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Microfluidic Systems for Cell Growth and Cell Migration Studies

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Abstract: Lab-on-a-chip devices for cell culturing are becoming imperative in order to satisfy the needs for point-of-care or decentralized laboratory testing. Towards that goal we have developed miniaturized membrane based cell culture chambers for use within the field of chromosome analysis. The same principle was applied for the development of a miniaturized cell migration device. Flow and concentration gradient simulations have been conducted in order to evaluate the performance of the devices before their fabrication but also in order to confirm and explain the experimental results. This paper will present the idea behind membrane based culturing and the resulting designs, as well as the theoretical and experimental results.

Keywords: bioreactor, cell migration, diffusion, porous flow

1. Introduction

Lab-on-a-chip systems are becoming quite widespread for studying biological processes, such as cell growth or cell migration. Compared to their macroscale counterparts these systems have a great potential in presenting the cells with an environment very similar to that encountered inside the body in terms of surfaces, sizes and function, as surface functionalization and fabrication techniques are becoming better.

Cell bioreactors or cell growth chambers pose an interesting problem. Cells will grow on a flat surface, as is the practice in every biological lab today. However, that is far from their natural environment and important information and even cell functionality may be lost in vitro. We have fabricated and tested novel systems for cell culture of adherent and non-adherent cells [1], as well as systems for studies of cell migration [2] based on diffusion through membranes. By separating the flow of nutrients from the physical location of the cells and by only allowing nutrients to diffuse to the cells through the membrane, we don't disturb the intercellular communication by constantly removing the potential communication proteins excreted by the cells during the culturing, as is the case with traditional culture flasks with severe changes in the cells morphology, metabolism and growth rate [3]. At the same time the chamber can be used for non-adherent cell culturing, as opposed to other miniaturized systems that can only be used for adherent cells. In this paper we will concentrate on simulations of the filling and clearing of the cell chambers on two designs. Moreover, results of cell culturing and comparisons between experimental and theoretical results will be presented.

The idea of two flows separated by a membrane can also be used for the study of cell migration through microsized pores. The migration of cells inside the body is a process governing for example wound healing, cancer metastasis and embryonic development amongst others. Therefore, it is important to study how it takes place in vitro. In this paper we will present a device made for the study of cell migration and show simulations of the concentration gradient as well as experimental results of the migration following the achieved gradient.

2. Governing equations

As the designed systems are complex and exhibit little symmetry the simulations were conducted on a full scale 2D or even 3D model by use of COMSOL 3.5. We used the Incompressible Navier Stokes mode coupled with the Convection and Diffusion mode. The flow was solved in steady state and the solution stored and used for solving for the concentration with the time-dependent solver. Parameters for inlet velocity were taken from the experiments. The membrane was in all cases treated as a separate subdomain, where the flow was damped with a factor based on Darcy's law for porous flow, as we have previously utilized [4]. Moreover, the diffusion coefficient for the various molecules tested was corrected with the membrane porosity.

As the membrane used was the same for all systems, the parameters used for it will be described here to avoid redundancy. For the subdomain representing the membrane a term

of a volume force on the liquid given by $\vec{F} = -\alpha \cdot \vec{u}$ has been added, where $\vec{u}$ is the velocity of the fluid and α is a constant that can be roughly calculated using the Darcy number. This is given by $Da = \frac{\eta}{\alpha \cdot L^2}$, where η is the viscosity of the fluid and L is the characteristic length scale of the system. By assuming a value for the Darcy number equal to 10^{-4} and the typical length scale of our system to be 10 µm (i.e. the membrane thickness), we can calculate that $\alpha = 10^{11}$ kg/(m^{3}s) assuming that the viscosity of the liquid is equal to that of water, i.e. 0.001 Pa·s. This approximation is not far from the truth as most nutrient solutions are water based. This damping factor effectively separates the flows on the two sides of the membrane.

It should be noted that for the migration chamber a separate simulation was carried out, where the membrane was part of the geometry instead of a separate subdomain. This simulation was done in order to evaluate the validity of the porous flow approximation. Due to the complexity of the geometry, the simulations for the migration chamber were done in 2D.

3. Description of the various systems

In this section the various designs are going to be presented together with the detailed simulation parameters.

3.1 Circular cell culture chamber

In this first attempt the cell culture chamber was designed as a circular chamber with a depth of 200 µm and a diameter of 5 mm. The chamber was fed by a channel 2 mm wide and 200 µm deep. Figure 1 shows the three constituents of the cell culture chamber. The cells are inserted into the top chamber by a pipette while the bottom microfluidic channel feeds the system with nutrients. The membrane was 10 µm thick with pore size of 5 µm and a porosity of 0.14. The inlet volumetric velocity was 9.8 x 10^{-9} m^3/min, which is 9.8 µl/min. The simulation was done in 3D, though the channels feeding the cell chamber were only modeled to be 2 mm long for the sake of computational power.

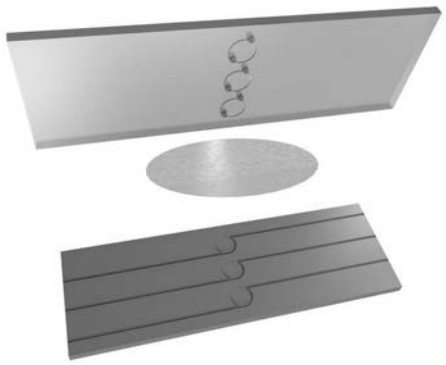

Figure 1. The three constituents of the circular cell culture chamber. The three parts are thermally bonded together. During perfusion the pipette holes on the top part are closed with PCR tape.

The device was simulated using three subdomains. The bottom subdomain was the nutrient flow. The inlet was set at the experimentally used volumetric flow mentioned earlier, while the outlet was set at a zero pressure condition. All other boundaries apart from the internal boundaries were set at no slip conditions. It was assumed that the device was initially filled completely with the nutrient solution and that at t = 0 a solution of streptavidin at a concentration of 1 M began to flow through the inlet. The diffusion coefficient of streptavidin was set to 130 µm^2/s based on the literature. After 1500 sec the concentration of streptavidin at the inlet was set back to 0 and the simulation was continued until t = 3000 s. For the concentration simulation the outlet boundary was set to convective flux. All other boundaries were set at the insulation/symmetry boundary condition.

3.2 Rectangular cell culture chamber

The cell culture chamber in this second design is designed to be rectangular, 250 µm in depth and fed by a meandering channel that is 500 µm wide and 300 µm deep. A schematic of the system is shown in figure 2 and is comprised of an inlet and outlet for the meandering channel as well as an inlet and outlet for the cell culture chamber. These are closed during cell feeding via the meandering channel.

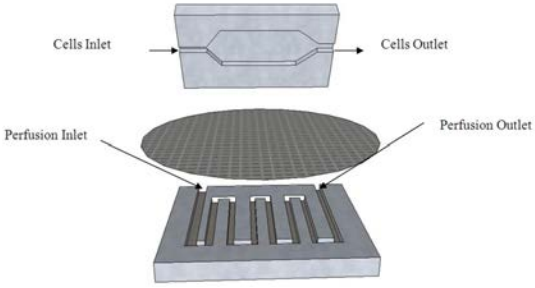

Figure 2. Schematic of the rectangular cell culture chamber with indications of the various inlets and outlets. The three pieces are bonded thermally together.

For this chamber we simulated the speed at which we can exchange the liquid in the upper rectangular chamber when perfusing with another liquid from the perfusion inlet. The perfusion inlet flow rate was set to 150 µl/h with the outlet at zero pressure. The cells inlet and outlet were closed and were therefore treated as walls, as was the rest of the boundaries. For the convection and diffusion part the perfusion inlet was set to a normalized concentration of 1 M while the outlet was set to convective flux. Simulations were conducted from t = 0 up to 2000 s. We considered a solution of KCl entering from the perfusion inlet, with a diffusion coefficient of 6.7×10^{-10} m^2/s.

3.3 Migration chip

The migration chip needs to meet two requirements for use in cell migration studies. It should maintain a constant concentration gradient and introduce shear flow action on migrating cells. At the same time the device should be as close as possible to the natural cell environment. To that end we have designed a device with two channels, one on each side of the membrane, each with its own inlet and outlet. The bottom channel contains the solution of the so called chemoattractant, a chemical that the cells migrate towards. The top channel contains the cell solution. A schematic of the device is shown in figure 3. Both channels are 500 µm wide, 100 µm deep and 2 cm long at their meeting point.

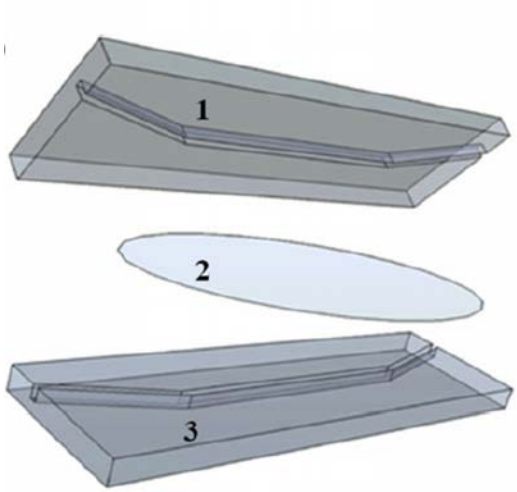

Figure 3. Schematic of the migration chip before thermal bonding.1 – Cell suspension channel, 2 – Membrane, 3 – Chemoattractant channel

The inlets of the two channels were set at a velocity of 1.67×10^{-3} m/s and we have simulated the achieved concentration gradient on the top channel of the chemoattractant, with a diffusion coefficient of 1.76×10^{-10} m^2/s.

The simulation was conducted in 2D for this system. For that reason this system was used also to evaluate the use of the Darcy law for flow through the membrane by making the same simulation but this time by physically designing a 10 µm thick membrane with a large number of 5 µm holes to achieve a porosity of 0.14 over the 2 cm channel.

For the diffusion problem the chemoattractant channel concentration was set to 1 M while the solution concentration to 0. Both channel outlets were set to convective flux. All other boundaries were considered to be walls. The simulation was run from 0 to 25 sec.

4. Results

In this section the simulation results will be shown for all three systems.

4.1 Circular cell culture chamber

The concentration inside the circular chamber is plotted for three different times: at t=250 s, i.e. briefly after the filling of the channels and chamber with streptavidin starts, at t = 1500 s, i.e. at the starting point for the emptying of the channels from streptavidin and at the end of the experiment at t = 3000 s. The result can be seen in figure 4.

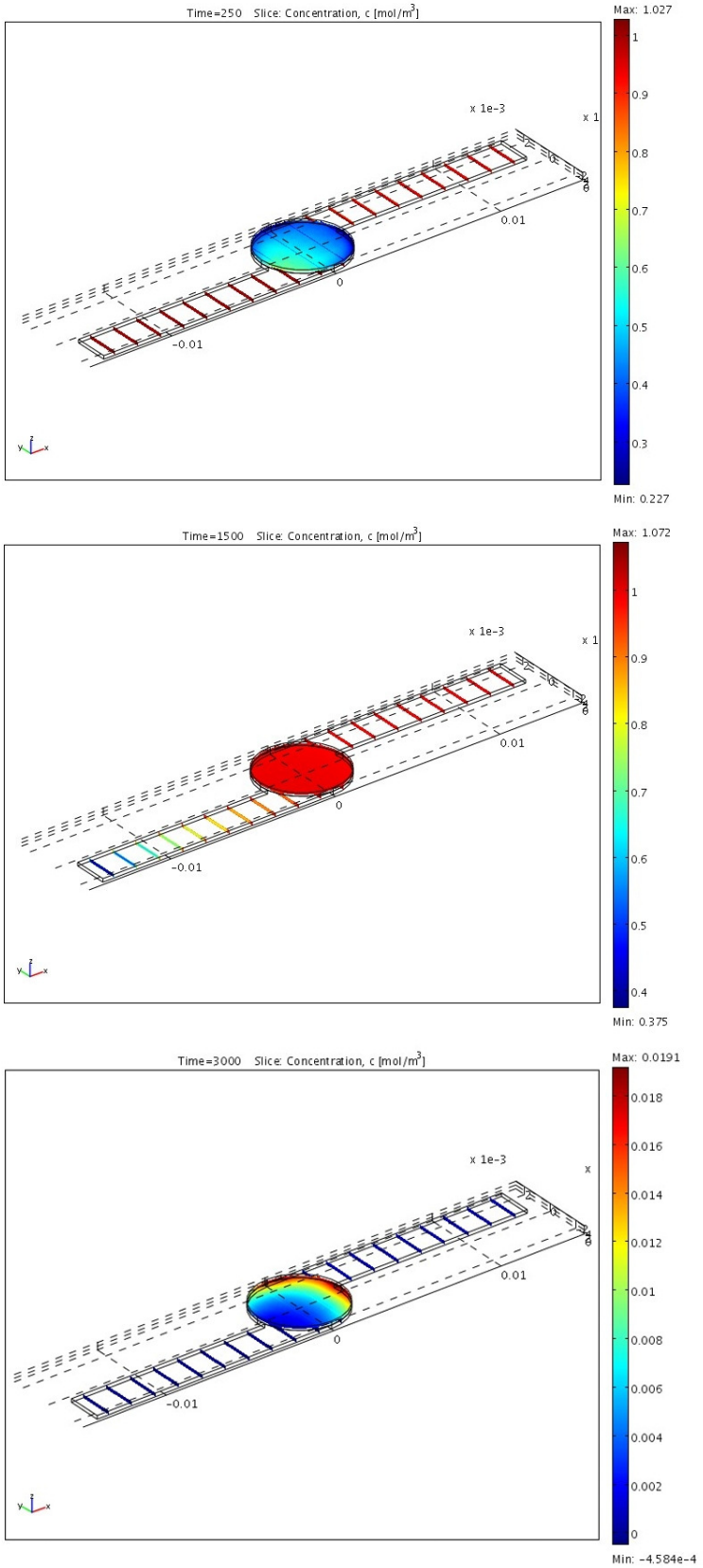

Figure 4. The concentration of streptavidin inside the chamber for three different times. Note the different scales in the colormap.

A plot of the concentration of streptavidin inside the circular chamber close to the outlet as a function of time can be seen in figure 5. The concentration is taken close to the top of the channel by integrating the simulated concentration values in a small volume of the chamber.

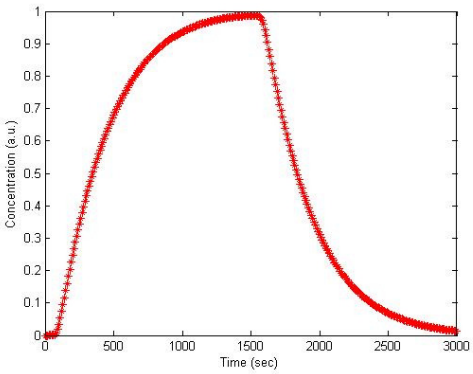

Figure 5. The concentration of streptavidin at the top corner of the chamber as a function of time.

4.2 Rectangular cell culture chamber

The filling of KCl inside this second system is shown in figure 6. The figure shows the filling at t = 1500 s after the start of the perfusion, which corresponds to the experimental time used for exchanging the fluid.

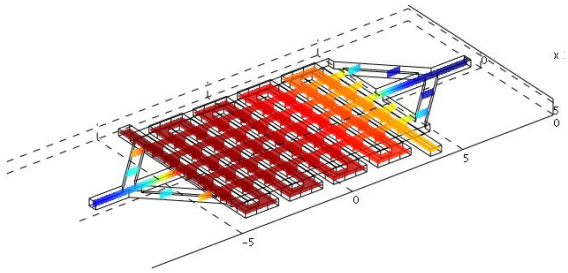

Figure 6. The concentration of KCl inside the rectangular chamber t = 25 min after the start of the experiment plotted for various planes. Blue indicates a concentration of 0 and red a concentration of 1.

4.3 Migration chip

A plot of the concentration profile in the cells channel 5 µm above the membrane is shown in figure 7. In 7(a) the results from the simulation using the Darcy law are shown, while in 7(b) the results from the simulation utilizing the geometrical representation of the membrane are shown.

As can be seen from the figure, ´the concentration of the chemoattractant in the upper channel increases with time and length from the inlet, reaching a steady state after about 20 sec. The maximum concentration achieved is about 0.3 of the bottom channel concentration.

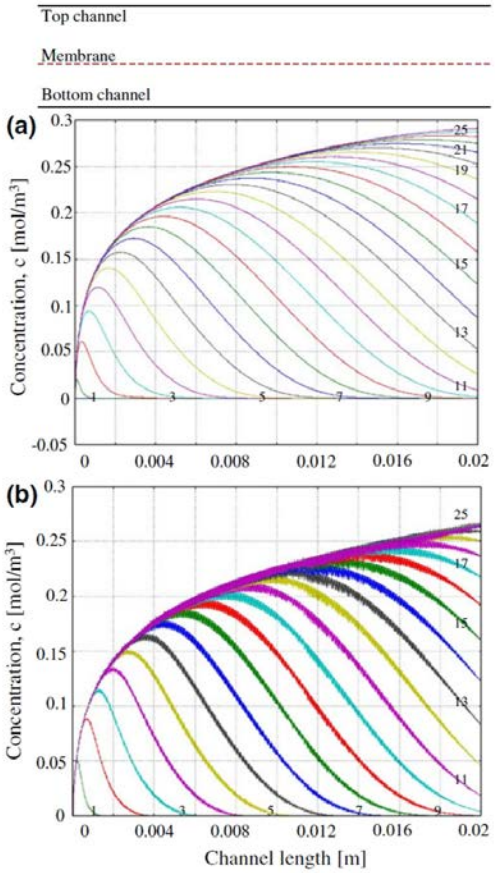

Figure 7. The concentration of chemoattractant 5 µm above the membrane as a function of distance from inlet and time. (a) Simulation of the membrane as a subdomain utilizing Darcy's law. (b) Geometrical representation of the membrane.

The only observable difference between figures 7(a) and 7(b) is the slightly oscillating nature of the concentration in 7(b), with local maxima right above the pore openings. There is also a slight discrepancy in the maximum steady state concentration.

5. Discussion

We have presented simulations from three different membrane based systems, two for cell culturing and one for cell migration studies. The simulations have been used to design and mostly validate the experimental results.

In the case of the circular cell culture chamber it was observed that the concentration of streptavidin reached the maximum value after about 20-25 min, taking at least 25 minutes to return to zero upon switching to clearing mode. Experimentally this was evaluated by attaching a fluorescence marker on streptavidin and measuring the fluorescence intensity while focusing at the top of the chamber close to the outlet. The results show that the clearing of streptavidin from the channel takes roughly 25 min, as predicted from theory, though due to background fluorescence it is hard to establish the exact clearing time.

In the case of the rectangular cell culture chamber the simulations show that after 25 minutes the liquid in the chamber is mostly exchanged with that coming from the perfusion inlet. However, the parts of the chamber closest to the cell outlet have only reached about 80% of the inlet concentration. Experiments were conducted using 25 min as the allowed time for fluid switching and as cell culturing was successful and even better than cell culturing with traditional methods, we can conclude that 25 minutes is indeed enough time for the purpose. The discrepancy can be attributed to some erroneous simulation parameters such as the fluid viscosity (which is assumed to be that of water) and the diffusion coefficient of the KCl, as well as the fabrication accuracy of the device.

Finally, the cell migration chip simulations show that the design is indeed capable of providing a stable concentration gradient in the cell channel, with a maximum concentration of about 0.25-0.3 of the chemoattractant concentration. We have observed experimentally that the cells migrate towards the middle and end of the 2 cm channel, which is also confirmed by the simulations that show that the chemoattractant concentration is increasing with distance from the inlet, as is also intuitively expected. Moreover, the simulations of the geometrical representation of the membrane show no significant differences from those utilizing the Darcy law for porous flow, which validates its use in such systems and greatly simplifies the modeling and simulations.

6. Conclusions

In conclusion we have shown how simple COMSOL Multiphysics simulations can contribute to the understanding of experimental results as well as to the planning of these experiments. The discrepancies observed between experiments and simulations were relatively small and can be explained by the uncertainties involved in the definition of simulations parameters as well as geometrical discrepancies between the design and the actual devices. In the future, simulations taking these uncertainties into account will also be performed.

7. References

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8. Acknowledgements

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