Simulating Fluid Flow through a Culture Chip for Cell Migration Studies in Microgravity

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Abstract: Exposure to microgravity is known to cause genomic and proteomic alterations and modulate immune cell activity. This may influence cancer development. SUNY Polytechnic Institute and SpacePharma, Inc. have teamed up to develop a system to study migration of metastatic cancer cells in microgravity. Initial development will include lab-scale functional tests and progress to using the system in an on-ground simulator and Low Earth Orbit (LEO). Modeling fluid flow through the system using the Computational Fluid Dynamics (CFD) module of COMSOL Multiphysics® will assist with design considerations. Simulations conducted include a single phase flow (spf) study under laminar conditions for cell growth media and a transport of diluted species (tds) study for a chemoattractant (EGF). Results are utilized for choosing operational flow parameters and testing the efficiency of a chemical gradient for cell migration.

Keywords: Single Phase Flow, Transport of Diluted Species, Migration, Culture Chip, COMSOL Multiphysics®.

1. Introduction

Microgravity provides an opportunity to study systems without effects such as sedimentation, hydrostatic pressure and non-diffusive types of mass transfer [1, 2]. Examining biological processes under such conditions has potential applications in fields such as tissue engineering, drug testing, vaccine development, stem cell propagation and protein crystallization [3, 4]. Exposure to microgravity is known to cause genomic and proteomic alterations in *C. elegans* [5, 6], transgenic OT II mice [7] and human cancer lines, DLD-1 and MOLT-4 [8]. It can also suppress immune cell activity that may influence cancer development [9].

The Colleges of Nanoscale Science and Engineering (CNSE), SUNY Polytechnic Institute and SpacePharma, Inc. have teamed up to develop a system to study the migration of metastatic cancer cells by isolating gravity as an experimental variable and assessing its contribution to earth-based cellular function (Figure 1). Lab-scale functional tests will be followed by use of on-ground simulators with the ultimate aim being to build a system ready for space-flight to Low Earth Orbit (LEO).

Human cancer cells will be cultured in the cell culture chambers and maintained under microgravity conditions. During cell maintenance growth media will be supplied through media inlets. Cells will proliferate and double their cell number every 24-36 hours depending on the cell type. Cells will be lifted using trypsin and flushed out to avoid overcrowding.

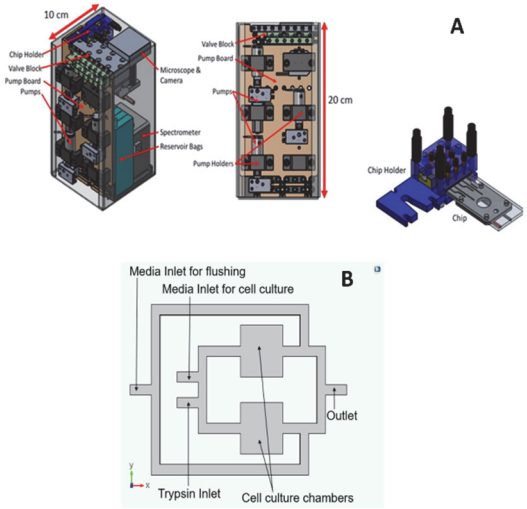

Figure 1. A) Illustration of the Migration Lab. Source: SpacePharma, Inc. B) An initial design of the culturing system with inlets for cell growth media and trypsin (to lift cells).

Cells migrate in response to chemoattractants. To conduct cell migration studies in microgravity, we will also need inlets for chemoattractants and models of how they will diffuse through the system. Deciding on operating flow rate, usage of

valves or nozzle-diffuser systems for these processes will be challenging. The Computational Fluid Dynamics (CFD) module of COMSOL Multiphysics will be utilized to simulate these processes and address such challenges. Here we present preliminary simulations on the flow of cell growth media and diffusion of chemoattractant in the system.

2. Governing Equations

For incompressible, single phase flow under laminar conditions, Navier-Stokes equations reduce to the following:

$$\boldsymbol{\rho}(u \cdot \boldsymbol{\nabla})u = \boldsymbol{\nabla} \cdot [-\boldsymbol{\rho}I + \mu(\boldsymbol{\nabla u} + (\boldsymbol{\nabla u})\mathbf{T}] + \mathbf{F}$$

and $\boldsymbol{\rho}\boldsymbol{\nabla} \cdot (u) = 0$

with $u = -U_0 n$ at the inlet

and $[-\rho I + \mu(\nabla u + (\nabla u)^T]n = -p_0 n$ at the outlet.

For transport of diluted species, Fick's law and conservation laws can be used to derive the following equations.

$$\partial c_i/\partial t + \nabla \cdot (-D_i \nabla c_i) = R_i$$

and $N_i = -D_i \nabla c_i$

The nomenclature section lists the symbols used in these equations.

3. Methods

Two types of studies were conducted - A single phase flow study under laminar conditions for the flow of cell growth media and a transport of diluted species study for the diffusion of chemoattractant.

3.1 Single Phase Flow (spf)

Spf studies under laminar conditions were run for cell growth media - Dulbecco's Modified Eagle Medium (DMEM) to simulate the following scenarios.

First, a simulation of flushing the outer channels (Figure 3) of the initial culturing system with media was run to test for backflow into the cell culture reservoir. Next, a simulation to test the effect of changing the shape of the cell culture chamber and the nature of flow into it was run (Figure 4). The square chambers, seen in Figure 1 and 3, produce dead zones with no flow. To avoid such zones, devices containing circular chambers, pinched inlet chambers (to promote circular backflow and remove dead zones) and elongated chambers were designed and tested. Lastly, a simulation of media flowing through a newer version of the culture chip (Figures 5, 6 and 7) with three chambers was run to identify an optimal flow rate range under operating conditions.

Viscosity and density of DMEM at 25 ºC are 0.89 mPa.s and 997.04 kg/m^3 respectively. These properties are almost identical to those for water.

3.2 Transport of Diluted Species (tds)

A tds study (Figure 8) was conducted to test the timescale of diffusion of chemoattractant (EGF) along the gradient generated. The diffusion coefficient for EGF was taken as 5.18 X10^{-11} m^2/s.

3.3 Culture Chamber Design

Chips for carrying out cell migration experiments were made by sealing PDMS (polydimethylsiloxane) housings to glass. Figure 2 illustrates the culture chambers constructed for both single chamber and triple chamber chips. Here V_{IC} and V_{OC} are defined as the input velocity for the inner and outer chambers respectively.

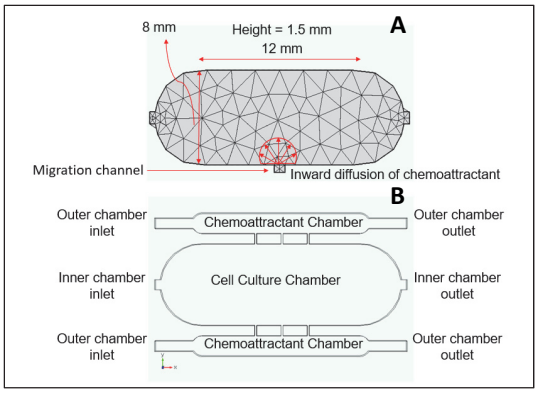

Figure 2. A) COMSOL drawing of a 250 µL culture chamber with a migration channel (0.6 X 1 X 1.5 mm) showing inward diffusion of chemoattractant (EGF). Inlet and outlet dimensions are 1 X 1 X 1.5 mm. B) A new chip with 3 chambers and longer migration channels (1 X 0.2 X 1.5 mm). Chemoattractant chambers are 4 times narrower than the cell culture chamber.

4. Results and Discussions

Figure 3 shows a top view of simulating DMEM flushing through the outer channels of the unit and points to the need to avoid backflow into the culture chambers. Backflow can be avoided by using valves or nozzle-diffuser flow.

Figure 4 shows the results for simulations with preliminary designs for the cell culture chamber. Uniform flow through a square chamber results in dead zones, which must be avoided to maintain healthy cells. Uniform flow through the circular (Figure 4B) chamber may be obstructed by pinching the flow at the inlet (Figure 4C). This helps promote circular backflow and prevents the formation of dead zones. Newer designs have an elongated middle with rounded edges (Figure 4D).

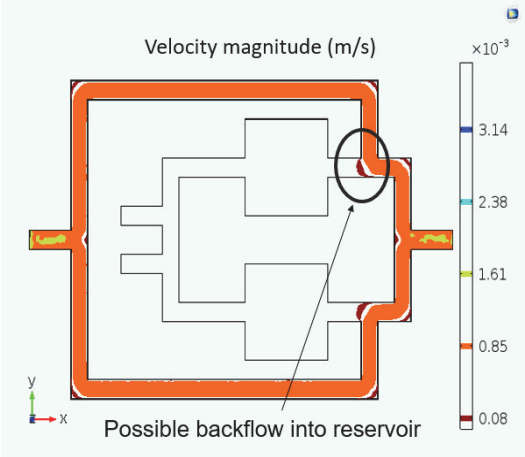

Figure 3. Simulation of flushing media through the outer channels of the culture unit. An iso-surface velocity plot is used to show the path taken by the media. Note that there is a chance of backflow into the cell culture chambers. Velocity is in m/s.

The optimal flow rate range for a three reservoir chip is demonstrated in Figure 5. Here the ratio of V_{IC} to V_{OC} is varied and the effect on flow is noted. When V_{IC} is less than or equal to V_{OC}, there is no chance of media leaking into the outer chambers. (Figure 5A and 5B). As V_{IC} increases further, media in the inner chamber starts to spread outwards (Figure 5C) and finally beyond a threshold V_{IC}, media begins leaking into the outer chambers through the third channel (Figure 5D).

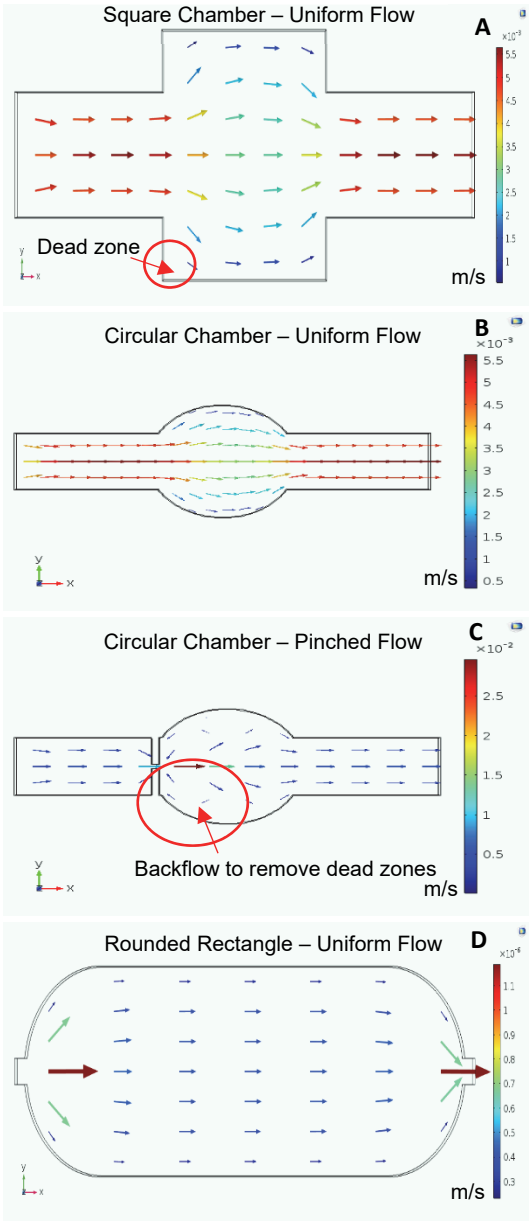

Figure 4. A) Simulation of fluid flow through a circular culture chamber to promote uniform flow and prevent 'dead zones' within the chamber. B) The same simulation with pinched flow at the inlet to aid with circular flow and removing 'dead zones' if present. C) Simulation of uniform flow through the latest version of the culture chamber at a flow rate of 180 µL/min. Note the elongated middle and rounded edges. All arrows represent velocities in m/s.

Figure 6 shows a plot between the ratio of V_{IC} to V_{OC} and the degree to which media from the inner chamber leaks into the outer chamber. The region to the right of the threshold ratio represents

the leaky region. Figure 7 demonstrates a special case where $V_{IC} = - V_{OC}$. Here media leaks through all the channels.

The diffusion of chemoattractant (EGF) with an initial concentration of 0.04 mM through a migration channel of length 0.6 mm is shown in Figure 8. This confirms that a gradient (similar to studies with chemotaxis devices [10, 11]) can be established at timescales that are practical for cell migration experiments.

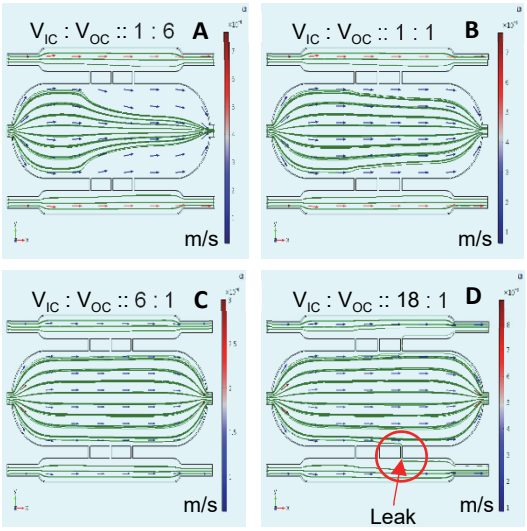

Figure 5. Simulation of cell growth media flowing through the 3 reservoir chip. Ratio of flow rates in the inner chamber (V_{IC}) to the outer chambers (V_{OC}) is increased from A) through D). A) Ratio of 1:6 leading to a narrowing stream in the inner chamber. B) Ratio of 1:1 broadens the stream C) Ratio of 6:1 broadens the stream further D) Ratio of 18:1 marks the threshold at which the stream is broad enough to leak through the third channel. All arrows represent velocities in m/s.

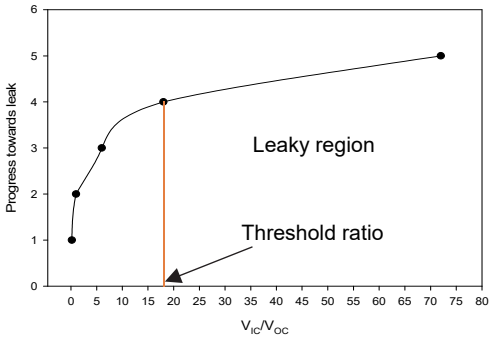

Figure 6. A plot of the progress towards a leak in the chip vs the ratio of input velocity for the inner chamber (V_{IC}) to input velocity for the outer chambers (V_{OC}). The chance of media leaking into the outer chambers keeps increasing with an increase in V_{IC}/V_{OC}.

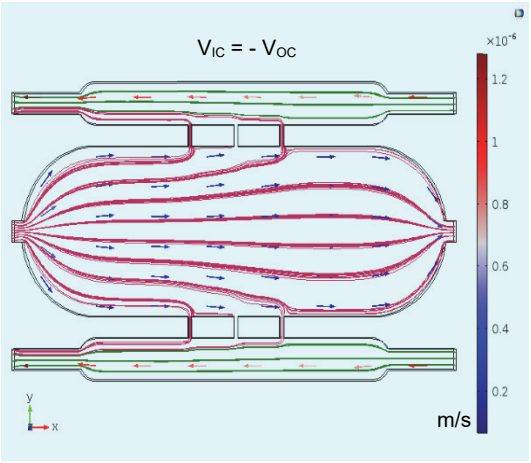

Figure 7. Simulation of cell growth media flowing through the 3 reservoir chip with equal and anti-parallel input velocities. In this case, media leaks through all 3 channels. All arrows represent velocities in m/s.

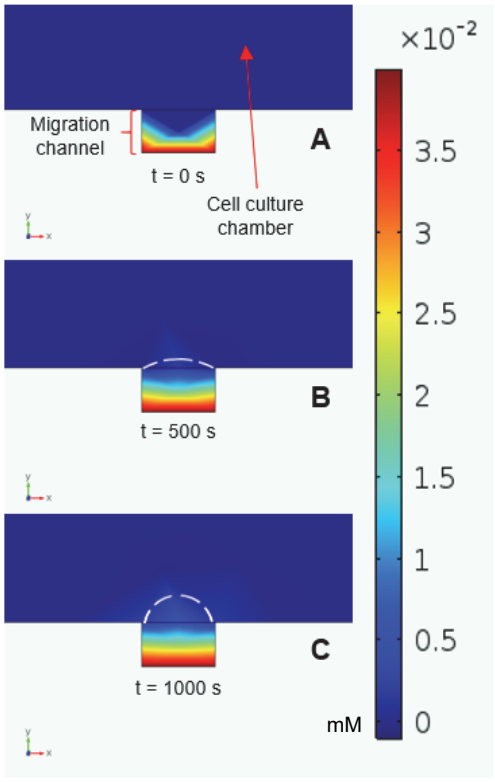

Figure 8. Surface concentration plots demonstrating diffusion of 0.04 mM EGF across a 0.6 mm migration channel. A), B) and C) show diffusion at times 0, 500 and 1000 s respectively. The white dashed curve represents a moving front of diffusion. Concentrations are in mM.

5. Conclusions

Migration studies on cancer cells in true microgravity can lead to the discovery of novel therapeutic targets for their metastatic behavior. Designing a robust system to study such migration is an essential starting point. Spf studies helped in gauging an optimal operating flow rate range and improved the shape of cell culture chambers to avoid dead zones. Issues such as backflow and leakage between chambers were resolved. Tds studies for EGF point to the timescale over which diffusion of chemoattractant take place and can provide an estimate of the migration rate. Further simulations using COMSOL Multiphysics will investigate the interactions between cell growth media and chemoattractant.

6. Acknowledgments

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7. Nomenclature

V_{IC}	Velocity through the inner chamber
V_{OC}	Velocity through the outer chambers
ρ	Fluid density
u	Flow velocity
μ	Dynamic viscosity
p	Pressure
F	External Force
N	Molar flux
c	Concentration of species
D	Diffusion coefficient
R	Production or consumption

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