Design of Cross Type of Flow Channel to Control Orientation of Cell

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ABSTRACT

A cross type of flow channel has been designed to control orientation of cell by direction of flow *in vitro*. The flow path (4 mm width, 0.2 mm height) is made between parallel transparent plates. Flow rate of 48 cm^3/hour is controlled by a syringe pump. The wall shear rate of 500 s^{-1} is calculated by the parabolic velocity profile between the parallel plates. The direction of the flow can be changed by alternative use of the inlet and outlet port of the cross type of the channel. The flow channel is placed in a chamber, where temperature and CO_2 content are kept 310 K and 5 percent, respectively. The chamber is placed on the stage of an inverted phase contrast microscope to observe the behavior of cells adhered on the wall under a flow. After several cells adhered to the disk, the wall shear rate was applied on the cells in the medium flow for 1 hour at each direction. C2C12 (mouse myoblast cell line) was exposed to the wall shear rate with the methodology. The experimental results show that the change of the direction of myoblasts can be observed by the cross type of flow channel.

Keywords: Biomedical Engineering, Flow Channel, Cell Culture, Myoblast, Shear rate, and Flow.

1. INTRODUCTION

A biological cell deforms not only passively but also actively. The flow deforms the cell along the stream line [1]. The cell deforms, on the other hand, to minimize the intra force.

Cells are exposed to mechanical stimulation *in vivo* [2-4]. The shear stress is one of the mechanical stimulations. The endothelial cells are exposed to the shear stress in the blood flow at the wall of the blood vessels [5-9]. The other cells are also exposed to the shear stress among the deformation of the tissue.

Cells adhere, migrate, deform, proliferate, and differentiate. The mechanical stress might affect the behavior of cells [10].

Cell culture technique has been developed and several methodologies have been clinically applied to regenerative medicine. The acceleration technique for orientation and proliferation of cells has been studied to make tissue *in vivo* or *in vitro* [11-15]. Control methodology for orientation and proliferation of cells would be applied to the regenerative tissue technology.

A flow can be used to apply a stress field to a cell [16-22]. The cell directly receives the shear stress in the shear flow. The high shear flow might deform cell, peel cells off the scaffold, and inhibit proliferation. The mild shear flow, on the other hand, might accelerate the active deformation, migration, and proliferation.

In the present study, a cross type of flow channel has been designed to control orientation of cell by direction of flow *in vitro*.

2. METHODS

Cross Type of Flow Channel
A cross type of flow channel has been designed to change perpendicularly the direction of the flow during cell culture (Figs. 1-4).

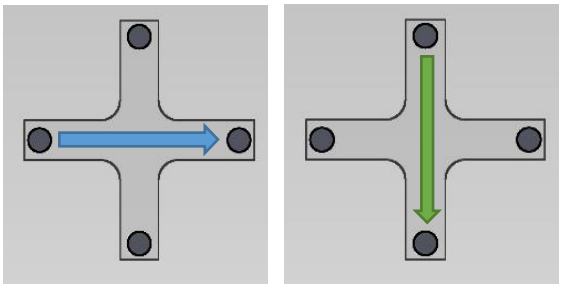

Fig. 1: Change direction of flow at cross type of channel.

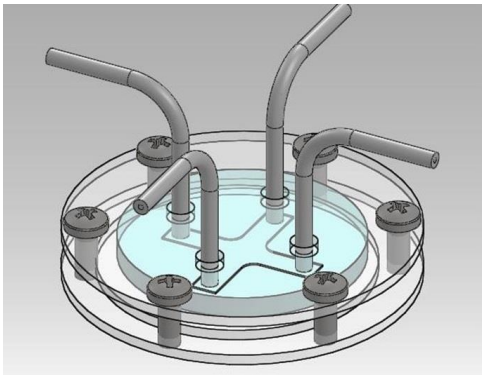

Fig. 2: Design of cross type of flow channel.

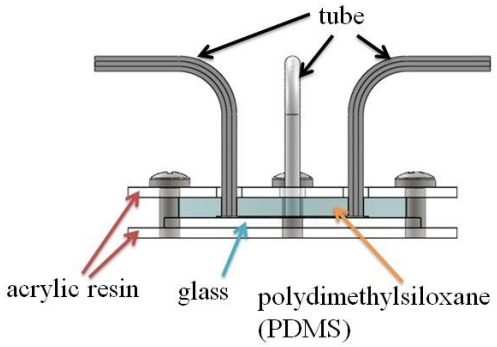

Fig. 3: Cross section of flow channel.

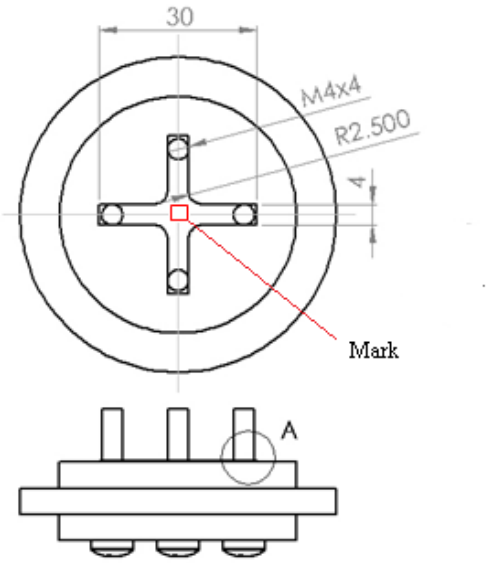

Fig. 4: Dimension (mm) of flow channel.

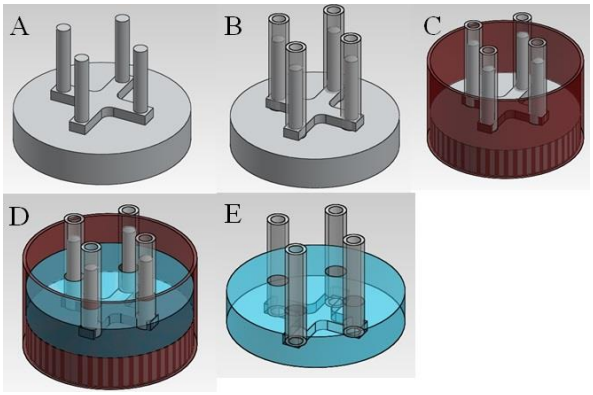

Fig. 5: Upper disk on mold: mold (A), mold with tubes (B), wall with mold with tubes (C), PDMS with tubes on mold (D), PDMS with tubes (E).

The flow channel consists of two disks of 50 mm diameter. The borosilicate glass (Tempax) disk (1.1 mm thickness) is used for the lower disk for the scaffold of the cell culture. The upper disk is made of polydimethylsiloxane (PDMS), which has concave pattern for the flow path (Fig. 3).

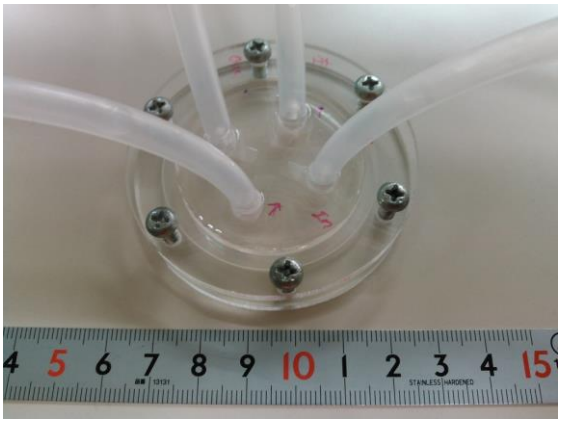

Fig. 6: Flow channel and tubes.

The square mark of groove (1 mm × 1 mm) was machined by the ultrashort pulse laser (IFRIT, Cyber Laser Inc., Tokyo, Japan) at the center on the rear surface of the scaffold of the lower disk, to trace the same cell (Fig. 4).

The mold for the upper disk is made of aluminum (Fig. 5A). The mold has a convex cross pattern with the following dimension: the width of 4 mm, the height of 0.2 mm, and the length of 30mm. At the crossing point, each corner has a fillet of curvature of r = 2.5 mm (Fig. 4). Each end of the cross has a hole with the female screw of M4. The dimension of the convex part was measured with a laser microscope (VK-X200, Keyence Corporation, Osaka, Japan).

In each hole with the female screw, the bolt with the male screw was inserted. Each bolt was covered with the silicone tube (internal diameter 2 mm, external diameter 4 mm) (Fig. 5B). After the mold was enclosed with a peripheral wall of polyimide tape, PDMS (Sylgard 184 Silicone Elastomer Base, Dow Corning Corp., MI, USA) was poured with the curing agent (Sylgard 184 Silicone Elastomer Curing Agent, Dow Corning Corp., MI, USA) on the mold (Fig. 5D). The volume ratio of PDMS to curing agent is ten to one. After degassing, PDMS was baked at 373 K for one hour in an oven (DX401, Yamato Scientific Co., Ltd). The baked disk of PDMS (3.1 mm thickness) with tubes was exfoliated from the mold, and sterilized in an autoclave.

The upper and lower disks were exposed to the oxygen gas of 30 milliliter per minute at power of 50 W for one minute in a reactive ion etching system (FA-1, Samco Inc., Kyoto) just before the flow test to be characterized as hydrophilic (oxygen plasma ashing).

The upper disk adheres to lower disk to make the flow path between them. To strengthen the contact between two disks, they were sandwiched between two transparent disks of polymethylmethacrylate (diameter of 65 mm, thickness of 2 mm), and fixed with six screws (Fig. 6).

In the present experiment, the shear rate on the wall of the scaffold is approximated with an estimated parabolic velocity profile between the parallel walls [19].

The shear rate (γ, [s^{-1}]) on the wall of the plate is calculated by Eq. 1, in which a parabolic velocity profile between parallel

plates is hypothesized.

$$\gamma = 6\, q / (b\, D^2) \qquad (1)$$

In Eq. 1, q is the flow rate [$m^3\ s^{-1}$], b is the width of the channel [m] and D is distance [m] between two parallel walls. In the present study, D is 0.2 mm, and b is 4 mm. When q = 48 cm^3/hour, γ= 500 s^{-1}.

The shear stress τ [Pa] is the product of viscosity η [Pa s] of the fluid and the shear rate γ [s^{-1}] of the flow (Eq. 2).

$$\tau = \eta\, \gamma \qquad (2)$$

When η = 0.002 Pa s [17], τ = 1 Pa.

The silicone tube connects the flow channel with the syringe pump, with the reservoir of the suspension of the cells, or with the reservoir of the medium (Fig. 7).

The direction of the flow can be changed by alternative use of the inlet and outlet port of the cross type of the channel. The channel is placed on the stage of an inverted phase contrast microscope (IX71, Olympus, Tokyo) to observe the behavior of cells adhered on the disk under the flow (Figs. 8&9). The CO_2 gas is introduced into the reservoir of the medium to maintain the carbon dioxide partial pressure at five percent in the medium through the channel (Fig. 10). The reservoir is placed in the thermostatic bath to maintain the temperature at 310 K.

Flow Test

C2C12 (mouse myoblast cell line originated with cross-striated muscle of C3H mouse) of the passage ninth was used in the tests.

D-MEM (Dulbecco's Modified Eagle Medium) containing 10% FBS (Fetal Bovine Serum) and 1% penicillin/ streptomycin was used for the medium. After the medium was drawn into the flow channel through the one inlet tube and through three outlet tubes by the syringe pump to remove the air bubble, the suspension (100000 cells/cm^3) of C2C12 in D-MEM was introduced into the channel through the one inlet tube by the syringe pump (Fig. 7).

After several cells adhered to the disk for 1.5 hour or for 3 hour, the wall shear stress was applied on the cells by the flow through one inlet tube and one outlet tube pulled with the syringe pump for 1 hour. To trace the same cell, the time-lapse pictures were taken with the constant interval of thirty seconds.

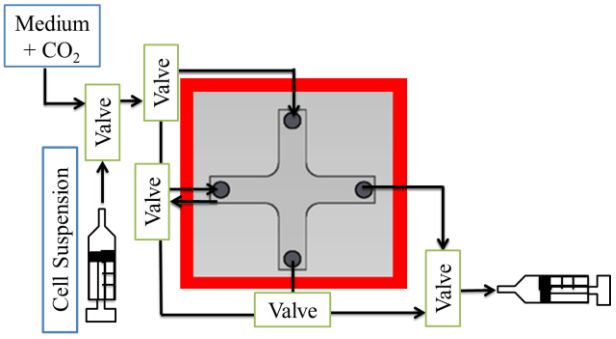

Fig. 7: Filling of medium before test.

On the microscopic image, the outline of each cell was traced, and the contour of each cell was approximated to ellipsoid. The length of the major axis (a) and the minor axis (b) were measured, and the ratio of axes is calculated as the shape index (I) by Eq. 3.

$$I = a / b \qquad (3)$$

At the circle, I =1. As the ellipsoid becomes flat, I approaches to zero.

The angles between the direction of the flow and the direction of the major axis of each cell are also measured at the microscopic image.

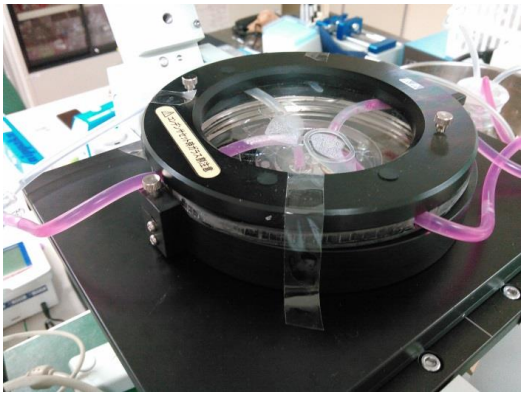

Fig. 8: Flow channel on stage of microscope.

Fig. 9: Experimental system.

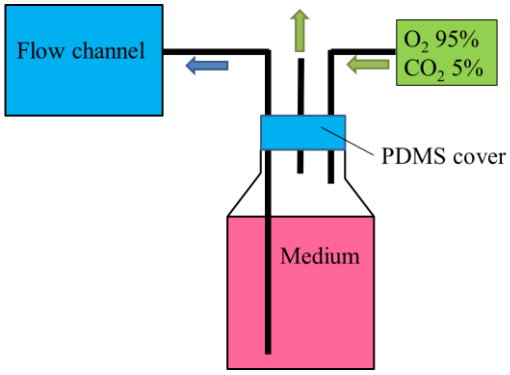

Fig. 10: CO_2 supply to medium.

3. RESULTS

The measurement by the laser microscope of the mold shows that the height is 0.2 mm (Fig. 11). By the movement of the flowing cells, the control of the alternating flow direction along the rim of the square of the mark is confirmed (Fig. 12).

Under the flow, the shape indexes (*I*) of adhered cells are 0.6 (mean) ± 0.2 (standard deviation).

Fig. 13 shows exfoliation of cell under the shear flow. Fig. 14 shows cell rounded after the change of the flow direction.

Fig. 15 shows cells under shear flow. The cells are exposed to the flow of respective direction for one hour each, after cultivation for three hours for adhesion to the bottom of the channel.

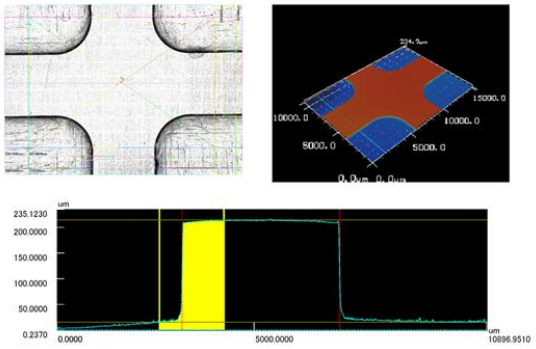

Fig. 11: Dimension of mold for upper disk of flow channel.

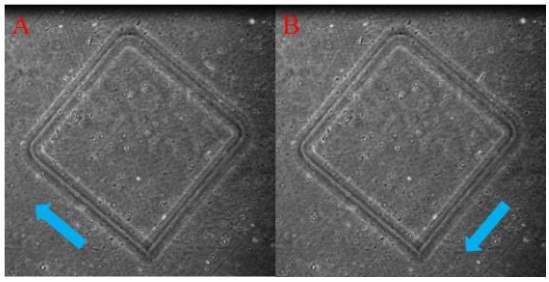

Fig. 12: Alternating flow direction (arrow).

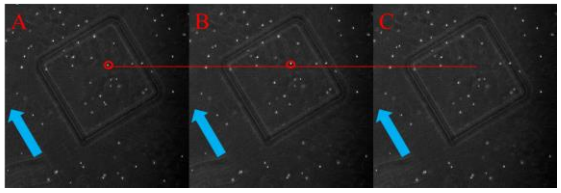

Fig. 13: Cell exposure to flow for 30 s (A), for 33 min (B), for 38 min (C), exfoliation of cell (C). Arrow shows flow direction. Square mark is 1 mm × 1 mm.

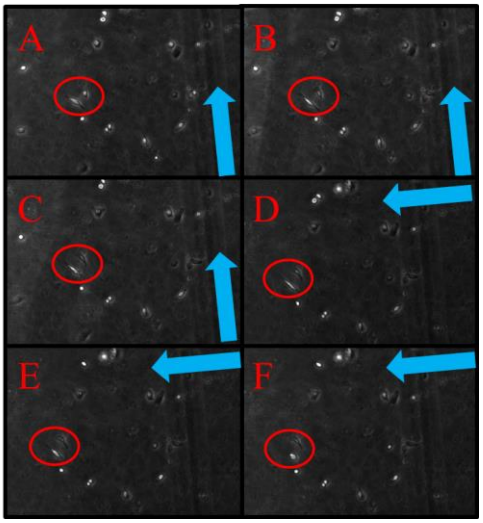

Fig. 14: Cell immediately after exposure to flow in the first direction (A), for 30 s (B), for 30 min (C), for 1 hour (D), immediately after exposure to flow in the second direction (E), for 30 s (F).

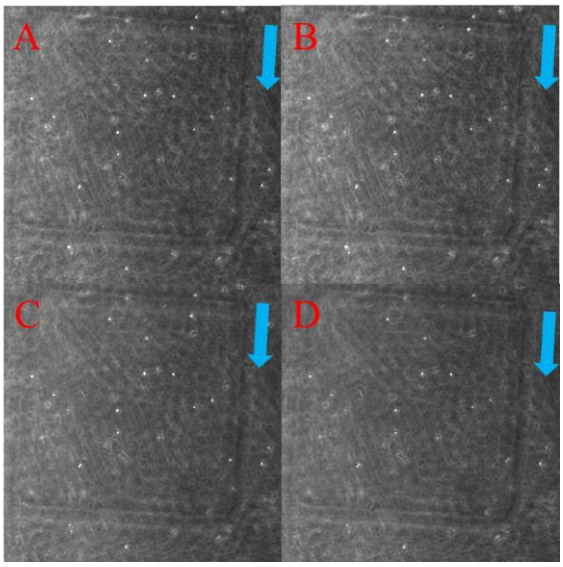

Fig. 15a: Cell in the first flow direction. Immediately after exposure to flow (A), for 30 s (B), for 30 min (C), for 1 hour (D). Square mark is 1 mm × 1 mm.

Fig. 16 shows the angle between the major axis of the cell and the flow direction. Data of angle (360 degree is 6.26 rad, zero degree is horizontal in the figure) are shown in ascending order in Fig. 16. Data would rise along the straight line at random distribution of the angles. When the data make curved line, the cell tends to tilt to the angle in the range of low slope. Fig. 16a and Fig. 16b show different distribution of the angles, which shows the change of orientation of cells after the change of the flow direction. Fig. 16a shows more cells tend to tilt to the angle around 90 degrees, which is flow direction in Fig. 16a, in 1 hour. Fig. 16b shows more cells tend to tilt to 45 and 135 degrees in 1 hour: the angle of zero is flow direction in Fig. 16b.

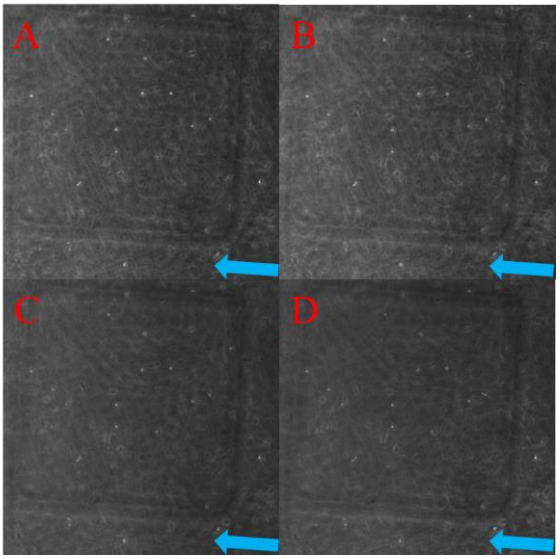

Fig. 15b: Cell in the second flow direction. Immediately after exposure to flow (A), for 30 s (B), for 30 min (C), for 1 hour (D). Square mark is 1 mm × 1 mm.

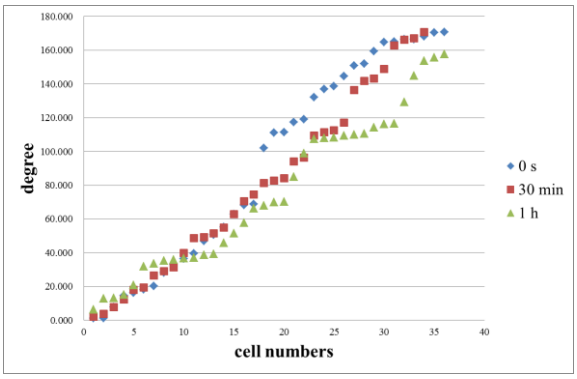

Fig. 16a: Cell orientation in the first flow direction.

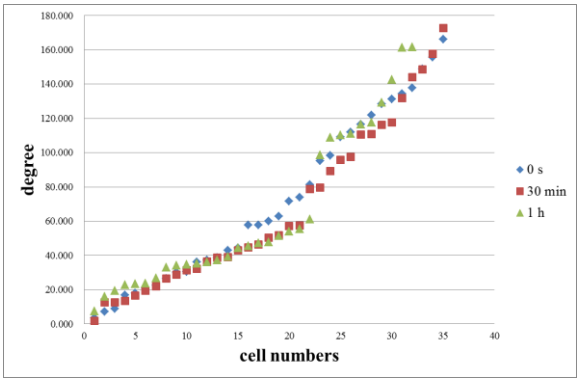

Fig. 16b: Cell orientation in the second flow direction, after 3 hours exposure to the first direction.

4. DISCUSSION

To decrease the turbulence of the flow around the crossing, the side wall has the curvature at the crossing (Fig. 17). The tubes for inlet and outlet are embedded in the PDMS disk during the processing to decrease the leakage.

In the present study, the inner surface of the flow channel was treated to be hydrophilic to avoid contamination with bubbles. The hydrophilic property of the wall might affect the migration of the cell. To avoid generation of bubbles, the internal pressure of the flow path was controlled by the opening of inlet tubes in the present study.

The extended cultivation after exposure to the shear flow might show the effect of the shear flow on proliferation and differentiation of cells. In the dense state of cell, the orientation of cells might depend on the interaction between cells.

The response of cell to the shear flow depends on point of adhesion to the scaffold. Enough points of adhesion are necessary for the active response to the shear field.

Several elongated cells recover to the rounded shape after stopping exposure to the shear flow. The immediate recovery shows that the deformation of cell might be passive.

The cells might change the orientation at the differentiation. C2C12 made perpendicular orientation of myotubes to the flow direction in the previous study [1]. The orientation of C2C12 at diagonal direction of flow might be preparation to make perpendicular orientation of myotubes in the successive cultivation.

The wall shear stress of 1 Pa is selected in the present study as the typical value, which affected cells in the previous study [17].

The flow channel system of the parallel disks has been used in the previous experiments to study the effect of the shear stress on the cells. Although the sandwiched elastic film between the disks is convenient to avoid air leakage, the shape of the film may make the geometric fluctuation. The geometric accuracy of the wall of channel improves the laminar flow between the parallel walls.

It is not easy to estimate the shear stress value on the wall, when the medium has the free surface [1, 16]. The parallelepiped chamber is convenient to observe the response of cells under controlled shear stress [19].

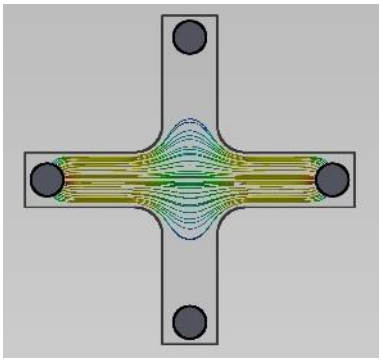

Fig. 17: Calculated stream line at the cross type of flow channel.

5. CONCLUSION

A cross type of flow channel has been designed to control orientation of cell by direction of flow *in vitro*. The flow path is made between parallel transparent plates. The direction of the flow can be changed by alternative use of the inlet and outlet port of the cross type of the channel. The flow channel is placed in a chamber, where temperature and CO_2 content are kept 310 K and 5 percent, respectively. The chamber is placed on the stage of an inverted phase contrast microscope to observe the behavior of cells adhered on the wall under a flow. The experimental results show that the change of the direction of myoblasts can be observed by the cross type of flow channel.

6. ACKNOWLEDGMENT

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