Dissolution Modeling of Uniform Aqueous Droplets in Two-Phase Flow in a Microfluidic Device

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Abstract

Safe preservation of mammalian cells for long term is essential for medical and pharmaceutical industries. Cryopreservation by conventional slow freezing and by vitrification below freezing temperatures are the most effective biopreservation methods compared to in vitro culture at physiological temperatures and hypothermic storage at low temperatures above the freezing point. Cells contain about 80% cytoplasmic water by volume, which requires cooling rates faster than 1,000,000 C/s to transform into glass, and respond atypically to osmotic fluctuations. Freezing at cryo temperatures by slow cooling causes cell injury due to ice formation and cell dehydration. Vitrification by rapid cooling requires the employment of carbohydrate based cryoprotectant agents (CPAs) to accommodate for the ultrafast cooling rates and to prevent intra- and extra-cellular crystallization by increasing the glass transition temperature at the cost of toxicity. An alternative approach for safer cryopreservation is to modify the protocols to control the loading and concentration of CPAs in and around the cell prior to freezing.
We developed a mathematical model to show that preconcentration of cells is possible by dehydrating picoliter aqueous droplets in an organic phase in a microfluidic channel by reducing the exposure time to CPAs, eliminating the multistep CPA loading-diluting procedures, and avoiding the mechanical and osmotic stresses caused by large concentration differences. The system consists of a two-phase laminar flow following droplet formation and is thermally controlled to allow for interphase diffusion of water into the organic phase. We solved the mathematical model based on unsteady state two-phase flow and mass transfer through a moving boundary layer using finite element analysis calculations. The COMSOL Multiphysics® software was used to create two- and three-dimensional models of the microfluidic system. We employed the Transport of Diluted Species interface for the calculation of mass transfer, Laminar Flow interface for the calculations of fluid flow, and the Moving Mesh interface to create the moving boundary during shrinkage of aqueous droplets. Thin Diffusion Barrier boundary condition under the Transport of Diluted Species interface was used to create the effect of a cell membrane. MathWorks®, MATLAB®, and Microsoft Excel® 2010 softwares were used

to create curve-fits for fluid flow parameters, such as relative velocity, from the resulting data imported from the COMSOL® software and exported back to the COMSOL® software for the sake of a controllable and less sophisticated calculation sequence.
The model showed that it is possible to preconcentrate mammalian cells with CPAs up to 10 times the initial concentration below 4 minutes via the microfluidic method. We determined the critical droplet sizes for specific channel widths for optimum dehydration. For a channel with a cross-section of 150 μm x 200 μm, the ideal initial aqueous droplet size to yield a maximum rate of increase in CPA concentration would be 90 microns. There is significant need for a mathematical model to include the volumetric response of the cell to concentration gradients ahead of the cell wall through an advanced model for diffusion through the cell membrane.