BIOMEDICAL AND ENVIRONMENTAL SCIENCES **19**, 163-168 (2006)

Numerical Simulation of Microcarrier Motion in a Rotating Wall Vessel Bioreactor[1]

ZHI-HAO JU[*], TIAN-QING LIU[*, 2], XUE-HU MA[*], AND ZHAN-FENG CUI[*,#]

[*]State Key Laboratory of Fine Chemical Engineering, Stem Cells & Tissue Engineering Laboratory, Dalian University of Technology, Dalian 116012, Liaoning, China; [#]Department of Engineering Science, Oxford University, Oxford OX1 3PJ, UK

Objective To analyze the forces of rotational wall vessel (RWV) bioreactor on small tissue pieces or microcarrier particles and to determine the tracks of microcarrier particles in RWV bioreactor. **Methods** The motion of the microcarrier in the rotating wall vessel (RWV) bioreactor with both the inner and outer cylinders rotating was modeled by numerical simulation. **Results** The continuous trajectory of microcarrier particles, including the possible collision with the wall was obtained. An expression between the minimum rotational speed difference of the inner and outer cylinders and the microcarrier particle or aggregate radius could avoid collisions with either wall. 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 model works well in describing the trajectory of a heavier microcarrier particle in rotating wall vessel.

Key words: Tissue engineering; Bioreactor; Rotating wall vessel; Numerical simulation

INTRODUCTION

A bioreactor refers to a system where conditions are closely controlled to permit or induce a certain behavior in living cells or tissues[1]. 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.

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. 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 minimized.

Rotating wall vessel (RWV) bioreactor is a new type of device to incubate cells or tissues, which was developed for cell growth under the condition of microgravity by American National Aeronautics and Space Administration (NASA) in 1992[2]. 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. Several experimental researches[3-4] showed that it is a promising bioreactor for tissue engineering.

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.*[5] simulated the motion of microcarrier particles inside a horizontally rotating bioreactor. Tsao *et al.*[6] developed a mathematical model to characterize cell-medium interaction in a Couette-flow bioreactor. Gao *et al.* [7] analyzed and calculated the movement of a

[1]This work was supported by Bureau of International Cooperation, NSF of China (2002008) and Science and Technology Funds of Liaoning Province (20022140).

[2]Correspondence should be addressed to Prof. Tian-Qing LIU, Tel: 86-411-84706360. E-mail: liutq@dlut.edu.cn

Biographical note of the first author: Zhi-Hao JU, male, born in 1980, Ph. D. candidate, majoring in the field of tissue engineering and neural stem cells.

0895-3988/2006
CN 11-2816/Q

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.*[8] 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[9] 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.*[10] studied the relationship between fluid dynamic conditions and the effect of RWV ractor 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.*[11] 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[12] reviewed the engineering principles which allow optimal suspension culture conditions to be established in the RWV bioreactor.

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 and densities between culture medium and the particle. In addition, we present an expression between the minimum difference of the rotational speeds of the two concentric cylinders and the microcarrier radius. Finally, the shear stress acting on the particle was analyzed.

MODEL DEVELOPMENT

The RWV bioreactor model was designed to provide a Couette flow field. The vessel consists of two concentric cylinders, with the outer cylinder having a radius of Ro and rotating at ωo, while the inner cylinder having a radius of Ri. and rotating at ω 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. 1 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, while the Couette flow can be obtained by rotating the two cylinders at different speed or even in opposite directions. 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.

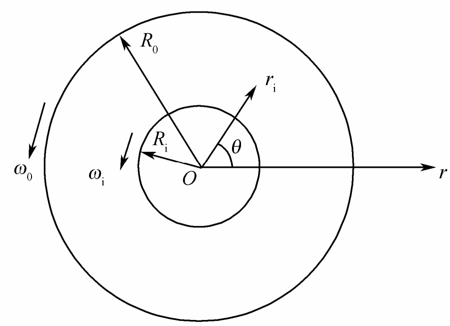

FIG. 1. Schematic drawing of totating wall.

In modeling the microcarrier particle motion, the following assumptions were introduced: 1) The physical properties of both liquid medium and solid particle are constants, since the operational temperature is maintained at 37℃. In this simulation the relative parameters were taken as ρ_l =1000 kg/m^3, 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[6]. 4) The acceleration of the fluid due to the acceleration of the micrcocarrier was approximated by the virtual mass concept[13].

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

In radial direction (r-direction):

$$-(m-mp)r\omega + 2Mr\phi'\omega + Mr\phi'^2 + g(m-mp) \sin(\phi+\omega t) - kr' - Mr'' - F = 0 \quad (1)$$

and in circumferential direction (θ-direction):

$$-g(m-mp)\cos(\phi+\omega t) + 2Mr\phi' + kr\phi' + 2Mr'\phi' + Mr\phi'' = 0 \quad (2)$$

$$\mathrm{M} = \mathrm{Cvm} + \mathrm{mp} = C_v\rho_l V_p + \rho_p V_p \quad (3)$$

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

$$k = \frac{1}{2}\pi * rp^2 * \rho_l * Cd * Vel \quad (4)$$

$$Cd = 18.5\,\mathrm{Re}^{-0.6} \quad (5)$$

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:

$$F = \pm\lambda m\omega^2(\mathrm{rp} + \mathrm{delta} - |\mathrm{R} - \mathrm{r}|) \quad (6)$$

where delta is a specified arbitrary small distance, according to the introduction of Gao[7], 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, R_o or R_i. 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) to (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.

RESULTS AND DISCUSSIONS

Particle Trajectory in Solid Body Rotation

A typical particle trajectory is shown in Fig. 2. 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 once a period, which may cause damage to cells.

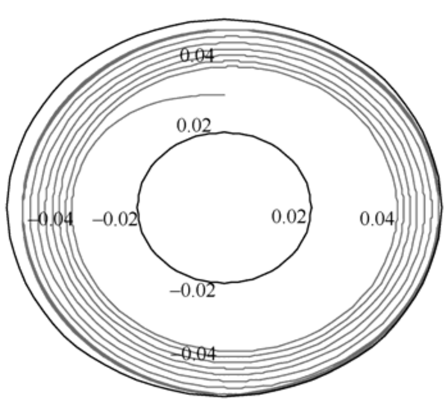

FIG. 2. Trajectory of a heavier microcarrier particle in Rotating Wall Vessel in the inertial frame of reference (rp =400 μm, $\omega_o = \omega_i$=60 rpm).

Our results showed that the microcarrier particle migrated 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. 3a). The value of the rotating speed only affected the migration time of the particle (Fig. 3b), 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. 3c). Otherwise, when the inner vessel rotated faster than the outer vessel, the particle would settle down just near the outer vessel (Fig. 3d).

To validate the model mentioned above, some experiments have been conducted[14]. The track of the microcarrier in RWV bioreactor was recorded with a video recorder. Then the data were analyzed and the final movement of microcarrier recurred.

The experiment data are shown in Fig. 4(b), while Fig. 4 (a) is the model simulation with the same parameters. The comparison showed that they were consistent to a great degree. 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. Our model works perfectly on simulating the movement of the microcarrier in RWV bioreactor. 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.

Optimization of Rotating Speeds for Different Microcarrier Sizes

There are two alternatives to be chosen in optimization: the outer cylinder rotates at different speed in the same direction and the inner clinder 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 analysis we could see that to avoid the collision between the microcarrier cylinder wall, the inner and outer cylinders should rotate in opposite direction and the rotating speeds of them should be matched properly.

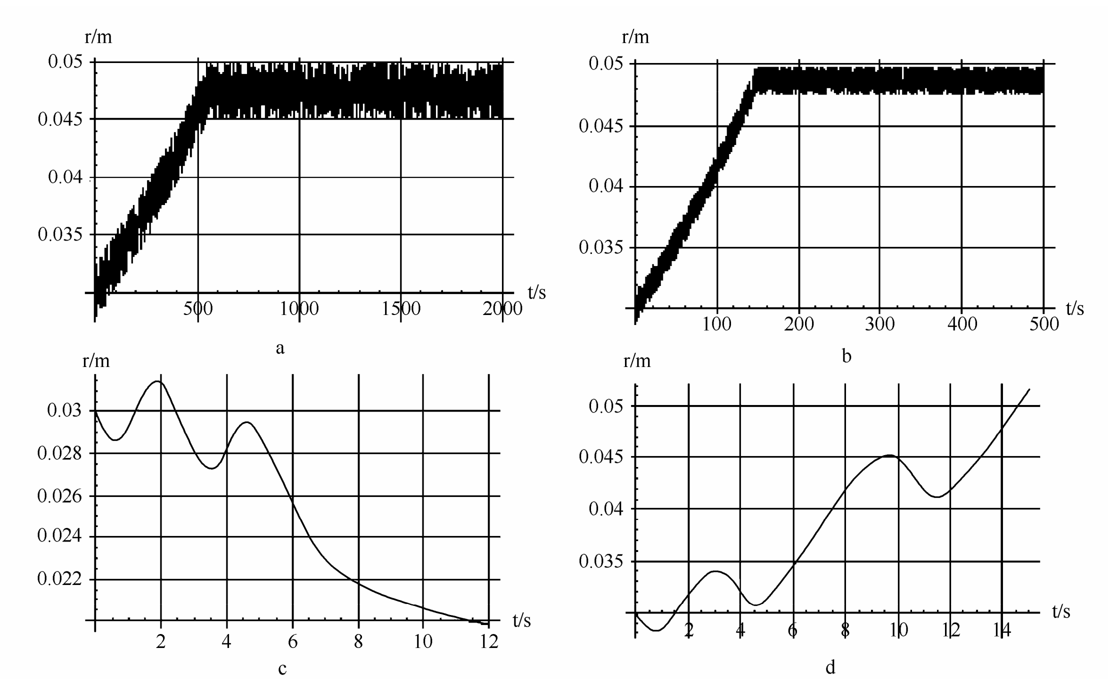

FIG. 3. Radial position of the particle *vs* the time (rp=100 μm). a: $\omega_o=\omega_i$=15rpm; b: $\omega_o=\omega_i$=30rpm; c: ω_o=30rpm, ω_i=10rpm; d: ω_o=10rpm, ω_i=30rpm.

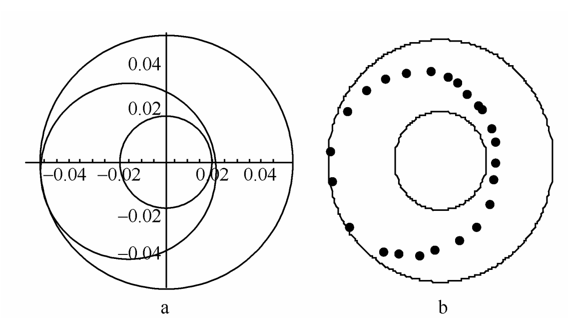

FIG. 4. Comparison of the trajectory of a microcarrier particle by model simulation results (a) and experiment data (b). (ρ=1308 kg/m^3, rp=730 μm, $\omega_o=\omega_i$= -40rpm).

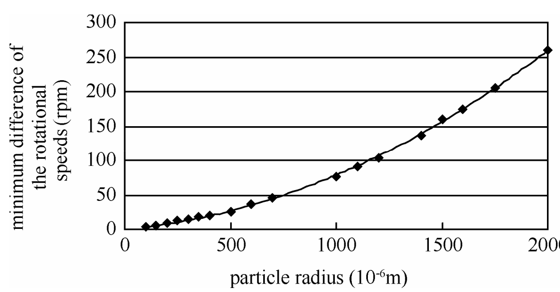

FIG. 5. The minimum difference of the rotational speeds of the outer and inner cylinders for different particles sizes.

In order to optimize the operating condition of the bioreactor, we changed the rotation speeds and the directions 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 results indicated that there existed a minimum difference in the rotational speeds (defined as $y=\omega_0-\omega_i$, here ω_i is negative since the inner cylinder vessel rotates at the opposite direction to the outer vessel) for a certain size of a microcarrier to avoid colliding with the cylinder walls. The results are shown in Fig. 5. From the figure we can see that the larger the particle was, the greater the difference of the rotational speeds was needed to avoid colliding. The relation between them is expressed as:

$$y= 0.00005(rp)^2 + 0.0274rp, \text{ with } R^2 = 0.9994, \quad (7)$$

where the unit of rp is μm. The rotating speed for each cylinder should be higher than y/2; otherwise, even the difference is larger than y, the particle would still settle down near the wall. Under these optimized conditions the particle could easily suspend in the RWV without colliding with the vessel walls. This could be easily verified as is shown in Fig. 6. Our simulation also showed that the above minimum difference in the rotational speeds of the outer and inner cylinders was independent of the radius of the cylinders, which was determined only by the size of the particle and the density difference between the particle and the medium.

Moreover, since the fluid flow between the cylinders is laminar, the rotational speed cannot be too high to avoid the turbulence. If the inner cylinder rotated at a speed less than 60rpm, the fluid flow was laminar (calculated from the formula in the literature[15] with the radius of our RWV). Thus, the upper-limited radius of particles to be safely cultured in RWV is about 1300 μm.

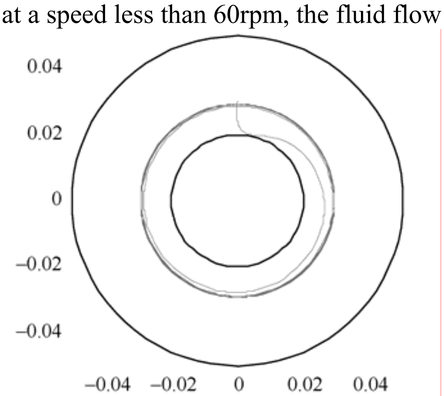

FIG. 6. Trajectory of a heavier microcarrier particle in rotating wall vessel in the inertial frame of reference (rp=300 μm, ω_o=7 and ω_i= -7rpm).

During the culture process the microcarriers may conglutinate, resulting in the increase of apparent radius. Then the match of the rotating speeds should be changed according to Fig. 3 to maintain the particle suspended in the RWV.

Shear Stress Acting on Microcarriers

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. Liquid shear stress acting on a microcarrier or incubated tissue in RWV is shown in Fig. 7, which clearly indicates that the shear stress acting on the cultivated tissue is linear with the particle size in RWV. For the mammalian cells, the mechanical shear stress should be lower than the value in the range of 0.3-1 N/m^2; otherwise severe

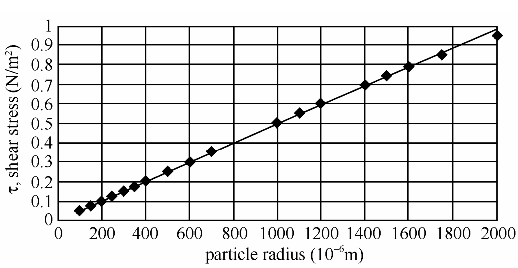

FIG. 7. Curve of shear stress *vs* particle radius τ = 0.0005rp, R^2=0.9982.

v damage to cells will occur and cell viability will be reduced[9]. There is a limit to the particle or tissue in size under which the particle can be cultured safely in the RWV.

From Fig. 7 we can see 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^2, 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. Gary *et al.*[16] 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.

An interesting phenomenon of a specified microcarrier particle in the RWV bioreactor is that the shear stress acting on the particle is independent of the rotating speeds, as is shown in Fig. 8, which is the same as that of solid rotation operations[7]. In fact, the shear stress inside the RWV bioreactors is dependent on both the size of cultured particles and the density difference between the particle and the medium.

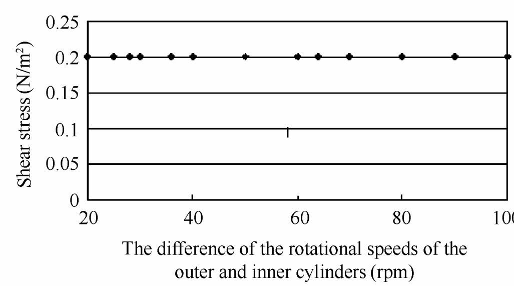

FIG. 8. The curve of shear vs the difference of the rotational speeds (radius of the particle is 400 μm).

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. The model works well in describing the trajectory of a heavier microcarrier particle in rotating wall vessel. Shear stress acting on a microcarrier is another 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

C_v	Virtual mass coefficient
F	Short range contact force near the wall
G	Acceleration of gravity, 9.81 $m \cdot s^{-1}$
K	Non-Stokes drag coefficient
M	Mass of particle, kg
mp	Mass of liquid with the same volume of particle
M	Total mass
R	Coordinate in RWV along radius, m
rp	Radius of particle, m
R_i	Radius of inner cylinder, m, designed to be 20 mm
R_o	Radius of outer cylinder, m, designed to be 50 mm
t	Time, s
Vel	Relative speed between particle and fluid
V_p	Volume of particle, m^3
y	Minimum difference of rotational speeds of outer and inner cylinder
ρ_l	Density of liquid medium, kg/m^3
ρ_p	Density of particle, kg/m^3
τ	Shear stress
ω	Rotating speed of liquid medium, rpm
ω_i	Rotating speed of inner cylinder, rpm
ω_o	Rotating speed of outer cylinder, rpm

REFERENCES

1. Godbey W T, Atala A (2002). *In vitro* systems for tissue engineering. *Ann N Y Acad Sci* **961**, 10-26.
2. http://microgravity.msfc.nasa.gov
3. Granet C, Laroche N, Vico L, *et al.* (1998). Rotating-wall vessels, promising bioreactors for osteoblastic cell culture: comparison with other 3D conditions, *Mecical & Biologltical Engineering & Computing* **36**(4), 513-519.
4. Mitteregger R, Vogt G, Rossmanith E, *et al.* (1999). Rotary cell culture system (RCCS): A new method for cultivating hepatocytes on microcarriers. *International Artificial Organs* **22**(12), 816-822.
5. Santos B D, Shiragami N, Unno H, *et al.* (1994). A model for microcarrier motion inside a horizontally rotating bioreactor for animal cell culture. *Bioprocess Engineering* **11**(3), 115-120
6. Tsao Y M D, Boyd E, Spaulding G (1994). Fluid dynamics within a rotating bioreactor in space and earth environments. *Spacecraft and Rockets* **31**(6), 937-943
7. Gao H X (2000). Numerical Studies of Microcarrier Particle Dynamics and Associated Mass Transfer in Rotating-Wall Vessels, PhD Dissertation, University of Pennsylvania, 2000.
8. Qiu Q Q, Ducheyne P, Ayyaswamy P S (1999). Fabrication, characterization and evaluation of bioceramic hollow microspheres used as microcarriers for 3-D bone tissue formation in rotating bioreactors. *Biomaterials* **20**, 989-1001.
9. Begley C M, Kleis S J (2000). The fluid dynamic and shear environment in the NASA/JSC rotating-wall perfused-vessel bioreactor. *Biotech and Bioengineering* **70**(1), 32-40.
10. Freed L E, Vunjak-Novakovic G (1995). Cultivation of cell-polymer tissue constructs in simulated microgravity. *Biotech and Bioengineering* **46**, 306-313.
11. Pollack S R, Meaney D F, Levine E M, *et al.* (2000). Numerical Model and Experimental Validation of Microcarrier Motion in a Rotating Bioreator. *Tissue Engineering* **6**(5), 519-530.
12. Milne-Thomson L M (1996). Theoretical hydrodynamics, New York: Dover Publications.
13. Hammond T G, Hammond J M (2001). Optimized suspension culture: the rotating-wall vessel. *American J Physiology-renal Physiology* **281**(1), F12-F25.
14. Xue L Y (2002). The measurement trajectory of microcarrier particles in rotating wall vessel bioreactor, Bachelor Thesis, Dalian University of Technology.
15. Bird R B, Stewart W E, Lightfoot E N (2002). *Transport Phenomena*. 2nd ed., New York, John Wiley & Sons, Inc.
16. Sanford G L, Ellerson D, Melhado-Gardner C, *et al.* (2002). Three-dimensional growth of endothelial cells in the microgravity based rotating wall vessel bioreactor. *In Vitro Cellular & Developmental Biology-Animal.* **38**(9), 493-504.

(*Received November* 12, 2004 *Accepted December* 25, 2005)