Rotating Disk to Apply Wall Shear Stress on Cell Culture at Microscopic Observation

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ABSTRACT

An experimental system of the Couette type flow with a rotating disk has been designed to apply wall shear stresses on the cell culture at the microscopic observation *in vitro*. The shear stress on the wall is calculated with an estimated Couette type of the velocity profile between the rotating disk and the culture plate. The constant rotational speed (lower than 100 rpm) produces the wall shear stress smaller than 3 Pa. The rotating disk system is mounted on the stage of an inverted phase contrast microscope to observe the behavior of cells adhered on the plate under the shear flow. The medium is refreshed at a slow speed by a syringe pump. The system is placed in an incubator, where temperature and CO_2 content are kept 310 K and 5 percent, respectively. The experimental system allows observation of cells during incubation under the wall shear stress. The designed system is useful to apply wall shear stresses quantitatively on cell culture.

Keywords: Biomedical Engineering, Cell Culture, Shear Stress and Couette Flow.

1. INTRODUCTION

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* [1-4]. The behavior of biological cells depends on electric and magnetic fields [1, 2]. Control methodology for orientation and proliferation of cells would be applied to the regenerative tissue technology.

The mechanical stress is one of the interested points in the environment of cells, because they receive mechanical force *in vivo*. The mechanical stress on cells might induce various responses: deformation, migration, proliferation, and differentiation. Several methods have been designed to apply the mechanical stress to cells [3, 4].

A transmission point of the stress to a specimen is important. In many studies, the stress is applied to a scaffold [4]. When fixation between the cell and the scaffold is not enough, the stress is not transmitted to the cell. A flow, on the other hand, can be used to apply a stress field to a specimen [5, 6]. The cells directly receive the shear stress in the shear flow (Fig. 1).

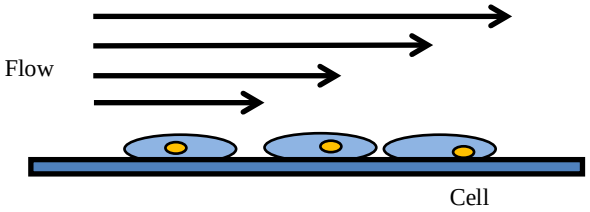

Fig. 1: Cells exposed to wall shear stress in shear flow.

The high shear flow might deform cell, peel cells off the scaffold, and inhibit proliferation as well as tissue formation. The mild shear flow, on the other hand, might accelerate migration, proliferation, and secretion of materials, which make the extra cellular matrix.

The volume of the bone tissue is controlled with osteoclasts and osteoblasts: osteoblasts form bone, although osteoclasts resorb bone. The volume of the bone decreases in the microgravity field.

In the previous study, osteoblasts were exposed to shear flow in a donut-shaped open channel, and the effect of flow stimulation on cultured osteoblast has been studied *in vitro* [6]. When the flow has an open surface, it is difficult to estimate the shear stress in the fluid.

Between two parallel walls, on the other hand, the velocity profile is easily estimated in the laminar flow. In the present study, an experimental system of the Couette type flow with a rotating disk has been designed to apply the wall shear stress quantitatively on the cell culture at the microscopic observation *in vitro*.

2. METHODS

Rotating Parallel Disk System

A cone and plate type of instrument is often used to apply Couette type of flow to a biological liquid. In the laminar flow in the space between a rotating cone and a stationary plate, a uniform shear field of Couette type is generated. The cone is rotating around the co-axis of the symmetry with the plate, while the apex of the cone is touching on the plate (Fig. 2). The shear rate (E [s^{-1}]) is constant regardless of the distance (r [m]) from the rotating axis (Figs. 3&4).

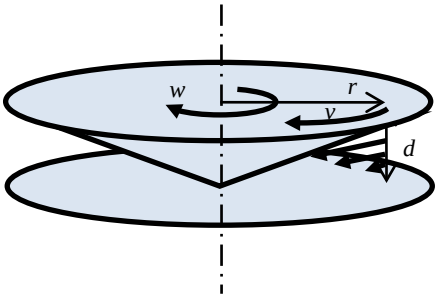

Fig. 2: Rotating cone and stationary plate.

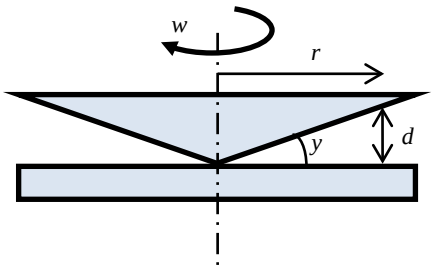

Fig. 3: Cross section of rotating cone and stationary plate.

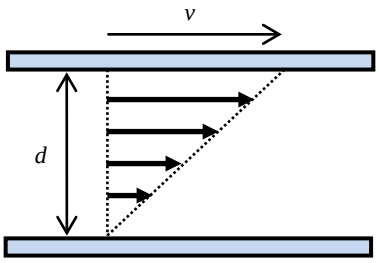

Fig. 4: Couette flow.

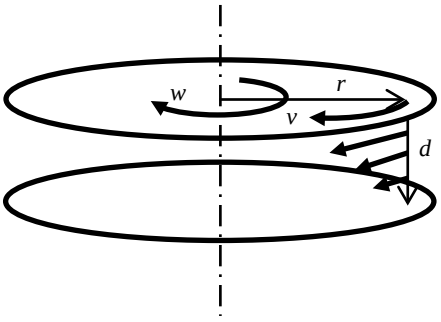

Fig. 5: Rotating parallel disk system.

$$E = v / d \quad (1)$$

In Eq. 1, v is the circumferential velocity [m s^{-1}] of the moving wall at the distance (r) from the rotating axis, and d is the distance between the moving wall and the stationary wall.

$$v = r\, w \quad (2)$$

In Eq. 2, w is the angular velocity [rad s^{-1}].

$$d = r\, y \quad (3)$$

In Eq. 3, y is the angle [rad] of clearance between cone and plate. Combination of Eqs. 1-3 makes Eq. 4, which shows the shear rate (E) does not depend on the distance (r) from the rotating axis.

$$E = w / y \quad (4)$$

In the present study, a rotating parallel disk system is chosen (Fig. 5). The fluid is sheared between a rotating disk and a stationary disk. The stationary disk is the bottom of the culture dish. In the system, the shear rate (E) is calculated by Eq. 5.

$$E = r\, w / d \quad (5)$$

In the parallel disk system, the shear rate (E) increases in proportion to the distance (r) from the rotating axis.

Shear Stress on Cell

The shear rate (E) generates the shear stress (F [Pa]) in a viscous fluid.

$$F = N\, E \quad (6)$$

In Eq. 6, N is viscosity of the fluid [Pa s]. The fluid is the medium of cell culture in the present study.

The rotating disk system is mounted on the stage of an inverted phase contrast microscope. The behavior of cells adhered on the plate under shear stress is observed with the microscope.

The experimental system is placed in an incubator, where both the temperature of 310 K and the carbon dioxide partial pressure of 5 percent are maintained. The medium is refreshed at a slow speed by a syringe pump. The system allows observation of cells during incubation under the wall shear stress.

3. RESULTS AND DISCUSSION

According to the methodology of the rotating parallel disk, the system has been designed to coincide with the microscope and the incubator (Fig. 6). The culture dish of 50 mm diameter has been selected to the stationary plate. The diameter of the rotating disk has been designed to be 40 mm (Fig. 7).

The shear stress is applied to cells on the bottom of the culture dish. The shear stress has been related to that of the blood vessel wall. The shear stress of 3 Pa is maximum value estimated as that is applied in the blood on the human blood vessel wall, when the wall shear rate is calculated by Poiseuille flow.

If the angular velocity (w) is selected to be 10 rad s^{-1}, the shear rate (E) is 3000 s^{-1}, when the distance from the axis (r) and the distance between the moving wall and the stationary wall (d) are 18 mm and 0.06 mm, respectively (Eq. 5).

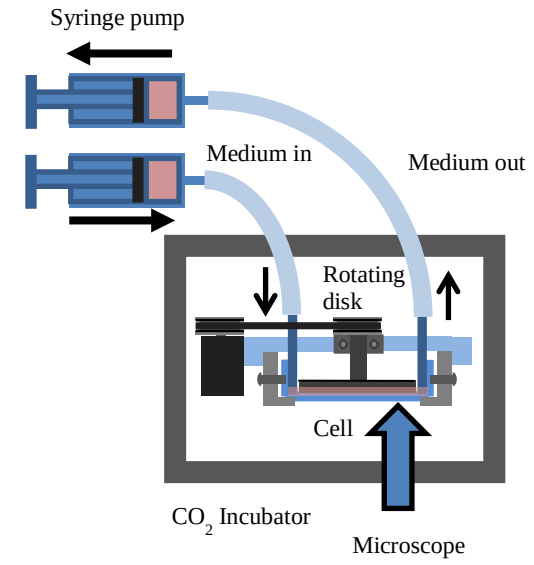

Fig. 6: Rotating parallel disks system in the incubator. Cells cultured in Couette type of flow are observed by microscope.

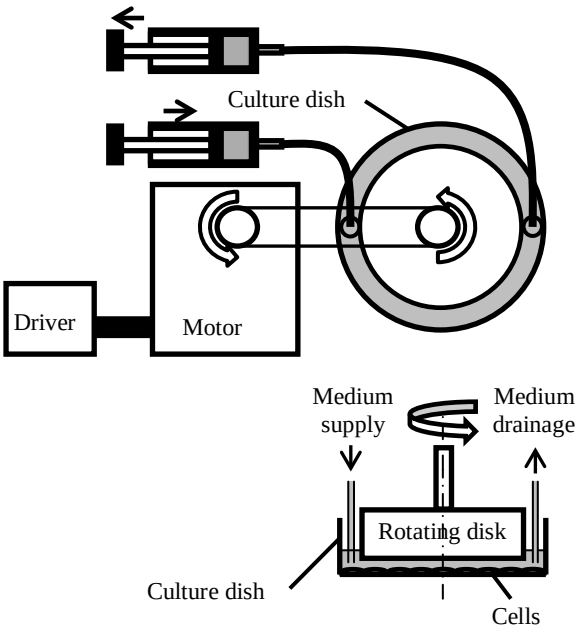

Fig. 7: Cells are cultured in shear flow generated by rotating disk. The lower figure shows the cross section of the culture dish.

At the constant angular velocity of 10 rad s^{-1}, the shear rate (E) increases from 2700 s^{-1} to 3000 s^{-1}, when the distance from the axis (r) increases from 16 mm to 18 mm in the observation area. The gradient of shear stress enables the simultaneous observation of the behavior of cells related to variation of the shear stress in the same view.

When the viscosity (N) of the culture medium at 310 K is 0.001 Pa s, shear stress (F) is 3 Pa at 3000 s^{-1} (Eq. 6)

Variation has been made on the angular velocity of the rotating disk from 1 rad s^{-1} to 10 rad s^{-1}, which makes variation of the shear stress from 0.3 to 3 Pa. The angular velocity is controlled by the motor.

Reynolds number is calculated by Eq. 7.

$$Re = D\,v\,d\,/\,N = D\,r\,w\,d\,/\,N \qquad (7)$$

In Eq. 7, D is density of the fluid. Re is 11, when D, r, w, d, and N are 1000 kg m^{-3}, 0.018 m, 10 rad s^{-1}, 0.00006 m, and 0.001 Pa s, respectively. The turbulent flow hardly occurs in the flow of small value of Reynolds number.

Finishing the surface is easier on the plane than on the circular conical surface. The roughness on the surface disturbs laminar flow between the cone and the plate. The system of parallel plane disks has advantage for the laminar flow than the system of cone and plate.

The distance between two parallel disks is more stable than the distance between cone and plate, because the viscous fluid sustains uniform narrow distance.

The donut-shaped open channel is convenient to study the effect of flow direction on the cell culture [5, 6], but it is not easy to estimate quantitatively the shear stress in the fluid because of the free surface.

Many kinds of the channels of Couette type flow were designed for quantitative experiments of biological fluid in the previous studies. The clot formation was quantitatively studied between a rotating cone and a stationary plate [7], and between a rotating concave cone and a stationary convex cone [8]. The erythrocyte destruction was studied between a rotating concave cone and a stationary convex cone [9]. The erythrocyte deformation was observed between counter rotating parallel discs [10, 11].

The rotating flow induces the secondary flow by the centrifugal effect. The effect is smaller in the system with the rotation of outer concave cone than with that of inner convex cone. The effect decreases with decrease of the rotational speed. The rotational speed of the disk is smaller than 0.2 m s^{-1} in the present system.

Poiseuille type of flow is also convenient to estimate the shear stress in the fluid. The behavior of cells was observed in the two dimensional Poiseuille type of flow between parallel walls in the previous study [12].

Several experimental systems were designed to observe the effect of shear flow on cells in the previous studies [13-16].

4. CONCLUSION

An experimental system with a rotating disk has been designed to apply wall shear stresses on the cell culture at the microscopic observation *in vitro*. The designed system is useful to apply wall shear stresses quantitatively on cell culture.

5. ACKNOWLEDGMENT

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