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Effect of Tilt Angle of Cone-Shaped Rotating Disk and Height of Medium on Shear Stress Distribution in a Cell Culture Plate

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Abstract

According to studies in cell biology, cell culture process development can be influenced by uniformity of shear stress distribution applied to cells. However, the method of applying a uniform shear stress in cell culture process development using commercial equipment requires a high volume of medium containing expensive growth factor agent. This study aims to design and develop a new method of producing a uniform shear stress applying to cells by using a cone-shaped rotating disk in order to reduce the needed medium volume. To achieve high uniformity of shear stress distribution at the bottom of a cell culture plate, effect of the tilt angle of a cone-shaped rotating disk and the height of the medium (in term of medium volume) were simulated using a computational fluid dynamics (CFD) technique (ANSYS FLUENT software). The simulation results revealed that higher medium volume (higher medium height) provided a more uniform shear stress distribution. When using 5 ml. medium volumes, the tilt angle of a rotating disk has little effect on the uniformity of shear stress distribution. As a result, a medium volume of 5 ml. and the tilt angle of 15 degrees was the most optimum condition, resulting in an average shear stress of 1.61 Pa and a standard deviation of 0.79 Pa.

***Keywords**:* Shear Stress Distribution, Cell Culture Plate, CFD, ANSYS.

1. Introduction

Periodontal ligament (PDL) is a group of connective tissue fibers which necessarily attach a tooth to the alveolar bone. During tooth movement, it receives mechanical loads such as compressive stress, tensile stress, and shear stress that transferred from the tooth [1]. The main functions of PDL cells is to maintain the width of the periodontal space which respond to such loads. It has been widely known that the PDL cells can respond to those mechanical loads which could profoundly affect the cell growth and repair [2-4]. However, shear stress is also loaded on the PDL cells since interstitial fluid is squeezed out of the space around the PDL cells and generates fluid shear stress on the PDL cells when teeth are load [5].

To study characteristics of PDL cell, the shear stress is usually applied to cell culture with several methods [6-7]. The proper shear stress (distribution) which is required to apply to PDL cell is between 0.1-2.4 Pa. When higher shear stress distribution is applied, it might peel cells off the scaffold, deform cell, and inhibit proliferation as well as tissue formation [8]. It has been reported that the shear stress generated by a flat (0 degree) rotation disk method can only reached a maximum shear stress of 1.5 Pa. In addition, the volume of medium and growth factor that costly are the main factors for PDL cell culture which still immoderate about the volume to apply to cell culture [8-10]. Furthermore, several cell culture grow system still consume large amount of the medium volume and growth factor over 10 ml.

Therefore, the purpose of this study is to design and develop a new method of producing shear stress on cells by using a cone-shaped rotating disk design in order to reach high shear stress with most uniform distribution and requires least medium volume. The effects of the tilt angle of cone-shaped rotating disk and height of the medium (in term of medium volume) on uniformity of shear stress distribution were simulated using computational fluid dynamics (CFD) technique (ANSYS FLUENT software).

2. Model Development

In this study, the relationships of the tilt angle of a cone-shaped rotating disk, volume of mediums, and rotational speed were investigated. The rotating disk was designed with the cone angle of 5, 10, 15 and 20 degree. The volumes of medium of 3, 4 and 5 ml were selected for the simulation since these were sufficiently small volumes. By using on the given tilt angles and the volumes of medium in cell culture plate, the possible conditions of the distance the cone-shaped rotating disk to the bottom of the cell culture plate (h) were determined as shown in table 1. The schematic diagram of the cone-shaped rotating disk is shown in Fig. 1. The diameter and height of the culture plate were 34 mm and 10 mm, respectively. The boundary condition of h was set between $1 \leq h \leq 5$ in mm to prevent the tip of rotation disk from touching the bottom of the cell culture plate. In addition, a larger volume than 5 ml. may cause medium fluid to overflow during rotation.

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The overall possible conditions are given in table 1. The rotational speeds of the cone-shaped rotating disk were set of 100, 200, 300, 400, and 500 rpm in order to investigate the shear stress.

The simulatiom model was generated by the fluid domain using ANSYS WORKBENCH. It was discretized into small computertional cells of 640,000 polyhedra cells in total as shown in Fig. 2. The density and viscosity of the medium fluid were set with 1012.95 kg $\cdot$m^3 and 0.00282 kg$\cdot$m$^{-1}\cdot$s^{-1}, respectively.

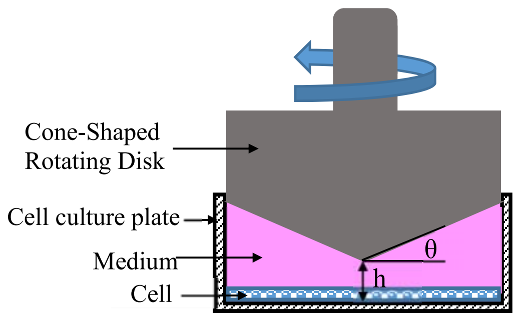


Fig. 1 Schematic diagram of the cone-shaed rotating disk with different cone angles (θ) and different medium volume resulting in different heights (h).

Table 1 The height of cone-shaped tip to the rest (h) of cell culture plate related to medium volume and tilt angle

Medium volume (ml)	Tilt angle of cone-shaped (θ)	h (mm)
3	5	2.32
	10	1.31
4	5	3.42
	10	2.41
	15	1.37
5	5	4.52
	10	3.51
	15	2.47
	20	1.39
5	0	5

In this study, based on the finite volume method, the mathematic model was developed. The governing equations of the steady-state mass conservation equation and Navier-Stoke's equations are included [1]. The genaral form of equations can be expressed as follow:

$$\nabla \cdot (\rho \phi \vec{V}) = \nabla \cdot (\Gamma_\phi \nabla \phi) + S_\phi \quad (1)$$

where ρ is the fluid density, ϕ denotes the transported quantity (mass and momentum), $\vec{V}$ is the velocity vector, Γ_ϕ is the transportation quantity diffusivity, and S_ϕ is the source term.

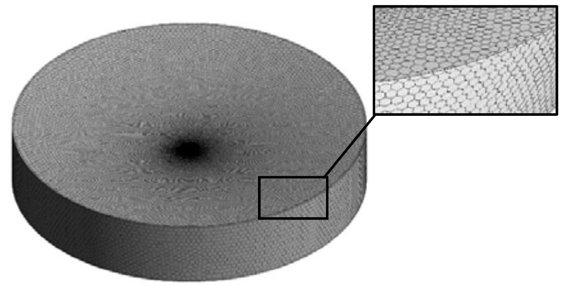


Fig. 2 computertional cells of 640,000 polyhedra cells

The following assumptions were applied on the model: (i) the system operated under the steady state and isothermal condition, (ii) the fluid velocity at all fluid-solid boundaries was equal to that of the solid boundary (no-slip condition), (iii) fluid medium was isotropic and homogeneous, and (iv) the cell height on the bottom of the cell culture plate was negligible.

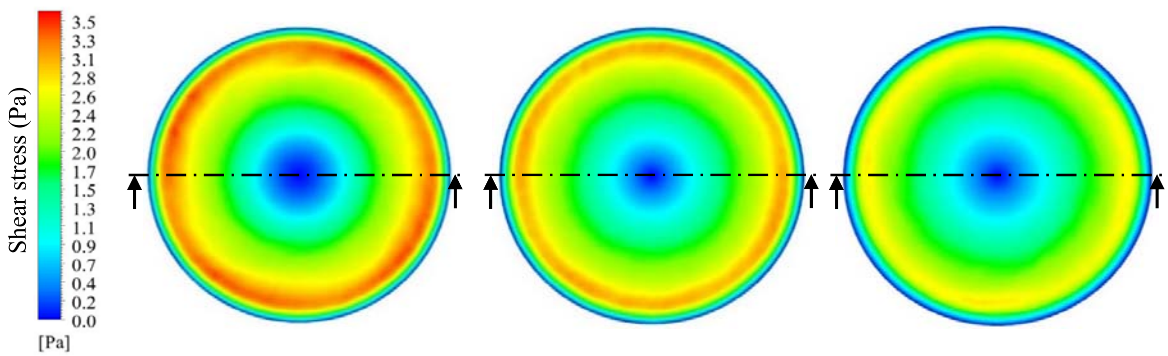
The SIMPLE (Semi-Implicit Method for Pressure Linked Equations) algorithm was used to solve the Navier-Stokes equations iteratively. The calculation in double digit precision was used to get highly accurate simulation results. To prevent the oscillation of the solution, the second-order upwind discretization scheme was selected. The under-relaxation factors of the pressure, density, body forces, and momentum were set at 0.27, 1, 1, and 0.55, respectively. The solutions were iterated until the specified convergence criterion of 10^{-5} was achieved.

3 Experimental Results and Discussion

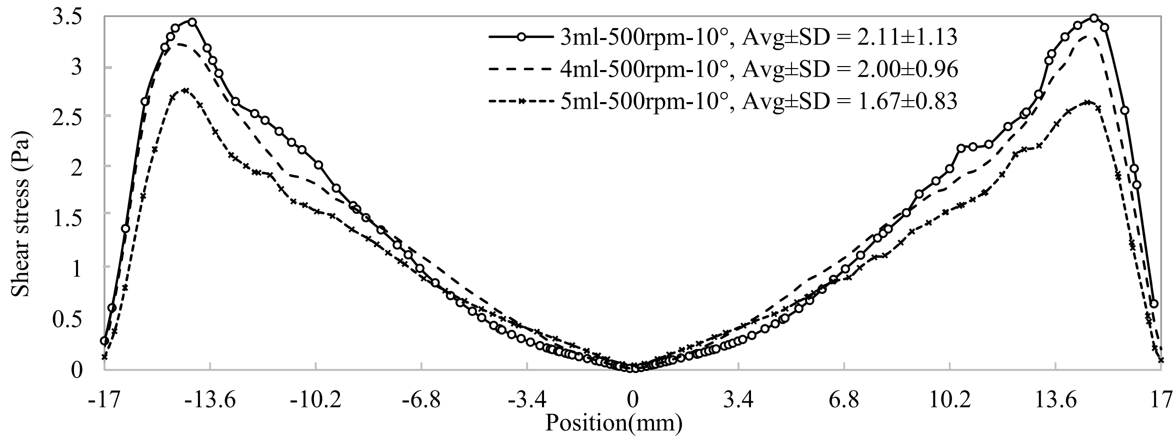
In this study, the investigation of the wall shear stress distribution was carried out on the cell culture plate since the typical height of the PDL cell was approximately 500 nm which was relatively small and thus it is negligible. From Fig. 3, the wall shear stress started from 0 Pa at the center of culture plate because of the boundary condition at the symmetry. Then, it tended to increase to maximum value when the distance increase and rapid decline to 0 Pa again at the wall of culture plate due to a non-slip flow condition. In addition, the characteristic of the wall shear stress distribution depended on the conditions of simulations. To compare the stress distribution of different conditions, the average stress over plate area (Avg), the maximum shear stress (Max), and the standard deviation (SD) of the shear stress were calculated for all conditions as shown in table 2.

Table 2 The average stress over all plate area (Avg), the maximum shear stress (Max), and the standard deviation (SD) of the shear stress

	100 rpm			200 rpm			300 rpm			400 rpm			500 rpm		
	Avg	Max	SD	Avg	Max	SD	Avg	Max	SD	Avg	Max	SD	Avg	Max	SD
3ml-5°	0.14	0.28	0.08	0.45	0.93	0.29	0.94	1.74	0.56	1.54	2.61	0.83	2.21	3.52	1.11
3ml-10°	0.13	0.26	0.07	0.43	0.88	0.26	0.88	1.69	0.53	1.45	2.58	0.82	2.11	3.50	1.13
4ml-5°	0.13	0.24	0.08	0.44	0.74	0.25	0.88	1.37	0.45	1.40	2.10	0.68	1.97	2.93	0.94
4ml-10°	0.12	0.24	0.07	0.43	0.77	0.25	0.88	1.46	0.47	1.41	2.24	0.71	2.00	3.04	0.96
4ml-15°	0.11	0.20	0.11	0.38	0.65	0.22	0.79	1.26	0.44	1.29	1.97	0.67	1.84	2.78	0.92
5ml-5°	0.12	0.21	0.06	0.40	0.63	0.20	0.77	1.22	0.38	1.21	1.92	0.60	1.71	2.68	0.83
5ml-10°	0.11	0.20	0.06	0.39	0.63	0.19	0.75	1.23	0.37	1.18	1.90	0.58	1.67	2.62	0.83
5ml-15°	0.10	0.19	0.06	0.36	0.58	0.19	0.72	1.11	0.35	1.13	1.76	0.55	1.61	2.49	0.79
5ml-20°	0.09	0.17	0.05	0.34	0.57	0.19	0.70	1.08	0.37	1.11	1.78	0.56	1.62	2.52	0.8
5ml-0°	0.12	0.21	0.06	0.10	0.52	0.10	0.59	0.85	0.21	0.85	1.19	0.28	1.13	1.53	0.35



(a) 3ml (b) 4ml (c) 5ml



(d)

Fig. 3 Shear stress distribution on the bottom of the cell culture plate at the rotational speed of 500 rpm when the medium volume were (a) 3 ml, (b) 4 ml, and (c) 5 ml. (d) Diameter section view of shear stress from (a), (b) and (c).

3.1 The Effect of the medium volume

Fig. 3 shows the example comparisons of the effect of medium volume of 3 ml, 4 ml and 5 ml on shear stress distribution for the tilt angle of 10 degree using a rotational speed of 500 rpm. Fig. 3d shows the diameter section view from the shear stress of Fig. 3a, 3b and 3c. As the results, the shear stress values raised from 0 Pa at the center (position r = 0 mm) to the side wall of culture plate (position r = 17 mm). For all cases, the shear stress values were almost similar from at the

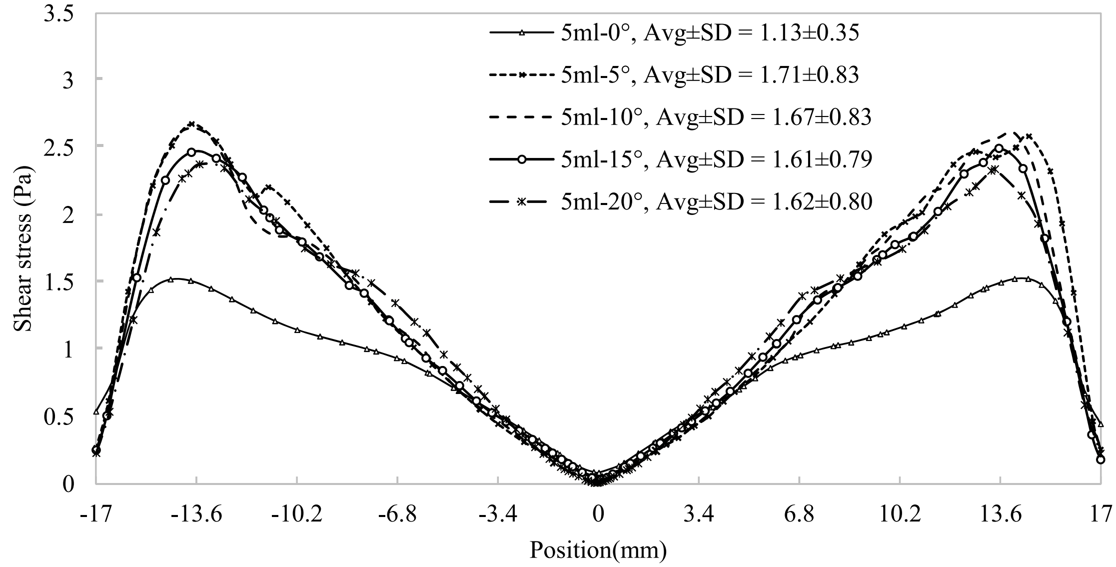


Fig. 4 Diameter section view of the shear stress when the tilt angle at 0°, 5°, 10°, 15° and 20° for a rotational speed of 500 rpm and 5 ml of medium.

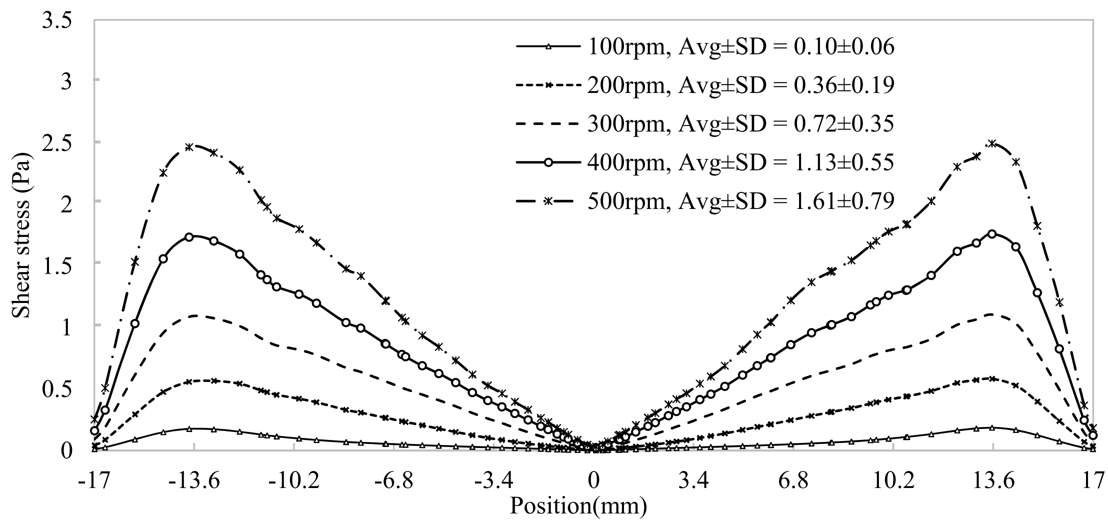


Fig. 5 Diameter section view of shear stress when the rotational speeds were 100 – 500 rpm for a tilt angle 15° and 5 ml of medium.

center to around r = 6.8 mm. This according to fluid shear stress equation of [11]:

$$\tau = \mu \frac{\omega r}{h} \tag{2}$$

where τ is the shear stress [Pa], ω is the rotational speed (rpm), r is distance from the rotating axis (mm) and the surface of the cone to the bottom of the cell culture plate (h). Where the r was small result to small difference in shear stress.

The simulation results showed that the uniformity increased (smaller SD) when the medium volume increased for all rotational speeds. Meanwhile, the maximum shear stress decreased when the medium volume increased. The increased volume resulted in larger height (h) between the cone tip and the bottom of the plate and allowed more space for the shear stress to become evenly distributed. As a result, the 5 ml volume had lower maximum stress as well as better uniformity of stress distribution.

3.2 Effect of tilt angle of cone-shaped rotating disk

The tilt angle (θ) of the cone-shaped rotating disk was investigated by comparing the small angles of 0°, 5°, 10°, 15° and 20° using 5 ml medium volume using a rotational speed of 500 rpm. As reported in Table 2, the simulation result of the tilt angle of 0° (flat disk) had been reported in our previous research [1]. The flat disk design was limited by the maximum shear stress of 1.5 Pa using the same condition. It was obvious that the flat disk was suitable for applications of the cell culture under shear stress 1.5 Pa. On the other hand, our simulation results showed that the cone-shaped disk can generate a higher shear stress with the maximum stress up to 3.5 Pa. For all conditions, when the tilt angles increased, the maximum shear stress increased. According Eq. 2 when the tilt angles increased, the *h* increase. It means that it provides better shear stress uniformity. Meanwhile, the stress variation (SD value) was smallest for the tilt angle of 15° but the SD values were similar for all tilt angles.

3.3 Effect of rotational speed

Fig. 5 shows the wall shear stress on different rotational speeds for a tilt angle of 15 degree and 5 ml medium volume. Base on the simulation results, the higher rotational speed produced higher shear stress because the shear stress is directly proportional the speed. Fig. 6 plots the relationship between the shear stress as a function of rotational speed. It was found that the rotational speed had the largest influence on the shear stress. Therefore, the shear stress function of rotational speed can be accurately predicted using a second degree polynomial function as follows:

$$\tau_{avg} = (9.8 - V) \times 10^{-6}\omega^2 + 8.1 \times 10^{-4}\omega \quad (3)$$

where τ_{avg} is the average shear stress [Pa] and V is the volume of the medium between 3 – 5 ml. This equation allows a user to achieve the required average shear stress by selecting the rotational speed and the medium volume used. Our results indicated that the tilt angle had smaller effect among all parameters and should be neglected for simplicity. The maximum shear stress can be estimated about 50% higher than the calculated average shear stress.

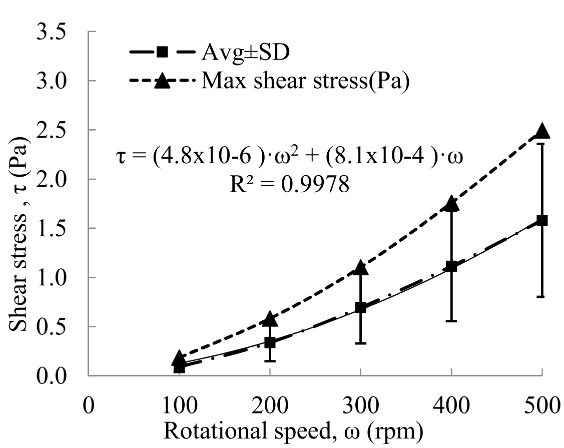


Fig.6 Maximum and area-average shear stress under five different rotational speed on medium 5 ml.

4. Conclusion

This study computational fluid dynamics (CFD) technique (ANSYS FLUENT software) was used to simulate the effects of the tilt angle of a cone-shaped rotating disk and height of the medium (in term of medium volume) on the uniformity of shear stress distribution. The results reported that the maximum shear stress can be generated between 0.17 to 3.5 Pa. The shear stress higher than 2.4 Pa should be neglected because it may peel off the cells from the scaffold, deform cell, and inhibit proliferation as well as tissue formation. The results suggested that when the requirement of the shear stress was under 1.5 Pa, a flat (0 degree) rotation disk provided better uniformity. When the requirement of the shear stress between was from 1.5 Pa to 2.4 Pa, a 5 ml medium volume and 15° cone-shaped rotation disk provided the highest uniformity for shear stress distribution.

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