Multi-scale Modeling of Sperm Cell Activation

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Introduction

Zebrafish are a model species for biological engineering applications (vertebrate development, DNA mutation, and human disease studies), and the cryopreservation of their reproductive cells allows for inexpensive cataloging and maintenance of particular strains [1]. This species has several key advantages: they reproduce rapidly, a large number of fish can be stored in a relatively small area, and finally external embryo development allows for easy visualization [2].

Zebrafish egg fertilization occurs externally and therefore sperm encounter a hypo- osmotic environment en route to the egg. It is this "osmotic shock" that is believed to activate the sperm cells after they are released from the male. Activation analysis is currently done manually and brings with it an inherent difficulty and error. Microfluidics offers a solution to this problem via automated, high-throughput, highly reproducible results.

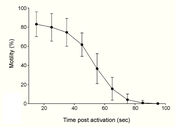
The manipulation of fluids on a sub-millimeter scale, microfluidics continues to emerge as a unique yet interdisciplinary field. Scientists from a variety of fields find microfluidics attractive for several reasons: 1) small, typically on the order of microliters, sample volume, 2) a number of sample control options, and 3) fast analysis time. Often biological samples can be either expensive or time-intensive to obtain; minimizing the sample volume needed for analysis is desirable. Because of their ability to scale down analysis, as well as their cross-over appeal between disciplines, these devices have earned names such as Lab-on-a-Chip (LoaC), µTAS (micro total-analysis-system), and bio-MEMS (biological-Micro-Electro-Mechanical) [4].

Modeling the transport of sperm cells in a microchannel will further the understanding of complex multi-phase flows in a microenvironment. Biomedical applications such as cell separation and analysis are plentiful in microdevices. However, while modeling these devices, the underlying physics of the discrete phase is often neglected. For this reason, microdevices are often developed iteratively – each prototype correcting for the deviation from expected behavior observed in its predecessor. Accurately capturing the dynamics of both the carrying fluid and the discrete phase will greatly reduce this number of iterations, making simulations extremely beneficial for their time and cost savings.

Challenges

1. Zebrafish motility lifespan

Once active, zebrafish sperm cells remain motile a little over 30 seconds (image taken from [3]).



We must get the cells from inlet to outlet as fast as possible to allow time for analysis. However, in traditional microfluidic channels increasing velocity decreases the amount of time diffusion has to take place.

2. Mixing (rapid dilution of sperm media) in a microchannel

Industrially, bulk mixing is usually achieved via bubblers or large propellers in stirred tanks. At high enough Reynolds numbers (a measure of inertial forces to viscous forces) turbulence will rapidly mix fluids in a pipe.

$$\mathrm{Re} = \frac{\rho U L}{\mu}$$

Fabricating scaled down moving parts is extremely difficult, and turbulence is non-existent at such small sizes. We are left with diffusion as the mixing mechanism. The Peclet number (a measure of convective transport to diffusive transport) is typically upwards of 1000 in microchannels. This indicates that diffusion is occurring on a much longer time scale than convection through the channel.

$$Pe = \frac{UL}{D_{ab}}$$

3. Membrane transport, motility, and microdevices are all on different scales

All three phenomena occur on different temporal and spatial scales.

Micromixer Development

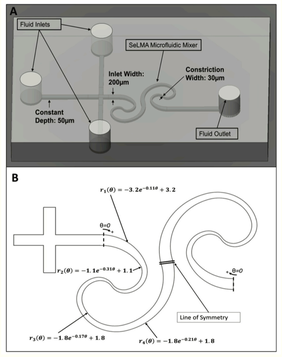
We have developed a Sequential Logarithmic Mixing Apparatus, SeLMA. The micromixer is inspired from logarithmic curves. The variable cross sectional area provides interfacial stretching while the changing radius of curvature provides secondary Dean vortices – the former being most effective in the low Reynolds number range and the latter driving mixing as Reynolds number is increased.

Here we solve the conservation of mass, conservation of momentum, and species conservation equations simultaneously. We use the finite volume method and ensure that our solutions are independent of grid size resolution.

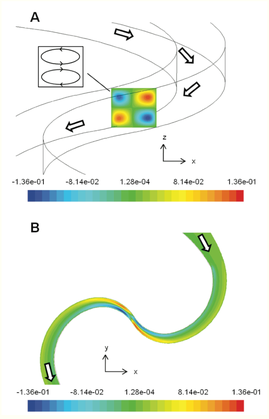
$$\frac{\partial(\rho\mathbf{u})}{\partial t} + \rho\mathbf{u}\cdot\nabla\mathbf{u} = -\nabla p + \mu\nabla^2\mathbf{u} + \mathbf{f}^{ext}$$

$$\nabla\cdot\mathbf{u} = 0$$

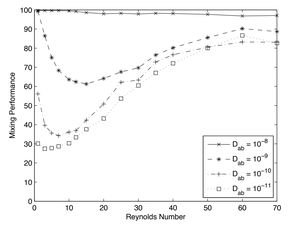
$$\frac{\partial C_A}{\partial t} + \mathbf{u}\cdot\nabla C_A = D_{AB}\nabla^2 C_A$$



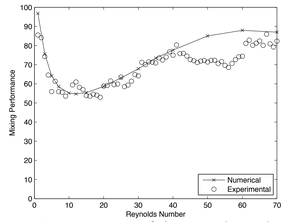
SeLMA has 3 inlets and 1 outlet. Its pathlength is 12 mm and it occupies 3 mm x 5 mm of chip space. The design can easily be repeated in series, making it "Sequential".



SeLMA uses a variable radius of curvature to create secondary Dean vortices (inset in A). These are figures of z-velocity (m/s) that illustrate the vortices.



At higher *Re*, SeLMA offers similar mixing performance over four orders of magnitudes of diffusivity.



SeLMA was fabricated and experimentally validated. The simulations match the experimental data very well.

Membrane Transport

The Kedem-Katchalsky (KK) equations [5,6] describe passive solute flux and water transport through a cellular membrane.

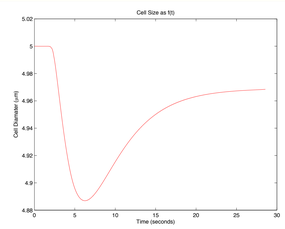
$$J_w = L_p\Delta P - L_p\sigma RT\Delta C$$

$$\frac{dV_w}{dt} = J_w A$$

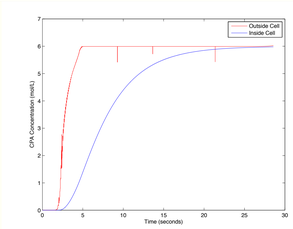
$$J_c = (1-\sigma)\overline{C}J_w + \omega RT\Delta C$$

$$\frac{dV_c}{dt} = J_c A$$

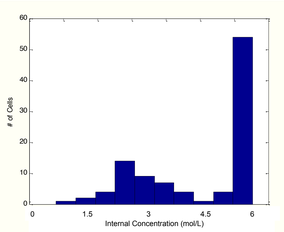
Our code takes input from the solution of the fluid flow and concentration profile (above). We release neutrally buoyant spherical particles and monitor the local concentration each particle encounters along its path. This concentration data becomes the input to the KK equations.



The cells display a "shrink-swell" response that is typical when they encounter an osmotic gradient. Minimizing this change in size offers the cells the best chance of survival after chemical loading. This profile varies based on external concentration, residence time, and membrane permeability parameters.



The solution rapidly mixes inside SeLMA and reaches its outlet value. Membrane transport is slower than the convection throughout the device, so the internal concentration lags behind the external concentration. Given a sufficient residence time in the channel, the cells will equilibrate.



Now we can offer insight into cellular conditions when they leave the mixer! This is what allows us to tune optimum device conditions for the best performance. At this flow rate, clearly not all of our cells had time to equilibrate – likely a result of the varying concentration field and non-uniform velocities across the mixer.

Microorganism Motility

The Micro-Swimmer Code Solution Algorithm

1. Distribute point forces along head and flagella
2. Prescribe flagellar motion
3. Solve fluid flow
4. Calculate cellular thrust by solving force balance
5. Move flagellum as result of thrust
6. Next time step

$$y(x,t) = A e^{\left(\frac{x}{\alpha}\right)}\sin(\omega t - \lambda x)$$

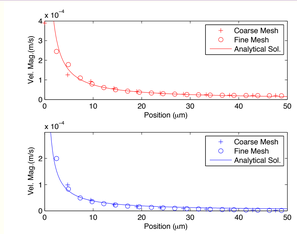
$$\mathbf{v}_i = \mathbf{v}_{i,r} + \mathbf{v}_c + \boldsymbol{\Omega}\times(\mathbf{x}_i - \mathbf{x}_c)$$

$$\frac{\partial(\rho\mathbf{u})}{\partial t} + \rho\mathbf{u}\cdot\nabla\mathbf{u} = -\nabla p + \mu\nabla^2\mathbf{u} + \mathbf{f}^{ext}$$

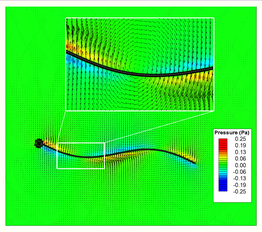
$$\nabla\cdot\mathbf{u} = 0$$

$$\mathbf{F} = \sum_i 3\pi\mu d_p\left(\mathbf{v}_{f,i} - \mathbf{v}_i\right) = 0$$

$$\mathbf{T} = \sum_i 3\pi\mu d_p\left(\mathbf{v}_{f,i} - \mathbf{v}_i\right)\times(\mathbf{x}_i - \mathbf{x}_c) = 0$$



An analytical solution does exist for a single point force. We get excellent agreement in our model.



The vortices inside the valleys of the flagellar wave and other perturbations due to the swimmer remain very localized.

Conclusions

As members of an emerging field, microfluidics researchers have largely been interested in pushing the realm of possibility with fabrication and device applications. Often it seems that intuition has been sufficient validation to fabricate a novel idea and adjustments can be made after initial testing. This has suited the field well to a point; it has generated attention while rapidly improving fabrication methods, analysis techniques, and identifying shortcomings across the field. However, as a result of this "shoot first, ask questions later" mentality, microdevices have often gone directly from concept to fabrication with minimal theory or preliminary simulation as validation. This is especially true of multi-phase (solid/liquid, liquid/liquid) systems that commonly exist in MEMS applications.

In our work, we investigate the behavior of sperm cells and their activation in microchannels. We have developed and characterized a micromixer that provides beyond adequate mixing to activate the cells. Our understanding of the membrane transport process allows us to tune operating parameters (flow rate, mixing concentrations) to load the cells as desired. Once the cells are motile, we have developed a method to model the hydrodynamics of the swimming.

As microfluidics is a highly interdisciplinary field, these studies certainly have wide applicability. SeLMA, our micromixer, can be inserted into any bio-MEMS device requiring a mixer. The membrane permeability code we have developed has uses ranging from cryo-biology to cellular homeostasis. Our micro-swimmer code can be used to investigate the hydrodynamics of any motile cell with a prescribed motion in a micro-environment.

Future Work

The membrane permeability code has many applications, one of which is the loading of cryoprotective agents into the cells. These improve cell viability after cryopreservation[6]. We would like to incorporate an on-chip freezing process along with the membrane transport of cryoprotective agents. With the micro-swimmer code we would like to investigate biflagellate hydrodynamics, chemotaxis, interactions with a non-Newtonian fluid, and the dynamics of a motile population.

References

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