Improved design of a cone-shaped rotating disk for shear force loading in a cell culture plate

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Abstract. In our previous study, a cone-shaped rotating disk had been designed and proposed for generating shear force on the cell in a cell culture plate. This study aims to improve the design of the rotating disk that could provide a better uniformity of shear stress distribution. The top of the cone was designed to be trimmed off to obtain a flat head area. The effect of tilt angle (θ) was numerically studied using computational fluid dynamics (CFD) technique in ANSYS-Fluent software. The results revealed that for 500 rpm, the new designed rotating disk with a height of cone-shaped top to the plate bottom h = 1 mm and θ = 25° provided the best uniformity of 0.820 which was better than that of the previously designed.

1. Introduction
Mechanical force loading like shear force can affect dental cell growth and regeneration. Shear stress, produced by torsion and shear between the teeth and alveolar bone, is the most common type of mechanical stress induced on periodontal ligament (PDL) cells [1]. PDL is also regarded as a porous tissue surrounded by a massive vascular network. When pressure builds up between the teeth and alveolar bone, pressurized interstitial fluid produces fluid shear stress at the PDL cell membrane [2]. Therefore, shear stress was thought to be the most appropriate biomechanical stress for PDL tissue engineering [1]. In previous studies, the proper shear stress required to apply to PDL cells was in the range of 0.1–6 Pa [3, 4].

Applying shear force onto the cells can be done in many ways. Force loading via angular velocity of the medium in a cell culture plate is one of the appropriate techniques as the loading apparatus does not directly contact the cells. However, the local angular velocity of the medium varies proportionally with the radius length of the rotating disk. Thus, the shear force exerted on the cell is not uniformly distributed throughout the bottom of the culture plate. To effectively study the effects of shear force on cell regeneration and its gene expression, the rotating disk must be carefully designed so as to obtain the shear force uniform distribution.

However, the cells are at the bottom of the culture plate and the rotating disk rotates at the surface of the medium. Therefore, this problem becomes three dimensional velocity profiles of which the angular velocity
at the bottom of the culture plate is not linearly proportional to the radius of the rotating disk. Hence, the numerical analysis is needed to estimate the shear stress distribution at the bottom.

In our previous work, a cone-shaped rotating disk design with different heights of cone-shaped tip to the bottom of the cell culture plate, tilt angles, medium volumes had been numerically investigated. The simulation results suggested that for 5 cm³ medium, the height of 2.47 mm and the tilt angle of 15° should be used in order to obtain the best uniformity of shear stress distribution at the plate bottom of 0.817 at 500 rpm [5]. Nevertheless, considering the geometry of the cone shape, the uniformity of the shear stress distribution can be improved by reducing the difference in radius of the top and the bottom of the cone. This can be done by trimming off the top of the cone by which the radius of the top will be increased at a closer distance to the bottom of the culture plate, as compared with the ordinary cone shape.

In this study, the height of the cone-shaped top (flat head) to the bottom of the cell culture plate \( h \) was fixed at 1 mm. This was because the smaller height provided the higher shear stress at the same rotational speed, and most importantly, the required volumes of the medium and the costly growth factor would become less. However, the height lower than 1 mm would be improper as the cells may be peeled off from the scaffold. Thus, the objective of this research was to numerically study the effect of the tilt angle of a top-flat cone-shaped rotating disk (new design) on the shear stress distribution, so that an optimum tilt angle under a given condition, i.e., \( h = 1 \) mm and the disk diameter of 34 mm, can be suggested with higher uniformity.

2. Model Development

The top of the cone was trimmed off to get a flat head (figure 1). The fluid model used to analyze the shear stress distribution was developed based on the finite volume method using ANSYS FLUENT software. The governing equations used in this work included the steady-state mass conservation equation and the steady-state Navier-Stoke’s equations which are coupled and can be expressed in the general form as follows.

\[
\nabla \cdot (\rho \phi \vec{V}) = \nabla \cdot (\Gamma_\phi \nabla \phi) + S_\phi
\]

where \( \rho \) denotes to the fluid density, \( \phi \) is the transported quantity (mass and momentum), \( \vec{V} \) is the velocity vector, \( \Gamma_\phi \) is the transportation quantity diffusivity, and \( S_\phi \) is the source term.

The fluid model was discretized into 291,132 computational polyhedral cells. The density and viscosity of the medium fluid were 1012.95 kg m\(^{-3}\) and 0.00282 kg m\(^{-1}\) s\(^{-1}\), respectively.

The assumptions conditions followed the previous work [5] which following: (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 at 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-Stoke’s 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.
Figure 1: Schematic diagrams of a) the previous and b) the new cone-shaped rotating disk design, respectively.

In this study, the diameter of the rotating disk was set at 34 mm according to the standard size of cell culture plate (34 mm diameter x 10 mm depth) and the height \( h \) was set at 1 mm. The tilt angle was set at 15, 20, 25, and 30 degrees. These criteria were selected based on the fact that the top of the rotating disk must not touch the bottom of the plate, and the surface of the respective medium must touch the cone bottom but must not overflow when the rotating disk is dipped into the medium. Table 1 presents the conditions of the tilt angles and their related medium volumes used in this simulation. The rotational speeds were varied from 100 to 500 rpm. In order to correctly and systematically analyze how uniform the stress distribution was in each case, an index called “uniformity” was introduced. The uniformity can be calculated using the equations (2) and (3). The higher value of uniformity means the shear stress is more evenly distributed in the entire bottom area.

The uniformity index presents how a specified field variable varies over a surface, where a value of 1 indicates the highest uniformity. The uniformity index can be weighted by area or mass: the area-weighted uniformity index captures the variation of the quantity (for example, the species concentration), whereas the mass-weighted uniformity index captures the variation of the flux (for example, the species flux) [6].

The area-weighted uniformity index \( \gamma_a \) of a specified variable \( \phi \) is calculated using the following equation:

\[
\gamma_a = 1 - \frac{\sum_{i=1}^n \left( (\phi_i - \overline{\phi_a}) A_i \right)}{2 \overline{\phi_a} \sum_{i=1}^n A_i}
\]  

(2)

where \( i \) is the facet index of a surface with \( n \) facets, and \( \overline{\phi_a} \) is the average value of the field variable over the surface:

\[
\overline{\phi_a} = \frac{\sum_{i=1}^n \phi_i A_i}{\sum_{i=1}^n A_i}
\]  

(3)
### Table 1
The simulation conditions of the tilt angles and their respective medium volumes

| h (mm) | Tilt angle of cone-shaped (θ) | Medium volume (cm³) |
|--------|-----------------------------|---------------------|
| 1      | 15                          | 2.8                 |
|        | 20                          | 3.7                 |
|        | 25                          | 4.8                 |
|        | 30                          | 5.9                 |

#### 3 Results and Discussion

The investigation focused on the shear stress distribution and its uniformity at the bottom of the cell culture plate as the typical height of the PDL cell was approximately 500 nm [7] which was relatively small and thus it is negligible. Figure 2 depicts the shear stress distribution at the bottom of the cell culture plate with different tilt angles. The results indicated that at the same rotational speed (500 rpm), the shear stress distribution seemed to be more uniform as the tilt angle increased. Thus, we further investigated the area-averaged shear stress and its uniformity at various rotational speeds. Figures 3 and 4 display the area-averaged shear stress and the uniformity index simulated with different tilt angles in the rotational speed range of 100-500 rpm. Obviously, the area-averaged shear stress was directly proportional to the rotational speed. For the effect of tilt angle, the average shear stress increased with the decreasing tilt angle decreased. This was because with the narrower tilt angle, the overall surface of the cone-shaped rotating disk rotated closer to the plate bottom, resulting in greater average angular velocity. Nonetheless, the increasing rate of the average shear stress slowed down as the tilt angle became lesser.

![Figure 2](image-url)  
**Figure 2** Shear stress distribution at the bottom of the cell culture plate at 500 rpm with the tilt angles of a) 15°, b) 20°, c) 25° and d) 30°
The relationship between the average shear stress and the tilt angle at various rotational speeds

For the uniformity, the flathead cone-shape rotating disks could produce the uniformity in the range of 0.73-0.82, which was quite good, considering the maximum value of 1.00. In contrast to the results of the average shear stress, the uniformity was lower when the tilt angles became narrower. This was due to the closer surface of the rotating disk to the plate bottom that contributed to the higher angular velocity. As a result, the difference in local shear stress around the center and the edge of the rotating disk became greater, leading to lower uniformity. Therefore, to select an optimum design of the flathead cone-shaped rotating disk, the balance in average shear stress and uniformity must be taken into consideration.

The relationship between the uniformity and the tilt angle at various rotational speeds

From figure 4, the uniformity of 25 and 30 degrees were almost identical at any rotational speeds. The maximum uniformity of 0.820 was reached with the 25 degree at 500 rpm. Therefore, the optimum condition suggested in this study was of θ = 25°, as it had a merit of using lesser medium volume vis-à-vis the 30° (see table 1), while maintaining the same high uniformity at any rotational speeds. With this
condition, the new design (flat head) was successfully improved in all viewpoints as compared with the previous design (normal cone head [5]) as at the same rotational speed (500 rpm), it generated greater average shear stress (1.69 Pa vs. 1.62 Pa), provided higher uniformity (0.820 vs. 0.816), and used less medium (4.8 cm$^3$ vs. 5.0 cm).

Figure 5 Relationship between the average shear stress and the rotational speed of the suggested design

Consequently, we further investigated the average shear stress generated by the suggested flathead cone-shaped ($\theta = 25^\circ$) at higher rotational speeds up to 900 rpm. This was to determine an empirical equation that facilitates a user to set up their experiments easily. From figure 5, the average shear stress can be expressed as a function of rotational speed using a second degree polynomial function with a high $R^2$ of 0.9997 as follows:

$$\tau = 3 \times 10^{-6} \omega^2 + 0.0021 \omega - 0.197$$

where $\tau$ is the area-averaged shear stress (Pa) and $\omega$ is the rotational speed between 0-900 rpm.

4 Conclusion
This study aims to improve the design of a rotating disk for shear force loading on cell in the cell culture plate using numerical simulation based on CFD techniques. The current design of flathead cone-shaped rotating disk with $25^\circ$ tilt angle outperformed the previous design in all key aspects. It achieved greater shear stress at the same rotational speeds, provided a better uniformity index and required less amount of medium and growth factor. Finally, an empirical equation representing the relationship between the average shear stress and the rotational speed was proposed for the simplicity sake in setting up the shear force loading experiment.

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