Published in final edited form as:

Cell Preserv Technol. 2008 June 1; 6(2): 125–132. doi:10.1089/cpt.2008.0002.

An Application of Stream Imaging Technique in the Study of Osmotic Behaviors of Multiple Cells

Hsiu-hung Chen¹, Edward H. Lin², Shelly Heimfeld³, and Dayong Gao¹

¹Department of Mechanical Engineering, University of Washington, Seattle, WA.

²University of Washington School of Medicine, Fred Hutchinson Cancer Research Center, Seattle Cancer Care Alliance, Seattle, WA.

³Bone Marrow Transplant Program, Fred Hutchinson Cancer Research Center, Seattle, WA.

Abstract

Light microscopy method offers unique abilities for the determination of membrane transport properties of either single or multiple cells. A stream imaging system composed of a microfluidic device, a charge-coupled device camera, and a microscope has been developed to study the osmotic behavior of multiple cells in response toward their extracellular environment. Cells of interest were first mixed with the desired extracellular medium and streamed into a microchannel. The microchannel confines the movement of the cells in a monolayer and allows cells to move along the flow direction only. The cells then pass through a sensing zone where the images of cells were capable of being captured under a microscope. Using mouse dendritic cells (mDCs) as a model system, the membrane transport properties were investigated. The kinetics volume changes of mDCs under various extracellular conditions at room temperature (22°C) were analyzed using a biophysical model to determine water and cryoprotectant transport properties of the cell membrane. This prototype system directly allows us to observe, trace, capture, and store the sample information in terms of number, concentration, dynamic size, or shape for further analyses and documentations. We believe that the system has the potential of being used as a stand-alone equipment, or integrated into a lab-on-a-chip system, or embedded into commercialized instruments.

Introduction

Osmotic response of cells toward their extracellular environment plays a critical role in the fundamental study of cryobiology.^{1–6} During cooling, warming, and addition or removal of cryoprotectants (CPAs), cells experience severe volumetric changes, which may cause cell damage or death.² In order to prevent cells from injuries, several biophysical parameters can be determined to predict optimal protocols for cryopreserving cells. These cell-type-dependent parameters include the water permeability coefficient to cell membrane (L_p), the CPA permeability coefficient (P_s) to cell membrane, and their activation energies to cell membrane (E_a).^{2,7–10}

Various approaches have been used to quantify the cell membrane transport properties aforementioned by means of nuclear magnetic resonance,¹¹ electron paramagnetic resonance,¹² electronic sizing,^{13–15} and light microscopy.^{16–19} Among these approaches, only the light microscopy allows the determination of membrane transport properties of either single cells

(<10) or multiple cells (>10).²⁰ Moreover, light microscopy method can fill a void in the field of sample characterization and documentation by storing captured images through digital cameras to computers [commercialized apparatuses available from Beckman Coulter Inc. (Fullerton, CA) and BrightWell Technologies Inc. (Ottawa, Ontario, Canada)], which, until recently, traditional methods have hardly been able to address.²¹ This technique makes it possible for the sample information in terms of number, concentration, dynamic size, or shape to be directly observed, traced, captured, and stored for further analyzing. In single-cell studies, cell membrane transport properties are determined by immobilizing suspended cells in a predefined environment so that the information of their osmotic behavior to the extracellular environment can be collected and analyzed under a microscope. The microdiffusion system,¹⁹ the microperfusion technique,¹⁷ microperfusion chamber,¹⁶ and microfluid perfusion system²² are proved to be effective to trap single cells and help determine their membrane transport properties. However, multicell analyses associated with cell osmotic behaviors that are frequently performed using electronic sizing methods have never been demonstrated using any light microscopy approaches, even though it is a practical technique, to statistically quantify membrane transport properties of a large group of cells. One reason is that it is impractical to analyze individual cells in the number of hundreds or thousands by the use of those single-cell techniques aforementioned for their time-consuming operations. Furthermore, not until recently can images be easily captured and stored in a digital way, and can the imaging information be processed by affordable hardware.

In this study, we present a prototype system that is able to help measure membrane transport properties of a group of cells by capturing and analyzing their two-dimensional images. In order to accomplish such kinds of measurements, cells of interest are first mixed with the desired extracellular medium and quickly flow into a microfluidic channel with the assistance of a negative suction from an infusion pump. The channel confines the movement of the cells in a monolayer in order to prevent image ambiguities and allows cells to move majorly along the flow direction. The cells then stream down and pass through a sensing zone, where the images of cells are capable of being captured by a charge-coupled device (CCD) camera under a microscope. By processing the collected images, the information regarding membrane transport properties of cells can be extracted. The results are statistically significant for their analyses based on multiple cells. In the following sections, we describe the experimental details and compare the measured membrane properties of mouse dendritic cells (mDCs) with published data, to further validate our prototype system.

Materials and Methods

Source of cells

mDC line were a gift from Dr. K.L. Rock (Department of Pathology, University of Massachusetts Medical School, MA). Cells were grown in complete RPMI 1640 medium (10% fetal bovine serum, 2 mM L-glutamine, 100 µg/mL streptomycin, and 100 U/mL penicillin) supplemented with nonessential amino acid (100 µM) and 2-mercaptoethanol (50 µM). Cells were maintained in an incubator at 37°C in a humidified atmosphere containing 5% CO₂.

Preparation of cell suspensions

On the day of the experiment, mDCs, with diameters in the range of 10–15 µm, were collected, counted by using a hemocytometer, centrifuged, and resuspended in 1× phosphate buffered saline (PBS) (300 mOsm) at a cell density of about 5×10^6 /mL, and used within 3 h.

Design and fabrication for microfluidic channel

In order to capture clear outlined images of cells along the optical path (z-axis), a microfluidic channel is designed to prevent cells from piling up on each other by restricting the volume

available above them, as shown schematically in Figure 1A. The area encompassed by dashed lines represents the sensing zone: Cells are mixed with the desired medium in the mixing well and quickly flow through this sensing area, where the two-dimensional images of cells are recorded. The cell suspension keeps flowing toward the outlet, where an infusion pump is connected to provide suction. The height of the microchannel (h) confines the movement of cells. To allow cells to flow freely from the inlet to the chamber, h should be larger than the diameter of cells (Φ). Since the diameter of the mDCs (Φ) ranges from 10 to 15 μm , the dimension for h was chosen to be 20 μm . The channel width was chosen to be 150 μm . Figure 1B shows a three-dimensional schematic overview of the experimental setup.

The fabrication of the microfluidic device in Polydimethylsiloxane (PDMS) (Sylgard 184; Dow-Corning Corp., Midland, MI), shown in Figure 1B, was done by soft-lithographic rapid prototyping and replica molding.^{23–28} Briefly, layouts of the design were created as a computer-aided design (CAD) file using AutoCAD (Autodesk Inc., San Rafael, CA). The CAD file was then sent to be printed on to a 2000 dpi transparency (CAD/Art Service Inc., Bandon, OR), which serves as a photomask. To make master structures, SU8–25 (SU8; MicroChem Corp., Newton, MA) were spun on a 3-inch single-side polished silicon wafer (Montco Silicon Technologies, Spring City, PA) at 500 rpm for 10 sec and 2000 rpm for additional 30 sec, soft-baked, UV exposed for 110 sec through a photomask ($\sim 3 \text{ mJ}/\text{cm}^2$, to polymerize the exposed region), and then developed. A surface profilometer (Alpha Step 200; KLA-Tencor, San Jose, CA) is used to measure the thickness of the fabricated structures and indicates 23.2 μm for h . The structures on the silicon wafer were exposed to the vapor of tridecafluoro-1,1,2,2-tetrahydrooctyl-1-trichlorosilane (UCT Inc., Bristol, PA) to prevent bonding with the PDMS. A silicone tube, served as a fluidic port, was glued on the expected position to form an outlet before a mixture of PDMS prepolymer and curing agent is poured over the positive structures on the wafer. After curing, a negative relief made in PDMS was peeled off, a cubical hole [around 10 mm (width) $\times$ 10 mm (length) $\times$ 3 mm (depth)] served as the mixing well was cut by hand using a scalpel, exposed to an oxygen plasma²⁴ at 150 W for 30 sec, and sealed to a glass slide.

Evaluation of mixing

The determination of cell membrane transport properties in previous multicellular measurements,^{13–15} as well as in the present study, was based on two assumptions: (1) Evenly distributed cell suspension and (2) a spontaneous response of cells toward extracellular environment. A mixing procedure that introduces samples containing cells of interest to another desired medium is essential and cells take time to disperse. Before, during, and after the sample is introduced to the mixing well, a programmable infusion pump (Aladdin AL1000; WPI Inc., Sarasota, FL) is connected through a silicone tube to the outlet of the device in order to provide a flow rate of 7.5 nL/sec (or 2000 $\mu\text{m}/\text{sec}$) through continual aspiration. Our strategy here to facilitate mixing is to apply a steady pumping from a pipette, which is also used to inject cell samples. In order to validate this mixing procedure, a 300- μL solution with fluorescein (Acid Yellow 73; Sigma-Aldrich Co., St. Louis, MO) was first filled up in the channel, followed by an injection of 20 μL 1 $\times$ PBS from a pipette (Pipetman P20; Gilson Inc., Middletown, WI), and then by continual pumping. The pumping was at a frequency of about two full cycles per second for 30 sec to assist mixing. Fluorescent images at the sensing zone were collected by using a fluorescent microscopy system, comprising a cooled CCD camera (DS-2MBWc; Nikon Co., Tokyo, Japan) and a compound microscope (LV-TI3; Nikon Co., Tokyo, Japan). Since the CCD camera used for the study is highly linear under nonsaturating conditions, the gray scale intensity of any pixel in the images can be quantified to demonstrate the fluorescein concentration of the sampled volume that their pixels represent.

System setup and experimental procedures

The microfluidic device, made in PDMS and irreversibly sealed on a glass slide, is mounted on the stage of an inverted microscope (TE2000-S; Nikon Co., Tokyo, Japan). The room temperature, during the experiments, was monitored using a thermometer (USB-TC; Measurement Computing Co., Norton, MA). The local temperature of our microfluidic device is assumed to be in the equilibrium state with its surrounding environment. Before, during, and after the sample is introduced to the mixing well, a programmable infusion pump is connected through a silicone tube to the outlet of the device in order to provide a flow rate of 7.5 nL/sec (or 2000 $\mu\text{m}/\text{sec}$) through continual aspiration. In experiments, 300 μL 3 $\times$ PBS (900 mOsm) is first injected into the mixing well to rinse the channel. Owing to the oxygen plasma treatment, the surfaces of glass and PDMS inside the channel are highly hydrophilic, and medium fills the channel quickly. An aliquot of 20- μL cell suspension is later injected into the same mixing well using a pipette. The pumping from the same pipette after the sample is squeezed out is maintained at a frequency of about two full cycles per second for 30 more seconds to assist mixing while cells flow through the channel for the continual suction by the infusion pump. Two-dimensional images of cells passing through the sensing zone are recorded at 10 fps (frames per second) with the help of a digital camera (UI-122 $\times$ LE; IDS Inc., Cambridge, MA), which is connected to a computer, until the osmotic equilibrium between the intracellular and extracellular environments is achieved inside the mixing well. Estimated cell volumes are taken in averages for a second-period to better illustrate the history of their volumetric changes.

Evaluation of water permeability coefficient of cell membrane

Values of L_p and V_b of mDCs were determined by measuring averaged changes in cell volume while cells were mixed with anisotonic PBS solutions, where only a nonpermeating solute was present, using light microscopy method. Two-dimensional images of cells taken from the camera were first transformed to other gray scale images by selecting a suitable threshold number that better differentiates the extracellular environment and cells themselves. The cells were assumed spherical and their equivalent circle diameters (ECD) were calculated from their thresholded images by counting the number of pixels that each cell occupied. Cell volumes were then estimated from their ECD. The image processing was assisted using Matlab (The Mathworks, Inc., Natick, MA). The data were then fitted to the following differential equation to determine L_p by the method of least squares, using Matlab, which describes the rate of water movement across the cell membrane:²⁹

$$\frac{dV_c(t)}{dt} = L_p \cdot A \cdot (C_n^i - C_n^e) \cdot R \cdot T, \quad (1)$$

where $V_c(t)$ is the cell volume (μm^3), t is the time (s), L_p is the water permeability coefficient of cell membrane ($\mu\text{m}/\text{min}/\text{atm}$), A is the cell surface area (assumed to be constant, μm^2), C_n^i is the intracellular osmolality [Osm/kg(water)], C_n^e is the extracellular osmolality [Osm/kg(water)], R is the universal gas constant [0.08207 atm L/(mole $\times$ K)], and T is the absolute temperature (K). The intracellular osmolality during hypertonic shrinkage can be determined using the Boyle–van't Hoff relationship applied to the osmotic responses of cells:³⁰

$$C_n^i = C_0 \left(\frac{V_0 - V_b}{V - V_b} \right), \quad (2)$$

where C_0 is the isotonic osmolality [Osm/kg(water)], V_0 is the isotonic cell volume (μm^3), and V_b is the osmotically inactive cell volume (μm^3). The value V_b was determined by a Boyle–van't Hoff plot^{18,30} after cells were mixed with the desired medium and osmotically

equilibrated with various PBS solutions with different salt concentrations [$2 \times (600 \text{ mOsm})$ and $3 \times (900 \text{ mOsm})$]. For mDCs, V_b has been determined and available in other reference.³¹ The L_p value of cells was determined by least-square curve fitting^{8,17–20,30} after cells were mixed with $3 \times$ PBS solution.

Determination of CPA permeability of cell membrane: Two-parameter transport formalism

The two-parameter transport model can be used to determine L_p and P_s when both permeable solute (e.g., permeating CPAs) and nonpermeating solute (e.g., salts) were present in a solution.^{32,33} The cell volume change is governed not only by nonpermeating solutes but also by the concentration of permeating solutes,

$$\frac{dV_c(t)}{dt} = \frac{dV_s(t)}{dt} + L_p \cdot A \cdot (C^i - C^e) \cdot R \cdot T \quad (3)$$

the solute flux is given by

$$\frac{dN_s(t)}{dt} = P_s A (C_s^e - C_s^i), \quad (4)$$

and $N_s(t)$ and $V_s(t)$ are interchangeable for

$$N_s(t) = V_s(t) / \bar{V}_s, \quad (5)$$

where $V_s(t)$ is the CPA volume (μm^3), C^i is the intracellular environmental osmolality [$\text{Osm/kg}(\text{water})$], C^e is the extracellular environmental osmolality [$\text{Osm/kg}(\text{water})$], $N_s(t)$ is the osmoles of CPA inside cell, P_s is the CPA permeability coefficient of cell membrane (cm/min), C_s^i is the intracellular osmolality of CPA [$\text{Osm/kg}(\text{water})$], C_s^e is the extracellular osmolality of CPA [$\text{Osm/kg}(\text{water})$], $\bar{V}_s$ is the partial molar volume of CPA (L/mol), and the remaining symbols are as defined previously. The L_p and P_s values of cells at 22°C were determined by least-square curve fitting after cells were mixed with 1.5 M Me_2SO solution isotonic with respect to NaCl.

Results

Concentration variation during mixing

As indicated previously, mixing procedure between two different media was evaluated from fluorescent microscopy images collected by using a CCD camera. The area under evaluation is located around $500 \mu\text{m}$ downstream away from the mixing well. The fluorescent image in Figure 2A shows the top-down view of the channel prior to introducing $20 \mu\text{L}$ of $1 \times$ PBS to the mixing well, and the dashed line represents the location of the chosen pixels in the monitoring area. Three experiments based on the same setup were performed and their relative concentration history of the pixels is shown in Figure 2B.

Determination of L_p and P_s

The water permeability coefficient of mDCs was determined by measuring volume changes of cells in response to the extracellular environment inside the mixing well. The photographs of mDCs passing through the sensing zone at 0 sec, 8 sec, 13 sec, and 68 sec are shown in Figure 3A, B, C, and D, respectively. The experiments were repeated three times and one representative result is shown in Figure 4: The history of volume changes of mDCs was processed using Matlab, and its L_p was then determined by curve fitting [using Eqns. (1) and

(2)] to be $0.25 \mu\text{m}/\text{min}/\text{atm}$ (correlation coefficient $r^2 = 0.50$) at 22°C . The total number of counted cells is 213 from $t = 0$ sec to $t = 72$ sec.

L_p and P_s of mDCs were determined by measuring volume changes of cells in response to the $1.5 \text{ M Me}_2\text{SO}$ solution isotonic with respect to NaCl inside the mixing well. The history of volume changes of mDCs was shown in Figure 5, and its L_p and P_s were determined by curve fitting [using Eqns. (3)–(5)] to be $0.16 \mu\text{m}/\text{min}/\text{atm}$ and $0.11 \times 10^{-3} \text{ cm}/\text{min}$ ($r^2 = 0.14$) at 22°C , respectively. The total number of counted cells is 179 from $t = 0$ sec to $t = 90$ sec.

Discussions and Conclusions

This study demonstrates the feasibility of applying the stream imaging technique on the determination of osmotic response of multiple cells. The procedure of mixing that introduces samples containing cells of interest to another desired medium is critical and needs to be evaluated. The fluorescein medium to $1\times$ PBS solution ratio is 15:1, which causes the diluted fluorescein medium to have a final concentration of 93.75%, if the original fluorescein concentration is treated as 100%. The history of the relative concentration shown in Figure 2B illustrate a clear switch at $t = 0$ (determined by eye) and develops smoothly without showing fluctuation $>6\%$.

As aforementioned, the fluid composed of $20\text{-}\mu\text{L}$ sample mixed with $300\text{-}\mu\text{L}$ fluorescent dye flow downstream and pass through the sensing zone due to the continual suction by an infusion pump. The photobleaching can be neglected for the following two reasons: (1) The fluid in the sensing zone where UV light is shooting at is continuously replaced by the fresh fluid flowing from the mixing well, and (2) photobleaching does happen at the sensing zone; however, this can be eliminated by normalization, if the photobleaching is assumed to be uniform throughout the whole process, which is reasonable.

The dynamic behavior of cell volume changed in response to the extracellular environment occurring in the mixing well of the microfluidic device and was recorded while cells were flowing through the sensing zone. There are two factors that may limit the image quality in our experimental setup: (1) The resolution of a camera under a microscope and (2) the shutter speed of a camera. For the first factor, our images were collected by using a digital camera (resolution of $0.28 \mu\text{m}/\text{pixel}$) under an objective lens with $20\times$ magnification (resolving power of $0.7 \mu\text{m}$) that has proven to be effective to capture the dynamic volume changes of trapped cells from their two-dimensional images.^{22,31} On the other hand, shutter speed can have a dramatic impact on the appearance of fast-moving objects. The exposure time provided by a camera must be short enough in order to display cell images without noticeable blurry outlines while cells are moving. Since the resolution our camera is better than the resolving power of our $20\times$ objective lens, the bottom line is to find a maximum exposure time which may cause a blur. If an object is moving at a speed of $2000 \mu\text{m}/\text{sec}$, the maximum exposure time that may result in the residual image of a cell to reside in the same photo when the same cells was $0.7 \mu\text{m}$ away is

$$0.7(\mu\text{m})/2000 (\mu\text{m}/\text{sec})=0.00035 (\text{sec})$$

or 350 nsec. In our experiments, the exposure time of our camera was chosen to be shorter than 350 nsec to prevent blurry effects and was adjusted in accordance with the intensity of light source provided by our microscopy system.

Table 1 shows several published data for other types and the same type of cells in other published papers.^{16,31,34–36} The water permeability of cell membrane for mDCs determined in the present study (averaged from three experiments) is similar to those reported for other

cell types and comparable to the same cell type. However, the kinetics of cell volume changes in response to 1.5 M Me₂SO isotonic with respect to salt at 22°C illustrates a vague trend of the two-parameter formulism for its poor correlation coefficient value, as shown in Figure 5. Because of the nonuniformity of mDCs' initial diameter (between 10 and 15 µm), their dynamic volume changes will be difficult to obtain if the response is short and abrupt. The microfluidic device demonstrated in this study may be unsuitable for cells with greater L_p since their dynamic volume changes may not be captured. In this case, a mathematical model involving cell size distribution has been developed to determine the membrane transport properties of a cell population.³⁷ Devices for the parameter determination on single cells can be an alternative.^{16–19,22} On the other hand, the trend can be relatively clear if such kind of kinetics measurement is performed at lower temperatures.³⁵

As part of the limitation inherited from light microscopy method, which is always been an arguable issue, the impact that the assumption regarding spherical geometry on the calculated volume and the resulting determinations of L_p and P_s is not discussed here in this study, but related discussion can be found in Mazur (1963).³⁸

The prototype system using stream imaging technique has shown that the microfluidic device combined with a light microscopy system is a useful tool to study the osmotic behavior of multiple cells. The dynamic response of hundreds of cells can be recorded in a single experiment for sample characterization and documentation. This stream imaging technique makes it possible for the sample information in terms of dynamic size or shape, to be directly observed, traced, captured, and stored for further analyzing, which electronic sizing or light scattering method is not able to perform. This technique in the study of cell kinetic response has potentials of being developed into a stand-alone system, integrated into a lab-on-a-chip system, or embedded into commercialized instruments, such as RapidVUE® (Beckman Coulter Inc., Fullerton, CA) and Micro-Flow Imaging™ (BrightWell Technologies Inc., Ottawa, Ontario, Canada).

Acknowledgments

The authors thank Dr. Hong Shen and Mr. Kenny K. Tran in the Department of Chemical Engineering at the University of Washington for preparing mDCs. This work is supported, in part, by a NIH pilot research grant from FHCRC/UW Center for Medical Countermeasures Against Radiation.

References

1. Mazur P. Role of intracellular freezing in death of cells cooled at supraoptimal rates. *Cryobiology* 1977;14:251–272. [PubMed: 330113]
2. Mazur P. Equilibrium, quasi-equilibrium, and nonequilibrium freezing of mammalian embryos. *Cell Biophys* 1990;17:53–92. [PubMed: 1704816]
3. Muldrew K, McGann LE. The osmotic rupture hypothesis of intracellular freezing-injury. *Biophys J* 1994;66:532–541. [PubMed: 8161706]
4. Toner M, Cravalho EG, Karel M. Thermodynamics and kinetics of intracellular ice formation during freezing of biological cells. *J Appl Phys* 1990;67:1582–1593.
5. Mazur P, Koshimoto C. Is intracellular ice formation the cause of death of mouse sperm frozen at high cooling rates? *Biol Reprod* 2002;66:1485–1490. [PubMed: 11967214]
6. McGann LE, Yang HY, Walteson M. Manifestations of cell-damage after freezing and thawing. *Cryobiology* 1988;25:178–185. [PubMed: 3396384]
7. Arnaud FG, Pegg DE. Permeation of glycerol and propane-1, 2-diol into human platelets. *Cryobiology* 1990;27:107–118. [PubMed: 2331885]
8. Gao DY, Liu J, Liu C, et al. Prevention of osmotic injury to human spermatozoa during addition and removal of glycerol. *Hum Reprod* 1995;10:1109–1122. [PubMed: 7657750]

9. Heymann JB, Agre P, Engel A. Progress on the structure and function of aquaporin 1. *J Struct Biol* 1998;121:191–206. [PubMed: 9615438]
10. McGann LE, Stevenson M, Muldrew K, et al. Kinetics of osmotic water-movement in chondrocytes isolated from articular-cartilage and applications to cryopreservation. *J Orthopaedic Res* 1988;6:109–115.
11. Paganelli CV, Solomon AK. The rate of exchange of tritiated water across the human red cell membrane. *J Gen Physiol* 1957;41:259–277. [PubMed: 13475690]
12. Kleinhans FW, Travis VS, Du JY, et al. Measurement of human sperm intracellular water volume by electron-spin-resonance. *J Androl* 1992;13:498–506. [PubMed: 1338068]
13. Gilmore JA, McGann LE, Liu J, et al. Effect of cryoprotectant solutes on water permeability of human spermatozoa. *Biol Reprod* 1995;53:985–995. [PubMed: 8527530]
14. Liu C, Benson CT, Gao DY, et al. Water permeability and its activation-energy for individual hamster pancreatic-islet cells. *Cryobiology* 1995;32:493–502. [PubMed: 7587287]
15. McGann LE, Turner AR, Turc JM. Microcomputer interface for rapid measurements of average volume using an electronic particle counter. *Med Biol Eng Comput* 1982;20:117–120. [PubMed: 7098550]
16. Gao DY, Benson CT, Liu C, et al. Development of a novel microperfusion chamber for determination of cell membrane transport properties. *Biophys J* 1996;71:443–450. [PubMed: 8804627]
17. Gao DY, McGrath JJ, Tao J, et al. Membrane-transport properties of mammalian oocytes—a micropipette perfusion technique. *J Reprod Fertil* 1994;102:385–392. [PubMed: 7861392]
18. Leibo SP. Water permeability and its activation-energy of fertilized and unfertilized mouse ova. *J Membr Biol* 1980;53:179–188. [PubMed: 7190193]
19. McGrath JJ. A microscope diffusion chamber for the determination of the equilibrium and non-equilibrium osmotic response of individual cells. *J Microsc (Oxf)* 1985;139:249–263.
20. McGrath JJ. Quantitative measurement of cell membrane transport: technology and applications. *Cryobiology* 1997;34:315–334. [PubMed: 9200819]
21. Allen, T. Particle Size Measurement. 5th ed.. London: Chapman & Hall; 1997. p. 327–348.
22. Chen HH, Purttman JJP, Heimfeld S, et al. Development of a microfluidic device for determination of cell osmotic behavior and membrane transport properties. *Cryobiology* 2007;55:200–209. [PubMed: 17889847]
23. Duffy DC, McDonald JC, Schueller OJA, et al. Rapid prototyping of microfluidic systems in poly (dimethylsiloxane). *Anal Chem* 1998;70:4974–4984.
24. McDonald JC, Duffy DC, Anderson JR, et al. Fabrication of microfluidic systems in poly (dimethylsiloxane). *Electrophoresis* 2000;21:27–40. [PubMed: 10634468]
25. Ng JMK, Gitlin I, Stroock AD, et al. Components for integrated poly(dimethylsiloxane) microfluidic systems. *Electrophoresis* 2002;23:3461–3473. [PubMed: 12412113]
26. Qin D, Xia YN, Whitesides GM. Rapid prototyping of complex structures with feature sizes larger than 20 μm . *Adv Mater* 1996;8:917–919.
27. Xia YN, Whitesides GM. Soft lithography. *Ann Rev Mat Sci* 1998;28:153–184.
28. Whitesides GM, Ostuni E, Takayama S, et al. Soft lithography in biology and biochemistry. *Annu Rev Biomed Eng* 2001;3:335–373. [PubMed: 11447067]
29. Davson, H.; Danielli, JF. The Permeability of Natural Membranes. Cambridge, UK: Cambridge University Press; 1970. p. 38–47.
30. Lucke B, McCutcheon M. The living cell as an osmotic system and its permeability to water. *Physiol Rev* 1932;12:68–138.
31. Chen H, Shen H, Tran KK, et al. A microfluidic study of mouse dendritic cell membrane transport properties of water and cryoprotectants. *IJHMT*. 2008 doi: 10.106/j.ijheatmasstransfer.2008.04.013.
32. Jacobs MH. The simultaneous measurement of cell permeability to water and to dissolved substances. *J Cell Comp Physiol* 1933;2:427–444.
33. Kleinhans FW. Membrane permeability modeling: Kedem-Katchalsky vs a two-parameter formalism. *Cryobiology* 1998;37:271–289. [PubMed: 9917344]

34. Hunter JE, Bernard A, Fuller BJ, et al. Measurements of the membrane water permeability (L_p) and its temperature-dependence (activation-energy) in human fresh and failed-to-fertilize oocytes and mouse oocyte. *Cryobiology* 1992;29:240–249. [PubMed: 1582230]
35. Liu J, Zieger MAJ, Lakey JRT, et al. The determination of membrane permeability coefficients of canine pancreatic islet cells and their application to islet cryopreservation. *Cryobiology* 1997;35:1–13. [PubMed: 9245505]
36. Takamatsu H, Komori Y, Zawlodzka S, et al. Quantitative examination of a perfusion microscope for the study of osmotic response of cells. *J Biomech Eng -Trans ASME* 2004;126:402–409.
37. Elmoazzen HY, Chen CCV, Acker JP, et al. The effect of cell size distribution on predicted osmotic response of cells. *CryoLetters* 2005;26:147–158. [PubMed: 16082441]
38. Mazur P. Kinetics of water loss from cells at subzero temperatures and likelihood of intracellular freezing. *J Gen Physiol* 1963;47:347–369. [PubMed: 14085017]

Fig 1.

System setup using stream imaging technique. (A) Schematic diagram shows that the cells are mixed with a desired medium inside the mixing well and stream toward the outlet. Cell images are recorded while passing through the sensing zone. It should be noted that the objective lens will be located under the microchannel if an inverted microscope is used. (B) An overview of the experimental setup.

Fig 2. Evaluation of the mixing procedure. (A) A top-down view of the channel filled with fluorescein medium. (B) The relative concentration history of pixels along the dashed line in the monitoring area of the 150- μm wide channel. The dashed line represents the location of the chosen pixels.

Fig 3. Magnified view in the sensing zone when cells were passing by. Four representative photos are shown at the moments of (A) $t = 0$ sec, (B) $t = 8$ sec, (C) $t = 13$ sec, and (D) $t = 68$ sec, when the cells were in response to $3\times$ PBS.

Fig 4.
Volume change of mDCs in response to $3\times$ PBS. All values are expressed as mean $\pm$ SEM.

Fig 5. Volume change of mDCs in response to 1.5 M Me₂SO + 0.9% NaCl. All values are expressed as mean $\pm$ SEM.

Table 1

A Comparison of Water Permeability for Mammalian Cells

Cell type	L_p ($\mu\text{m}/\text{min}/\text{atm}$)	Reference
Mouse dendritic cell	0.23 (averaged) (22°C)	Present
Mouse dendritic cell	0.16 ± 0.03 (22°C)	[31]
Mouse oocyte	0.48 ± 0.20 (20°C)	[34]
Golden hamster pancreatic islet	0.27 ± 0.08 (22°C)	[16]
Canine pancreatic islet	0.19 ± 0.05 (23°C)	[35]
Human prostate cancer cell	0.45 ± 0.14 (23°C)	[36]