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Determination of microcarrier radius that can be cultured in a rotational wall vessel bioreactor

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ABSTRACT

Introduction: Bioreactor are used as cell culture systems for growth of tissue – engineered, because strong shear stress effect on bioreactor damages delicate cells and is hypothesized to degrade the formation of three-dimensional tissue-like structures, such a stress should be analyzed. In this study, the forces acting on a small piece of tissue or a microcarrier particle and its movement in the RWV bioreactor with inner cylinder were analyzed. The tracks of a particle in RWV bioreactor were calculated under different inner and outer cylinder rotating speeds, different particle sizes. The shear stress acting on the particle was analyzed. Finally, an expression between the shear stress acting on the particle and the microcarrier radius is obtained.

Objectives: Determination of the range of microcarrier radius or tissue size, which could be safely cultured in the RWV bioreactor.

Materials and Methods: The forces of rotational wall vessel (RWV) bioreactor on small tissue pieces or microcarrier particles was calculated and the shear stress acting on the particle was analyzed.

Results: The range of microcarrier radius or tissue size, which could be safely cultured in the RWV bioreactor, in terms of shear stress level, was determined.

Conclusion: The upper safe size range for the particles cultured in RWV is 600-1300. µm in radius.

Key Words:

Tissue engineering, rotating wall vessel, bioreactor.

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INTRODUCTION

A bioreactor refers to a system where conditions are closely controlled to permit or induce a certain behavior in living cells or tissues. The behavior may simply be cell proliferation, or is as complex as several sets of cells that sense one or more variable parameters and produce specific chemicals accordingly. The concept of bioreactor is neither new nor restricted to tissue-engineered cells. Bioreactors have been used in the past to investigate other problems such as wastewater treatment, wine and even flavor production. The bioreactor intended to engineer tissue requires mimicking the in vivo microenvironment of cultured cells or tissue as closely as possible.[10]

Rotating wall vessel (RWV) bioreactor is a type of device to incubate cells or

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tissues, which was developed for cell growth under the condition of microgravity by American National Aeronautics and Space Administration (NASA) in 1992[16]. In this kind of bioreactor, the function of incubated cells and tissues is closer to natural ones because the shear stress acting on the cells and tissues is very low and the cells have chances to keep in three-dimensional touch with others.

Most previous studies focused on the effect of RWV bioreactor on the incubated cells or tissues while analysis on the forces acting on cells or microcarrier and their movement in RWV was not adequately described. Santos et al.[25] simulated the motion of microcarrier particles inside a horizontally rotating bioreactor. Tsao et al.[28] developed a mathematical model to characterize cell-medium interaction in a bioreactor. Gao et al.[9] analyzed and calculated the movement of a microcarrier in RWV bioreactor. They found that if the density of the microcarrier particles is greater than that of culture medium, the particles would migrate towards the outer cylinder wall and collide with it finally. The shear stress coming from liquid acting on the particles increases with the density difference between the microcarrier particles and liquid. Qiu et al.[21] recorded the motion tracks of microcarrier particles and found that the migrating speed in radial direction decreases with the particle radius. If the density of microcarrier particles is lower than that of the culture medium, the particles finally come to the center of the circle. Begley and Kleis[1] analyzed and calculated the velocity field and stress of liquid in RWV reactor with viscous pump, but they did not calculate the motion tracks of microcarrier particles or a small piece of tissue. Freed et al.[17] studied the relationship between fluid dynamic conditions and the effect of RWV reactor on incubated tissue. They derived a simple mathematical model of the forces acting on a small piece of tissue in a "static" place in RWV without considering the whole moving state of the tissue. Pollack et al.[24] presented a mathematical model of microcarrier motion in the rotating bioreactor. However, almost all the researches focused on the rotating wall vessel without inner cylinder. T. G. Hammond and J. M. Hammond[14] reviewed the engineering principles which allow optimal suspension culture conditions to be established in the RWV bioreactor.

This study was done to analyze the forces of rotational wall vessel (RWV) bioreactor on small tissue pieces or microcarrier particles, to determine the range of microcarrier radius or tissue size, which could be safely cultured in the RWV bioreactor.

MATERIALS AND METHOD

The Bioreactor System (Fig. 1) consists of a rotating cell culture vessel and external loop components (pump, manifold, oxygenator and valves) connected together with autoclavable tubing. Oxygenation is provided by a silicone rubber membrane oxygenator connected in the external loop. A simple peristaltic pump is used to recirculate the fluid and to fill and drain the system. When the pH of the medium becomes acidic, simply switch the valves to perfuse fresh medium. pH and glucose can be adjusted by manually injecting NaOH solution and glucose solution, as needed. This simple arrangement provide an operable system without complex pumps, electric valves, sensors or computers.

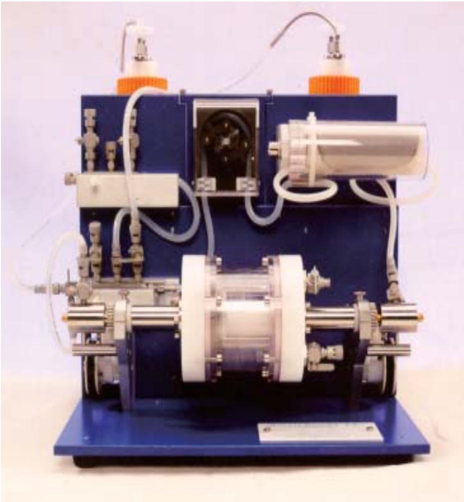

Fig. 1: Synthecm complete bioreactor system.

In external loop, there is a pump, a manifold, an oxygenator and manual valves. Medium stored in the medium vessel can be perfused through the reactor vessel by opening the medium valve and directing the outflow of the vessel to waste. Normal operation is for the pump to recirculate the medium through the oxygenator. Maximum circulation needed is only about 10 cc per minute for 10 million cells per ml. The fluid is circulated by a stepper motor driven peristaltic pump. The manifold provides connections for additional reservoirs of glucose solution or NaOH solution.

The reactor operates by rotating the entire vessel horizontally to suspend the vessel contents in the fluid. No air bubbles are present in the vessel. This minimizes damaging shear forces which inhibit the growth of cells. The central filter, (which can be made of different materials) is driven by a separate motor.

In general, the flow pattern and the structure of the bioreactor should be convenient to mix the culture medium in order to provide rigorous mass transfer control and the temperature should be maintained at a certain level for the good growth of cultured cells.

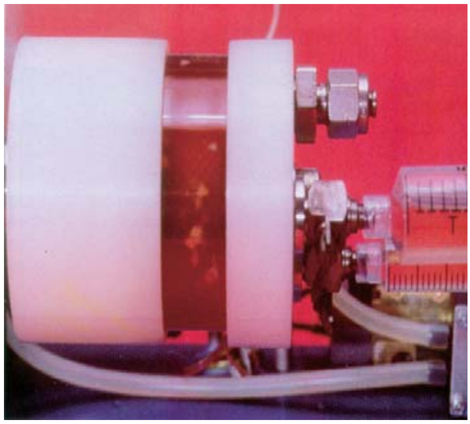

Fig. 2: Full vessel.

The bioreactor vessel consists of two concentric cylinders (Fig. 2), with the outer cylinder having a radius of Ro and rotating at .o, while the inner cylinder having a radius of Ri. and rotating at .i. The gap between the cylinders was completely filled with culture medium into which particles and microcarrier beads were introduced. In this study Ri=0.02 and Ro=0.05 were used for the calculation (Fig. 3) provides a schematic representation of the vessel. Cylindrical coordinates (r, θ) are used to indicate the positions of a cultured particle within the vessel, where r is the radial component measured outward relative to the cylinders, with Ri< r< Ro and θ. is the angular component measured positively in the direction of rotation of the two cylinders. In the rotating-wall vessel, the solid body rotation is accomplished by horizontal rotation of the two concentric cylinders at the same constant rotational speeds. The motion of microcarrier in the rotating flow is of interest and importance in the design of the rotating-wall vessel bioreactors and in the guiding of RWV cell culture experiments under optimal operating conditions.

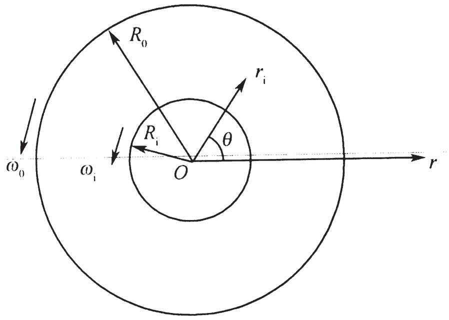

Fig. 3: Schematic drawing of rotating wall.

In modeling the microcarrier particle motion, the following assumptions were introduced:

1. The physical properties of both liquid medium and solid particles are constant, since the operational temperature is maintained at 37°C. In this simulation the relative parameters were taken as ρ_1=1000 kg/m3, μ.=0.001 kg/(m*s) and ρ_p.= 1040 kg/m.[3]

2. The flow field was not affected by the presence of the particle.

3. Secondary flows due to spinning up could be neglected and the fluid flow was steady.[28]

4. The acceleration of the fluid due to the acceleration of the micrcocarrier was approximated by the virtual mass concept.[14]

The force balance equations in the rotating reference frame of RWV for a particle are described as follows:

In radial direction (r-direction):

$$-(m-m_p)r\omega + 2Mr\emptyset'\omega + Mr\,\emptyset,^2 + g\,(m-m_p)\sin(\emptyset+\omega t) - kr` - Mr'' - F=0 \qquad (1).$$

and in circumferential direction (θ -direction):

$$-g\;(m-m_p)\cos((\emptyset+\omega t) + 2Mr\emptyset` + kr\emptyset + 2`Mr'\emptyset' + Mr\,\emptyset\,''=0 \qquad (2).$$

$$M=C_v m+m_p=c_v\boldsymbol{\rho}_1 V_p+\boldsymbol{\rho}_p V_p \qquad (3).$$

C_v is the virtual mass coefficient[13], equal to 0.5.

for a spherical particle; k is the non-Stokes drag coefficient, defined as

$$k=1/2\pi * r_p^{\,2} * \rho_1 * cd * Vel \qquad (4).$$

$$Cd.=18.5Re\;(-0.6) \qquad (5).$$

Re is the Reynolds number and Vel is the relative speed between the particle and the liquid.

When a particle is migrating close to the wall of the vessel, a fictitious short range contact force on the particle will be activated in the radial direction. This force is similar to the centripetal force in the form.

$$F= \pm\,\lambda m\omega^{2''}(r_p + delta - |R - r|) \qquad (6).$$

where delta is a specified arbitrary small distance, according to the introduction of Gao[9], 0.02rp was used in our simulation. This force is activated only when the particle moves very close to the wall, and R represents the radius of the wall, Ro or Ri. The positive sign is for the collision with the inner wall and the negative sign is for the collision with the outer wall; λ is an adjustable constant ranging from 1 to 2000, which represents the intensity of the collision, and the larger the constant the more intense the collision is.

The simultaneous equations from 1-6 can describe the motion of particle in the RWV with inner cylinder. Solving them numerically can obtain the continuous trajectory of the microcarrier particles.

Shear stress was then calculated $\boldsymbol{\tau}$ (N/m^2).

RESULTS

If the outer and inner vessels rotated synchronously, after a period the particle with density greater than the culture medium would migrate near the outer wall.

The particle travelled in epitrochoidal-like trajectories. In the end, the particle collided with the wall which may cause damage to cells.

The microcarrier particle will migrate towards the outer or inner wall of the rotating wall vessel. This is inevitable if the two cylinders rotate in the same direction, no matter under what matched rotational speeds of the two cylinders. If the inner cylinder rotated with the same speed as the outer vessel, after a period the particle would move to the outer vessel (Fig. 4-A). The value of the rotating speed only affected the migration time of the particle (Fig. 4-B), but could not change the fate of the particle. If the rotating speed of the outer vessel was greater than that of the inner vessel, the particle would migrate to the inner cylinder in a very short time (Fig. 4-C). Otherwise, when the inner vessel rotated faster than the outer vessel, the particle would settle down just near the outer vessel (Fig. 4-D).

Figure 5-A shows the trajectory of microcarrier particle with the model simulation and (Fig. 5-B) is shows the Parameters of Xue[31] experiment. The comparison showed that they were consistent to a great degree.

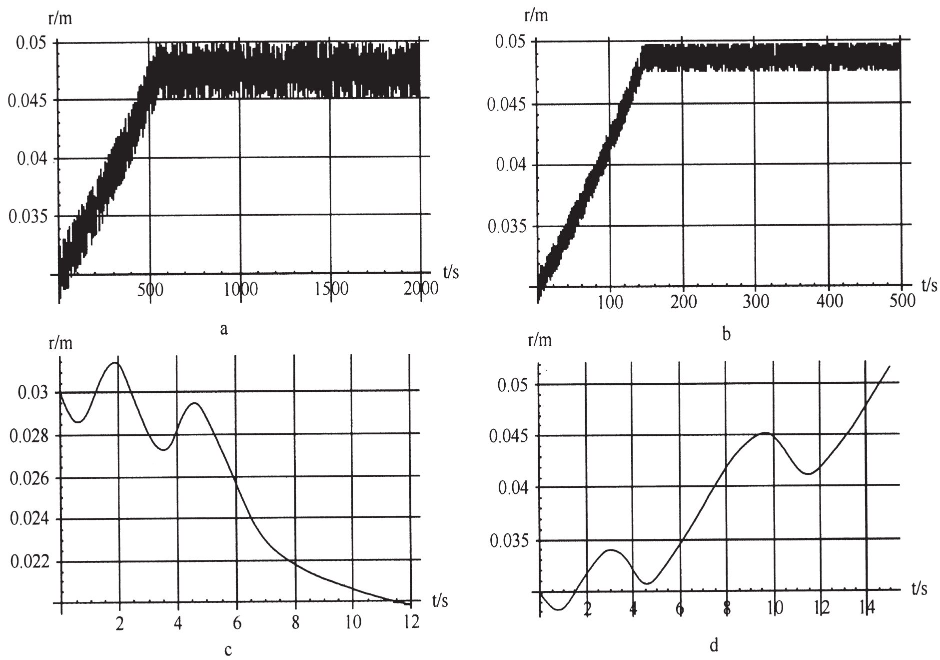

Fig. 4: Radial position of the particle vs the time (r_p=100 μ.m). a:$\omega_o=\omega_i$=15rpm; b: $\omega_o=\omega_i$=30rpm; c: ω_o=30rpm, ω_i=10rpm; d: . ω_o=10rpm, ω_i=30rpm.

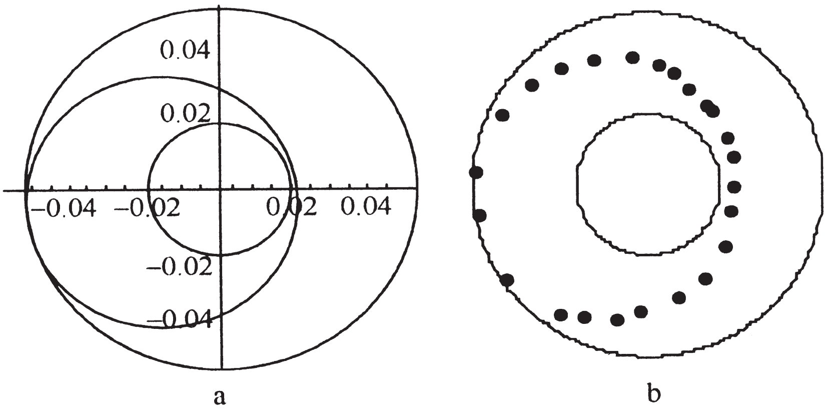

Fig. 5: Comparison of the trajectory of a microcarrier particle by model simulation results (a) and experiment data (b). (r=1308 kg/m³, r_p=730 µm, . ω_o=ω_i= -40 rpm).

Liquid shear stress acting on a microcarrier or incubated tissue in RWV is then calculated τ (N/m²) and plotted in (Fig. 6), which clearly indicates that the shear stress acting on the cultivated tissue is linear with the particle size in RWV.

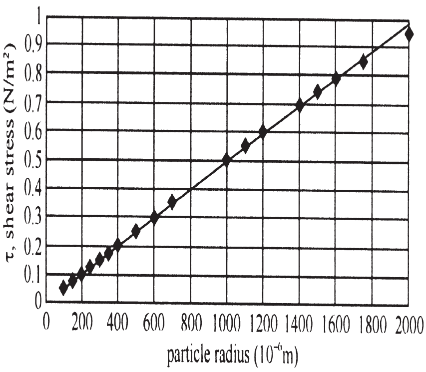

Fig. 6: Curve of shear stress vs particle radius $\tau = 0.0005r_p$, R^2=0.9982.

DISCUSSION

In a rotation period the microcarrier moved to the outer cylinder wall and collided with it, then it bounded back and rotated towards the inner cylinder wall. The microcarrier would collide with the wall of RWV bioreactor during each period, which may damage the cells at the outside layers of the microcarrier. This is not the desired circumstance for the culture of mammalian cells, especially stem-like cells. Optimization should be done to avoid the collision between the microcarrier and the wall as possible

There are two alternatives; the outer cylinder rotates at different speed in the same direction and the inner cylinder rotates at opposite direction. Our experiment also indicated that when the two cylinders rotated at different speeds, the microcarrier would still repeat what the microcarrier did when the two walls rotated at the same speed.

From the above to avoid the collision between the microcarrier cylinder wall, the inner and outer cylinders should rotate in opposite directions and the rotating speeds of them should be matched properly.

In order to optimize the operating condition of the bioreactor, the rotation speeds and the directions was predicted to be changed of the inner and outer cylinders to find if there was a proper match for the rotation of the two cylinders to avoid the collision.

the larger the particle was, the greater the difference of the rotational speeds was.

As strong shear stress in bioreactor could damage delicate cells, and is hypothesized to degrade the formation of three-dimensional tissue-like structures.

Therefore, the low shear stress is of vital importance for cell culture. For the mammalian cells, the mechanical shear stress should be lower than the value in the range of 0.3-1 N/m2; otherwise severe damage to cells will occur and cell viability will be reduced[1]. There is a limit to the particle or tissue in size under which the particle can be cultured safely in the RWV.

From (Fig. 6) it can be shown that all the particles with their radius less than 2 mm could be cultured in the RWV. When the particle radius was shorter than 600 μm, the level of the mechanical shear stress was less than 0.3 N/m'', which is suitable for culturing some stress-sensitive cells such as stem cells. Considering this together with the effect of rotating speeds, the upper safe size range of the cultured particles with shear-sensitive cells is 600-1300 μm in radius. If the cultured cells could endure larger shear stress, the size could exceed this value. Granet et al.[12] reported that the tissue-like aggregates of endothelial cells can grow with their size as large as 540 μm after 30 days in rotating wall vessel, which agrees with the size range mentioned above.

CONCLUSION

The motion of microcarrier particles in the rotating wall vessel with inner cylinder can be simulated with this model, by using a non-Stokes drag force component and the contact force near the wall of the RWV. If the two cylinders rotate in the same direction, the particles inside will move towards the outer cylinder wall and collide with the wall finally. When the two cylinders rotate in opposite direction, a minimum rotating speed difference exists for different particle size to avoid the collision between the particles and the walls. The results have been validated by experimental data. Shear stress acting on a microcarrier is important factor to be considered for the particles cultured in RWV. The upper safe size range for the particles cultured in RWV is 600-1300 μm in radius.

NOMENCLATURE

Cv- virtual mass coefficient.
F- short range contact force near the wall.
g- Acceleration of gravity, 9.81 m/s-1.
k- non-strokes drag coefficient
M- mass of particles, Kg.
mP- mass of liquid with the same volume of particles.
M- total mass.
R- coordinate in RWV along radius, m.
rp- radius of particle, m.
Ri- radius of inner cylinder, m designed to be 20 mm.
Ro- radius of outer cylinder, m designed to be 50 mm.
t- time, s
Vel- relative speed between particle and fluid.
Vp- volume of particle.

$p1$- density of liquid medium kg/m³.
p_p- density of particle kg/m³.
τ- shear stress.
ω- rotating speed of liquid medium.
ωi- rotating speed of inner cylinder.
ωo- rotating speed of outer cylinder.

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