostics, and regenerative medicine.

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### Keywords

Scaffold of cell culture, surface morphology, photolithography, orientation.

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