Effect of Medium Flow on Cultured Cells

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ABSTRACT

An effect of the flow on migration and on deformation of cultured cells has been studied *in vitro*. A flow chamber was designed to observe behavior of adhered cells on a plane in a culture medium flow under a microscope. A thin sheet of silicone rubber was sandwiched by two plates of transparent glass to form a rectangular flow channel of 2 mm width × 52 mm length × 0.1 mm depth. The chamber was tilted to make a slope of 0.17 rad perpendicular to the longitudinal flow direction. Five kinds of cells were used in the experiment: C2C12 (mouse myoblast), L6 (rat skeletal muscle cell), A7r5 (rat aortic smooth muscle cell), CS-2P2-C75 (porcine aortic endothelial cell), and L929 (mouse fibroblast). After several cells adhered to the glass plate, the medium steady flow between 4 and 60 mL/hour was applied on the cells with the syringe pump, and behavior of adhered cells in the flow was observed. The flow generates wall shear stress between 0.3 and 5 Pa. The experimental results show that cells tend to tilt, deform and migrate to the direction of downstream and downward before exfoliation. CS-2P2-C75 and L929 did not adhere to the wall of the glass chamber.

Keywords: Biomedical Engineering, Muscle Cells, Cell Culture, Flow, Migration and Orientation

1. INTRODUCTION

Behavior of biological cells depends on various environmental factors, such as electric [1-3], magnetic [4, 5] and mechanical [6-8] fields.

Cell culture technique has been progressed and myoblasts have been clinically applied to ischaemic cardiomyopathy in the field of regenerative medicine. Acceleration technique for orientation and proliferation of cells has been studied to make muscle tissue *in vivo* and *in vitro* [2, 6]. Control methodology for orientation and proliferation of cells would be applied to regenerative tissue technology.

Several methods have been designed to apply mechanical stress to cells [6-8]. The transmission point of stress to a specimen is important. When fixation between the cell and the scaffold is not enough, stress is not transmitted to the cell. A flow can be used, on the other hand, to apply stress to a specimen [9-13]. The specimen receives shear stress in the shear flow.

In the present study, the effect of flow and slope on orientation of cultured muscle cells has been studied *in vitro*.

2. METHODS

Culture Medium Flow System

A one-way flow system was designed to observe an effect of flow and slope on cells adhered to a plate. The system consists of a flow chamber, a syringe pump, tubes and a microscope (Fig. 1). TE-331S (Terumo Co., Ltd. Tokyo) was used for the syringe pump. A plastic tube of 2 mm internal diameter and of 3 mm external diameter was used for the flow channel.

The flow chamber consists of two transparent glass plates and a thin silicone rubber sheet (Fig. 2). The dimension of two glass plates is 76 mm length, 26 mm width and 1.5 mm thick, each. A rectangular open space of 2 mm × 52 mm is cut off in a thin sheet of silicone rubber of 0.1 mm thick, and sandwiched between the supporting plastic plates (Fig. 3). The sheet is sandwiched between two plates of glass to form a rectangular channel of 2 mm width × 52 mm length × 0.1 mm depth. The three plates stick together with their surface affinity without bond. At the upper glass plate, two holes of 2.5 mm diameter is machined by a grinder, and adhered to the plastic tube by a bond of polyurethane resin (Fig. 3). On the outer surface of the lower glass plate, grooves of dotted line were machined to make the marks of position in the chamber.

The tube is connected to the plastic syringe pump. The chamber is placed on the tilted plate to make a slope of 0.17 rad perpendicular to the longitudinal flow direction (Fig. 3). The tilted plate is heated at 37 degrees Celsius to maintain temperature of the medium. The chamber is placed on the inverted phase-contrast microscope (IX71, Olympus Co., Ltd., Tokyo).

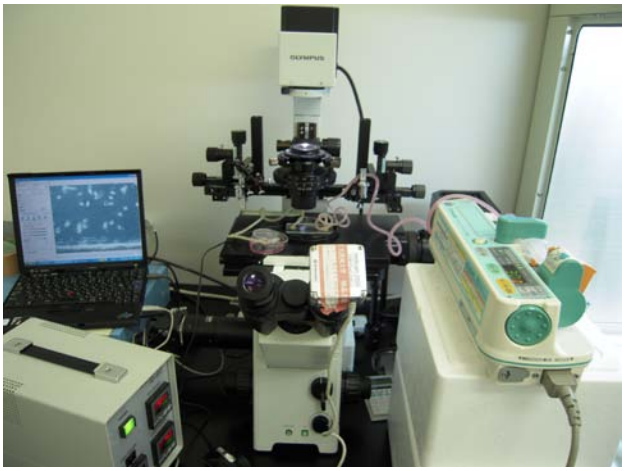

Fig. 1: Experimental system.

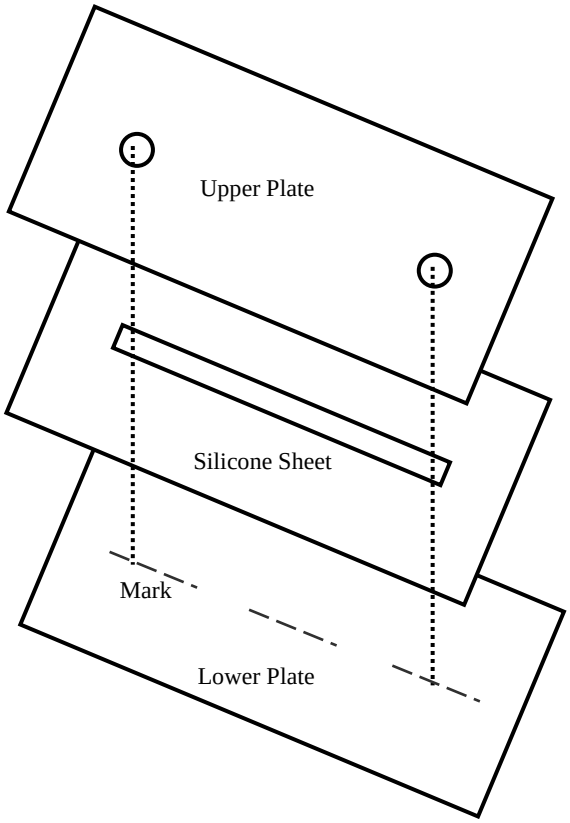

Fig. 2: Flow chamber of three plates; upper plates of glass, silicone sheet, lower plates of glass with mark.

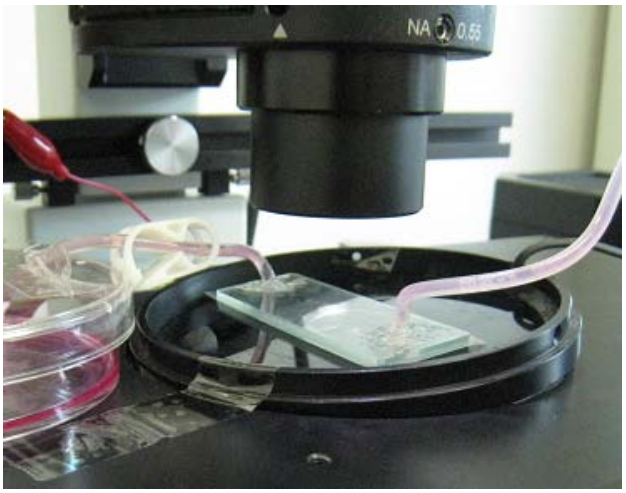

Fig. 3: Chamber placed on the tilted heating plate.

Cell Culture

Five kinds of cells were used in the experiment: C2C12 (mouse myoblast), L6 (rat skeletal muscle cell), A7r5 (rat aortic smooth muscle cell), CS-2P2-C75 (primary normal porcine aortic endothelial cell), and L929 (mouse fibroblast). The cells of each kind were cultured on a dish with the Dulbecco's Modified Eagle's Medium (D-MEM) in an incubator for one week. Then, Cells were exfoliated from the plate of the culture dish with trypsin, and suspended in the D-MEM. The suspension was introduced to the chamber and cultured in the incubator for 24 hours to make cells adhere to the glass plate of the chamber before the flow test.

Flow Test

After the chamber was set on the microscope out of the incubator, the constant flow of the medium was applied to adhered cells with the syringe pump (Fig. 1). The flow path was carefully examined to avoid inclusion of air bubbles, which might stir the medium in the flow chamber and induce exfoliation of cells. The behavior of cells on the plate of the chamber was observed with the microscope. The photo of cells was taken during the flow test for one hour. Variation was made in flow rate between 4 and 60 mL/hour. The flow rate started with 4 mL/hour and was escalated to 60 mL/hour: 4, 5, 8, 20, 30, 60 mL/hour.

Shear Rate on Wall

The shear rate (G, s^{-1}) on the wall of the glass plate is calculated by Eq. 1, which is assuming a parabolic velocity profile between parallel plates.

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

In Eq. 1, q is flow rate ($m^3\ s^{-1}$), b is width of the canal (m) and D is distance (m) between two parallel walls. In the present study, b is 2 mm, and D is 0.1 mm.

The shear stress (T, Pa) is product of viscosity (N, Pa s) of the fluid and the shear rate (G, s^{-1}) of flow (Eq. 2).

$$T = N\,G \qquad (2)$$

The viscosity of the medium was measured with the cone and plate type of viscometer (TVE-22L, Toki-Sangyo Co., Ltd. Tokyo).

3. RESULTS

The result of measurement with the viscometer shows that the viscosity of the medium is 0.0010 Pa s at 37 degrees Celsius at the shear rate of 600 s^{-1}. The calculated shear rate on the glass wall of the flow chamber by Eq. 1 varies between 330 and 5000 s^{-1}, when the flow rate varies between 4 and 60 mL/hour. The calculated shear stress, thus, varies between 0.3 and 5 Pa for viscosity of 0.001 Pa s, when the shear rate varies between 330 and 5000 s^{-1}.

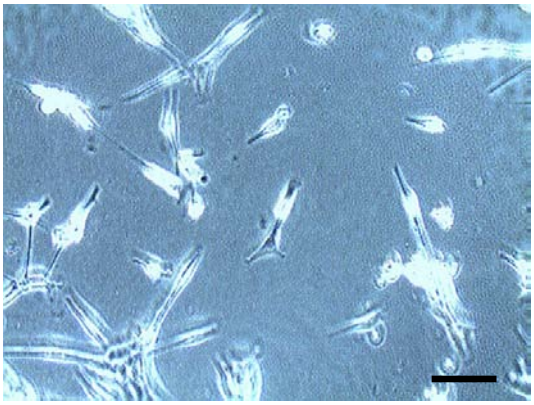

Fig. 4: C2C12 on the glass wall of the chamber before flow test. The bar shows 0.1 mm.

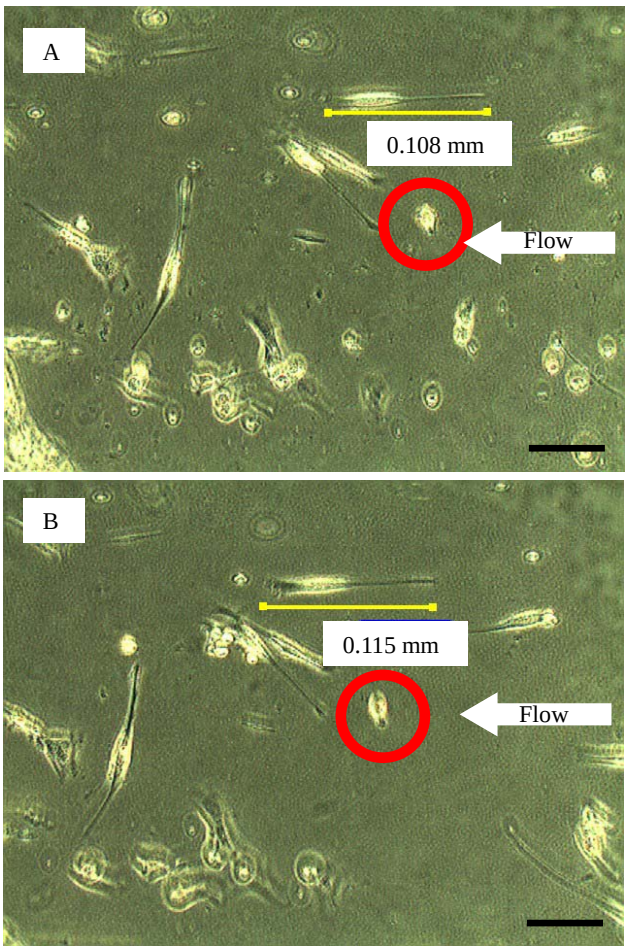

Fig. 4 exemplifies C2C12 adhered to the plate of the chamber in 24 hours without medium flow.

The slope goes down from upper to lower in Figs. 5-10. The medium flows right to left in Figs. 5-10. C2C12 elongates to the downstream along the streamline of the flow (Fig. 5). The C2C12 marked with the line in Fig. 5 elongates from 0.108 mm to 0.121 mm in thirty minutes under flow of 4 mL/hour, which generate a wall shear stress of 0.3 Pa estimated by Eqs. 1 & 2. Another C2C12 marked with the circle in Fig. 5 rolls down along the slope. The cell marked with the circle in Fig. 6 tilts for 30 minutes. Under steady flow of 30 mL/hour, most of C2C12 cells rolled downstream. Fig. 7 shows cells before and after flow stimulation for three minutes. Almost all of C2C12 cells are exfoliated in three minutes under steady flow of 60 mL/hour. The calculated wall shear rate and wall shear stress are 5000 s^{-1} and 5 Pa, respectively (Fig. 7).

Fig. 8 shows L6 migration for eleven minutes. L6 marked with the circle in Fig. 8 migrates downstream under steady flow of 4 mL/hour. In Fig. 9, L6 marked with the circle elongates downstream in 20 min under steady flow of 5 mL/hour.

Fig. 10 shows A7r5 under steady flow of 4 mL/hour. The most of cells are exfoliated in fifteen minutes. The rest of cells elongates downstream in sixty minutes. CS-2P2-C75 (Fig. 11) and L929 (Fig.12) does not adhere to the wall of glass in the chamber.

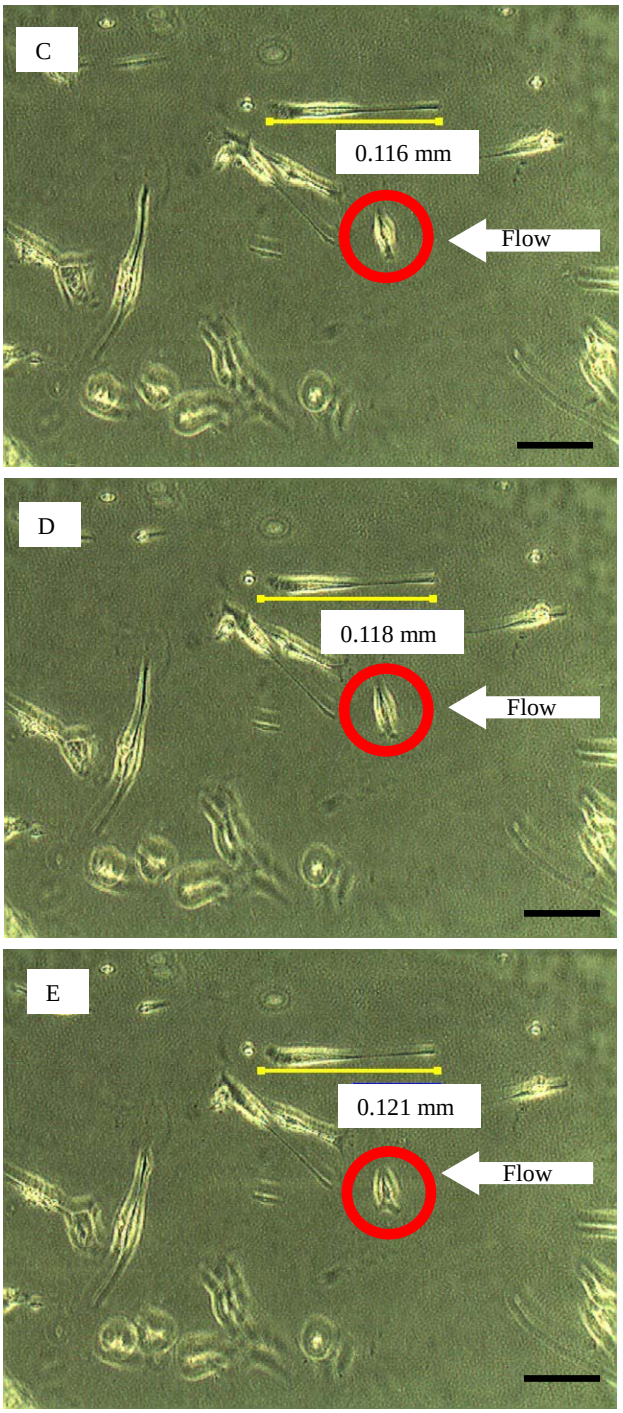

Fig. 5: C2C12 under steady flow (arrow: from right to left) of 4 mL/hour. Slope goes down from upper to lower. Cells were observed every 15 minutes: A, B, C, D, E.

4. DISCUSSION

Several movements occur on adhered cells in the flow: deformation, tilting to downstream, tilting to downward (Fig. 13 (c)), elongation along the streamline (b), deformation to be rounded (d), exfoliation (e), rolling to downstream (Fig. 13). The deformation and migration of cells in the flow depend on the adhering point of the cell to the wall. The free part of the

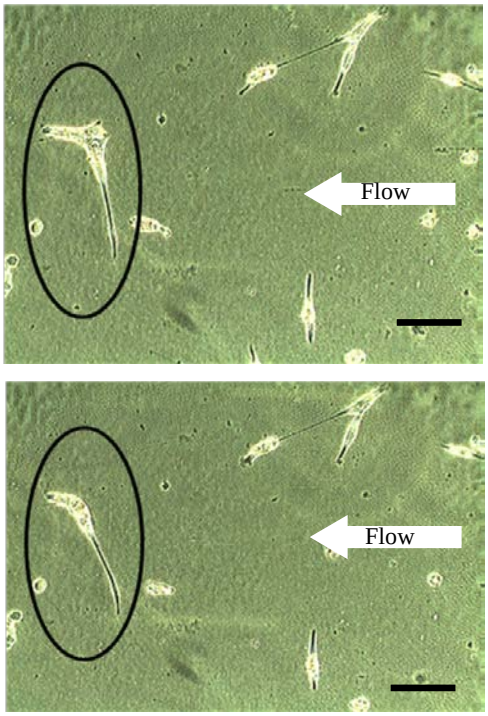

Fig. 6: C2C12 movement in 30 min (from above to bottom) under steady flow (from right to left) of 4 mL/hour. The bar shows 0.1 mm.

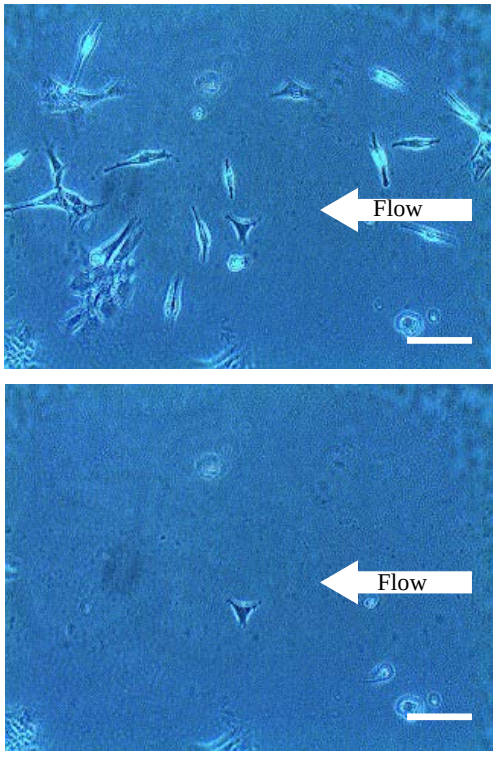

Fig. 7: C2C12 exfoliated in 3 min under steady flow (from right to left) of 60 mL/hour. The bar shows 0.1 mm.

cell might rotate around the adhering point. Collagen coating might be necessary for the flow test of CS-2P2-C75 and L929.

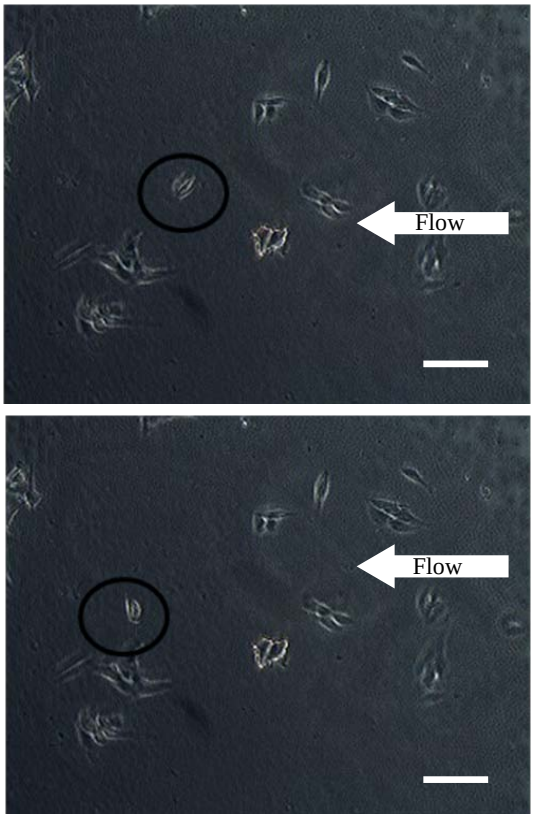

Fig. 8: L6 migrates downstream in 11 min under steady flow (from right to left) of 4 mL/hour. The bar shows 0.1 mm.

Acceleration of proliferation and orientation of cells are important targets in the research field of regenerative medicine on cultured biological tissue. The previous study shows that electric stimulation enhances differentiation of muscle cells [1]. Another study shows mechanical stimulation improves tissue-engineered human skeletal muscle [6]. Another previous study shows that muscle cells can adhere and proliferate under electric stimulation with periodical pulses, and that adhesion of muscle cells can be controlled with the amplitude of pulse [3].

Bioreactors have been developed to control the environment around the cultured cell [14].

The previous studies show that mechanical field, on the other hand, affects on cells' behavior. Erythrocytes are very flexible, and are rolled and deformed in the shear flow [9]. The shear flow also affects on the orientation of endothelial cells [10]. The shear stress affects on the orientation of smooth muscle cells in the biological tissue [7]. The direction of mechanical field affects on fibroblast [8]. The previous study shows that the micro-grooves affects on the orientation of cells [15].

The effect of slope has been examined in the present experiment, because a tilted rotating disk was used for another study of cell culture environment [16]. To evaluate the effect of slope independently of the effect of flow, the direction of slope has been set perpendicularly to that of flow. The present study shows that the shear flow and slope affects on behavior of cells adhered to the plate. The combined effect of flow and slope

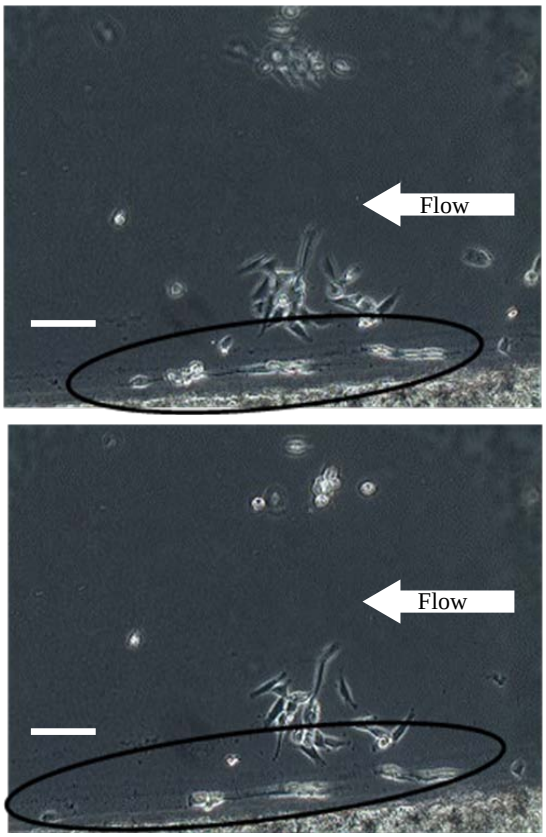

Fig. 9: L6 elongates downstream in 20 min under steady flow (from right to left) of 5 mL/hour. The bar shows 0.1 mm.

on each cell might govern the orientation of cells in the vortex flow on the tilted rotating disk.

5. CONCLUSION

The effect of flow on cultured muscle cells has been studied *in vitro*. The experimental results show that cells tend to elongate along the streamline and tilt to the direction of downstream and downward.

6. ACKNOWLEDGMENT

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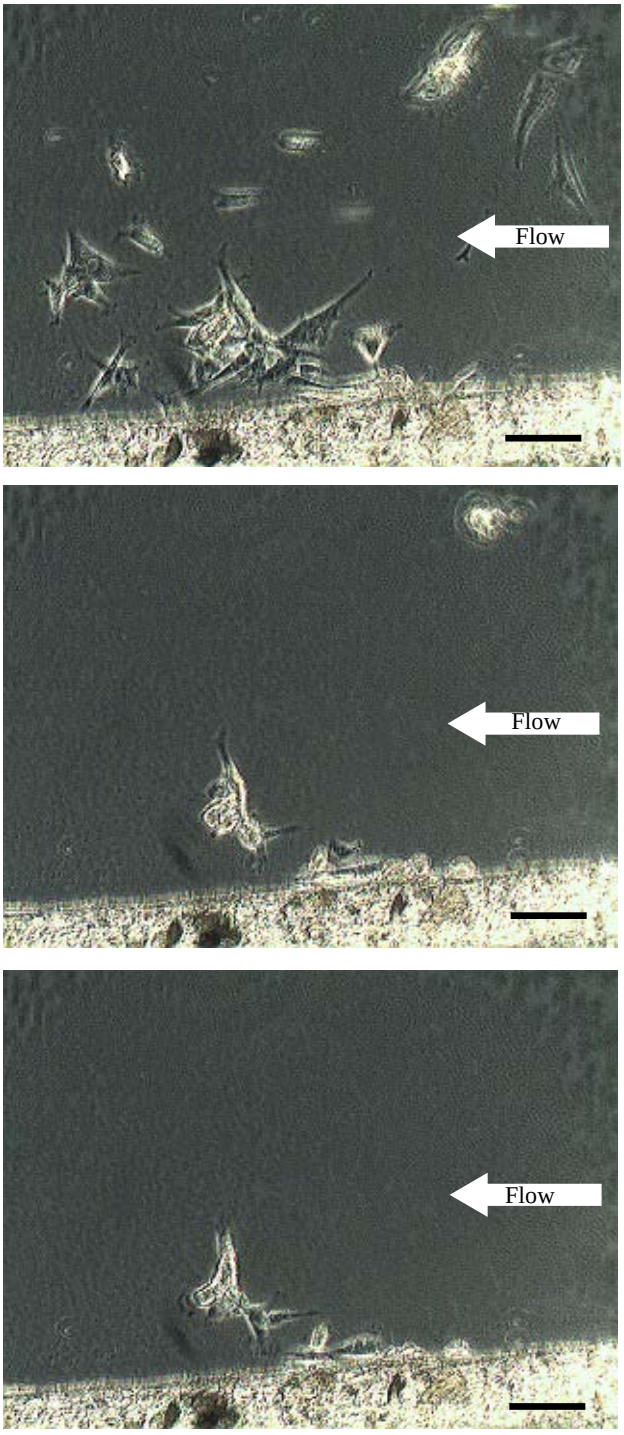

Fig. 10: A7r5 cells are exfoliated and elongated downstream under steady flow (from left to right) of 4 mL/hour. Middle, 15 min; Bottom, 60 min. The bar shows 0.1 mm.

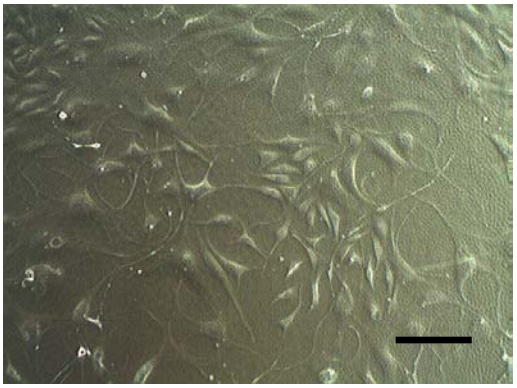

Fig. 11: CS-2P2-C75 in culture dish. The bar shows 0.1 mm.

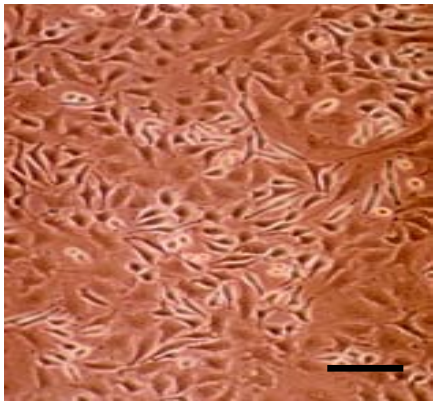

Fig. 12: L929 in culture dish. The bar shows 0.1 mm.

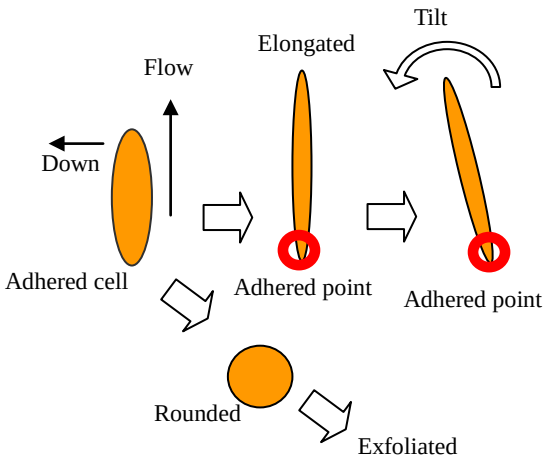

Fig. 13: Cells deformation and migration under flow.

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