Introduction of Cryopreservation Solutions using a Microfluidic Device

Andrew Fager[1], Rohini Bala Chandran[1], Katie Glass, PhD and Allison Hubel,PhD[1]

Departments of [1]Mechanical Engineering, University of Minnesota and University of Outer Mongolia, Ulan Bataar, Mongolia

BACKGROUND & OBJECTIVES

Cryopreservation solutions must contain some type of cryoprotective agent. Conventional methods of cryopreserving blood cells for therapeutic applications involve the use of dimethyl sulfoxide (DMSO). Current methods of cryoprotectant introduction require trained lab technicians and are labor intensive. It is proposed that a microfluidic device can be used to introduce a cryopreservation solution into a cell suspension.

Microfluidic devices for this application use low Reynolds number (laminar) flows to control cell motion and diffusion of DMSO from one stream to the other.

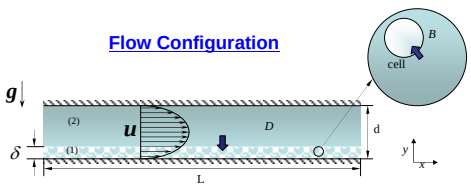
Numerical simulations suggest that diffusion-based introduction of DMSO into cells using a microfluidic device is viable.

In order to validate the theoretical model used in these simulations, a prototype was built and the flow of two parallel streams, (1) a cell suspension without DMSO or a PBS solution stream and (2) a donor stream with DMSO, was characterized experimentally. Desired outlet concentration is 10% DMSO v/v in cell suspension.

THEORETICAL MODEL

The rational design of a microfluidic device capable of introducing DMSO to cell suspensions for cryopreservation requires the development of a computational model that can be used to predict introduction and facilitate device design and scale-up.

Flow Configuration



Two streams enter at left and flow in parallel toward the right. The lower stream contains a cell suspension (with no DMSO). The upper stream is a donor solution that contains DMSO. Exploded view illustrates the diffusion of DMSO from the extracellular to the intracellular space.

Governing Equations

$$\frac{\partial C_f}{\partial t} + (\boldsymbol{u}\cdot\nabla C_f) = D\nabla^2 C_f \quad \text{for } 0<y<d$$

$$\frac{\partial C_i}{\partial t} + (\boldsymbol{u}\cdot\nabla C_i) = B(C_i - C_f) \quad \text{for } 0<y<\delta$$

Assumptions

- Steady, fully developed flow.
- Channel width, w, is much greater than the depth, d.
- The viscosity is constant and uniform across the two streams.
- Variations across the stream are much stronger than the variations along the stream, such that $\partial^2 C/\partial x^2 << \partial^2 C/\partial y^2$.

Model

$$u_x \frac{\partial C_f}{\partial x} = D\frac{\partial^2 C_f}{\partial y^2}, \quad \text{for } \delta<y<d$$

$$\left.\begin{aligned} u_x \frac{\partial C_f}{\partial x} &= D\frac{\partial^2 C_f}{\partial y^2} + \frac{V_i}{V_t} B(C_i - C_f) \\ u_x \frac{\partial C_i}{\partial x} &= B(C_i - C_f) \end{aligned}\right\} \text{for } 0<y<\delta$$

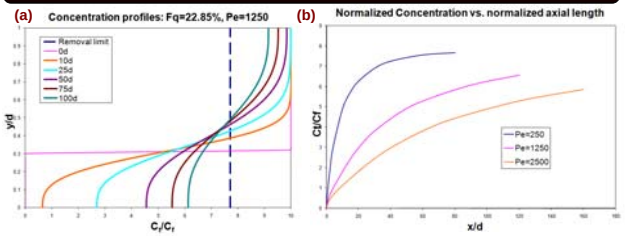
These equations were scaled using the mean velocity within the channel *U*, the channel depth *d*, and the initial DMSO concentration *Co*. They were solved using a forward-marching finite difference algorithm and MATLAB (MathWorks, MA).

Dimensionless Parameters

Peclet #: $Pe = \frac{dU}{D}$ Reynolds #: $\mathrm{Re} = \frac{Ud}{\upsilon}$ $B^* = \frac{Bd}{U}$ and $\frac{V_i}{V_t}$ and $\frac{\delta}{d}$

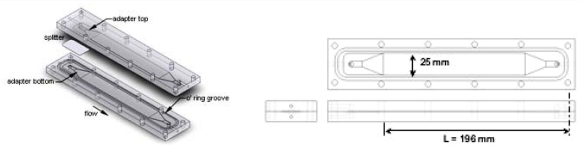
Flow rate fraction: $f_q = \frac{q_c}{q_t}$ Introduction limit: $C_0(1 - f_q)$

SELECTED NUMERICAL RESULTS

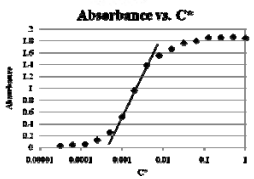


(a) Distribution of normalized cell stream (only PBS) concentration of DMSO (Ct/Cf) for various locations downstream from the inlet for Pe = 1250 (b) Normalized average cell stream concentration of DMSO Ct/Cf as a function of x/d for various Pe numbers and fq=0.23 for both these cases.

EXPERIMENTAL SET UP



- Two streams (cell and donor) enter the device through opposing ports separated by a splitter plate.
- Flow rates of cell and donor stream controlled using single syringe pump.
- An adapter with constant area cross section feeds both streams into a rectangular channel of 500 micron depth, 2.5 cm width, and 19.6 cm length.
- Downstream of this section, a constant area adapter is again used to transition flow from the channel to the outlet were the cell and donor stream split.
- Spectraphotometry allows DMSO concentration to be determined.

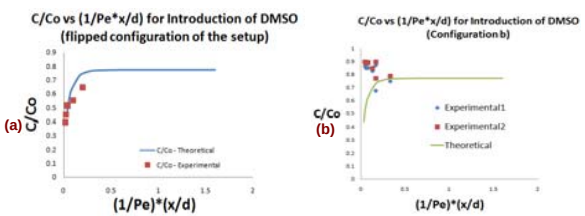


Absorbance at 209nm from spectraphotometry readings of a known serially diluted DMSO solution. Linear region allows unknown concentration to be found.

EXPERIMENTAL STUDIES WITH DMSO LADEN DONOR STREAM

The relationship between outlet concentration and flow parameters (Pe) was determined experimentally for a stream without cells for two different configurations of flow. The first configuration has the donor stream flowing on the bottom (DMSO laden and heavy) of the channel and the cell stream (less dense) flowing on top. The second configuration has the two streams switched in position.

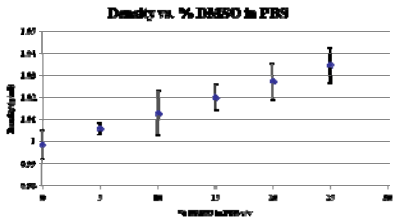
Cell Stream concentration as a function of Pe



(a) Normalized DMSO-laden stream concentration (C/Co) as a function of l/(Pe)*(x/d) for the heavier DMSO donor stream in the bottom and cell stream on top; (b) (C/Co) as a function of l/(Pe)*(x/d) for the reverse configuration(as shown in the flow configuration in the figure by the side). Differences are noted due to presence of free convection in (b)

DENSITY CONSIDERATIONS

Density differences exist between the two streams in the experimental studies. DMSO is heavy than PBS. Therefore the configuration (top or bottom) of the streams in the microfluidic device matters. For example, if the configuration is such that the donor stream is on top and the cell stream on the bottom free convection is a factor in addition to diffusion as a mode of DMSO transfer. To characterize free convection due to a density gradient the dimensionless Rayleigh number represents a good parameter to study.



Range of DMSO in PBS solutions is for donor stream solutions that are of interest for introduction to a cell stream. Density increases linearly for this range. Error bars represent 95% confidence interval in density measurement.

CONCLUSION

The objective of the ongoing investigation is to develop a clinical-scale microfluidic device capable of introducing dimethylsulfoxide (DMSO) to a concentration of 10% v/v in a cell suspension to be cryopreserved.

Preliminary experimental results based on visual and quantitative data set the path to the model validation. Further work include optimization of dimensionless numbers (Peclet, Rayleigh, Reynolds), the flow rate fraction, and initial donor stream concentration. Specific attention will be paid to understand free convection of DMSO and the dimensionless Rayleigh number that characterizes it.

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