Supporting Information

A non-PDMS microfluidic perfusion approach for on-chip characterization of the transport properties of human oocytes

Supplementary Method

Flow and replacement of CPA solution in the microchannel:

The laminar incompressible flow in the microfluidic channel was modeled using the Navier-Stokes (NS) equations as follows:[1]

$$\rho\frac{\partial \mathbf{u}}{\partial t}+\rho(\mathbf{u}\cdot\nabla)\mathbf{u}=\nabla\cdot\left[-p\mathbf{I}+\eta\left(\nabla\mathbf{u}+(\nabla\mathbf{u})^{T}\right)\right]+\mathbf{F} \quad \text{(S1)}$$

$$\rho\nabla\cdot\mathbf{u}=0 \quad \text{(S2)}$$

where ρ is density, η is viscosity, t is time, $\mathbf{u}$ is velocity vector, p is pressure, $\mathbf{I}$ is identity matrix, $\mathbf{F}$ is volumetric force vector ($\mathbf{F}$=0 in this study).

CPA transport is described using the following transient convection-diffusion equation:[1]

$$\frac{\partial M_s^e}{\partial t}+\nabla\cdot\left(-D\nabla M_s^e\right)+\mathbf{u}\cdot\nabla M_s^e=0 \quad \text{(S3)}$$

where M_s^e is extracellular CPA osmolality in Osmol/kg_{H2O}, and D is CPA diffusion coefficient. D was estimated using the Stokes-Einstein equation [2]:

$$D=\frac{k_B T}{6\pi\eta a_0} \quad \text{(S4)}$$

where k_B is the Boltzmann constant, T is absolute temperature, and a_0 is the apparent hydrodynamic radius of a water molecule.

An average flow velocity (calculated from the injection flow rate of the syringe pumps) and a zero pressure boundary condition for NS equation were established for the inlet and the

outlet, respectively; a no-slip condition was set for all the other solid boundaries. For the convection-diffusion equation, a constant concentration boundary condition and the convective flux condition were set for the inlet and outlet, respectively. The diffusion normal to all the solid boundaries was negligible.

The finite element method solver (COMSOL, Inc, USA) was used for simulation. The total number of the elements in the computational domain was 479569, 475127 and 464987 for the three different oocyte locations P1, P2 and P3. The self-adaptive time step of backward differentiation formula was used for the time-dependent solver. Grid and time step independent results were obtained with a convergence tolerance 1%.

CPA concentration profile in extracellular solution:

For most of the existing studies on measurement of cell membrane permeability, the extracellular CPA concentration profile ($M_s^e(t)$) have been assumed to be a step function,[3-5] while it has been recently shown that this assumption may introduce significant error in estimation of the permeability coefficients (L_p and P_s).[6] For this reason, the experiment-simulation approach developed by Liu *et al*.[6] was used with improvements. The effect of the presence of cells in the microchannel on extracellular CPA concentration distribution was not considered in Liu *et al*.,[6] because the size of the cells (~15 μm in diameter) was negligible compared to the depth of the microchannel (100 μm). However, human oocytes (~120 μm in diameter) are comparable in size to the depth of the microchannel (150 μm) developed in this study. As a result, the space taken up by the oocyte could not be ignored during modeling the microfluidic flow in the microchannel. Specifically, the integral-averaged CPA concentration

across the surface of the cell was calculated as the extracellular CPA concentration using the following equation:

$$M_s^e(t) = \frac{1}{S}\iint M_s^e(x, y, z; t)dS \tag{S5}$$

where S is cell surface area.

Mass transport across cell membrane:

The two-parameter (2-*p*) model was used to predict the water and CPA transport across the cell plasma membrane as follows:[7, 8]

$$\frac{dV_w}{dt} = -L_p ART\left[(M_s^e + M_n^e) - (M_s^i + M_n^i)\right] \tag{S6}$$

$$\frac{dN_s}{dt} = P_s A(M_s^e - M_s^i) \tag{S7}$$

where V_w is cell water volume, t is time, L_p is hydraulic conductivity, A is cell surface area, R is the universal gas constant, T is absolute temperature, M is osmolality, N_s is intracellular moles of permeable solute, P_s is CPA permeability coefficient. The superscripts e and i refer to extra- and intracellular, while the subscripts s and n denote membrane penetrating and non-penetrating solutes, respectively.

The total cell volume is calculated as follows:

$$V = V_w + V_s + V_n + V_b \tag{S8}$$

where V_w, V_s, and V_n are volumes of intracellular water, CPAs, and salts, while V_b is the osmotically inactive volume.

The cell membrane permeability coefficients (L_p and P_g) at a given temperature can then be determined by least-square curve-fitting of Eqs. S6 and S7 to the cell volume responses upon CPA addition.

The temperature dependence of the transport parameters can be described using the the following Arrhenius relationship:[9, 10]

$$L_p(T) = L_{pg} \cdot \exp\left[\frac{E_{L_p}}{R}\left(\frac{1}{T_0} - \frac{1}{T}\right)\right] \tag{S9}$$

$$P_s(T) = P_{sg} \cdot \exp\left[\frac{E_{P_s}}{R}\left(\frac{1}{T_0} - \frac{1}{T}\right)\right] \tag{S10}$$

where L_{pg} and P_{sg} are the reference values of L_p and P_s at the reference temperature T_0, and E_{Lp} and E_{Ps} are the activation energies for water and CPA transport across cell membrane.

Taking the natural logarithm on both sides of the equations S9 and S10 yields:

$$\ln[L_p(T)] = -\frac{E_{L_p}}{R} \cdot \frac{1}{T} + [\ln(L_{pg}) + \frac{E_{L_p}}{RT_0}] \tag{S11}$$

$$\ln[P_s(T)] = -\frac{E_{P_s}}{R} \cdot \frac{1}{T} + [\ln(P_{sg}) + \frac{E_{P_s}}{RT_0}] \tag{S12}$$

The activation energies for water and CPA transport across cell membrane, E_{Lp} and E_{Ps}, can then be determined by fitting Eqs. S11-S12 to the above obtained L_p and P_s values at three different temperatures: 4, 15 and 25 °C in this study.

All the parameters used in the above equations are listed in Table S1 and S3; the L_p and P_s values obtained from Eqs. S6 and S7 at 4, 15 and 25 °C are listed in Table S2 along with the published data.

Statistical Analysis:

For each combination of temperature and CPA concentration, 6-10 cells were measured for determination of L_p and P_s. The statistical analysis was performed using the two-tailed Student's *t*-test. The results were reported as mean ± standard deviation (SD). A *p*-value less than 0.05 is taken as statistically significant.

Supplementary Figures

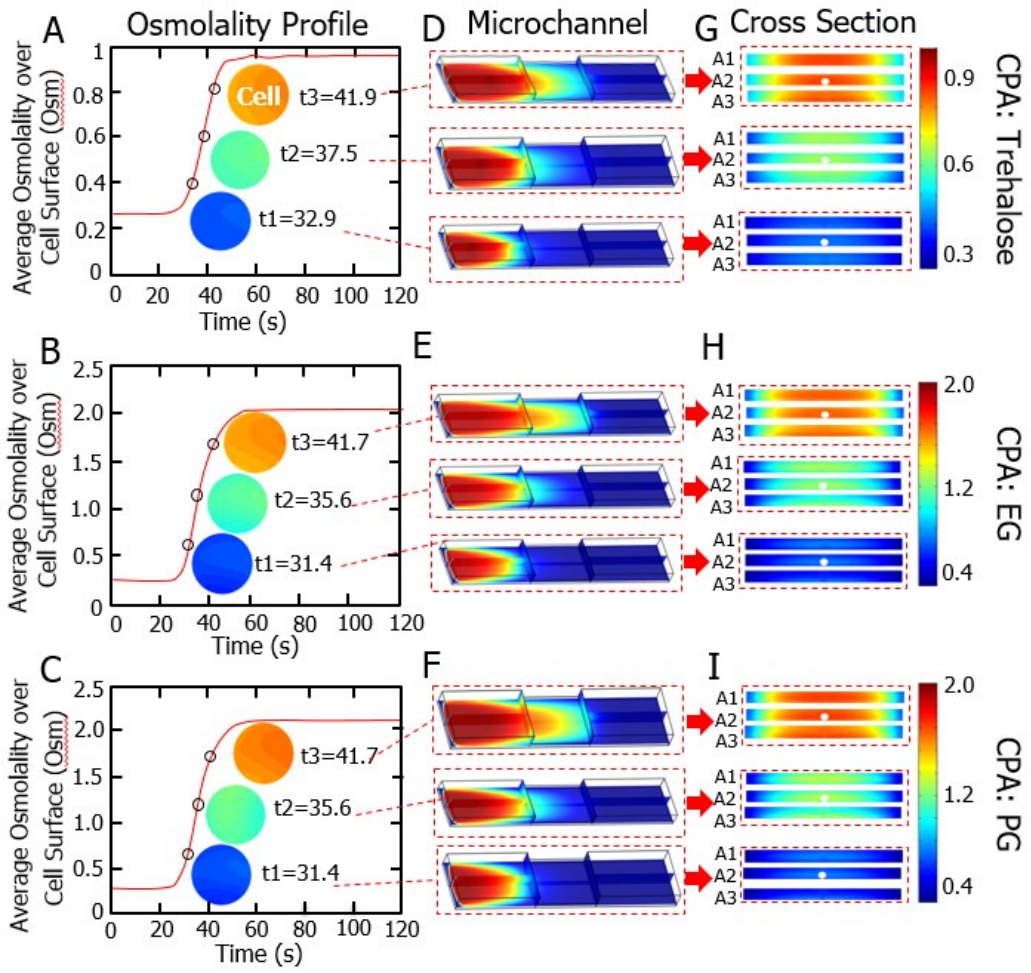

Figure S1. Predicted osmolality over the surface of a cell located at P1 and in the solutions in the microchannel after introducing the hypertonic solution made of cell culture medium with 0.56 M trehalose (A), 1.5 M EG (B), and 1.5 M PG (C). (A-C) average osmolality over cell surface *versus* time; (D-F) transient osmolality distributions in the microchannel at three typical times t1-t3 to achieve 20, 50 and 80% of osmotic shift over the cell surface; and (G-I) osmolality distributions of the characteristic cross sections (A1-A3, see inset ii in Figure 1B) at the typical times t1-t3. The solution in the microchannel was isotonic (0.3 Osm) initially. CPA: cryoprotective agent

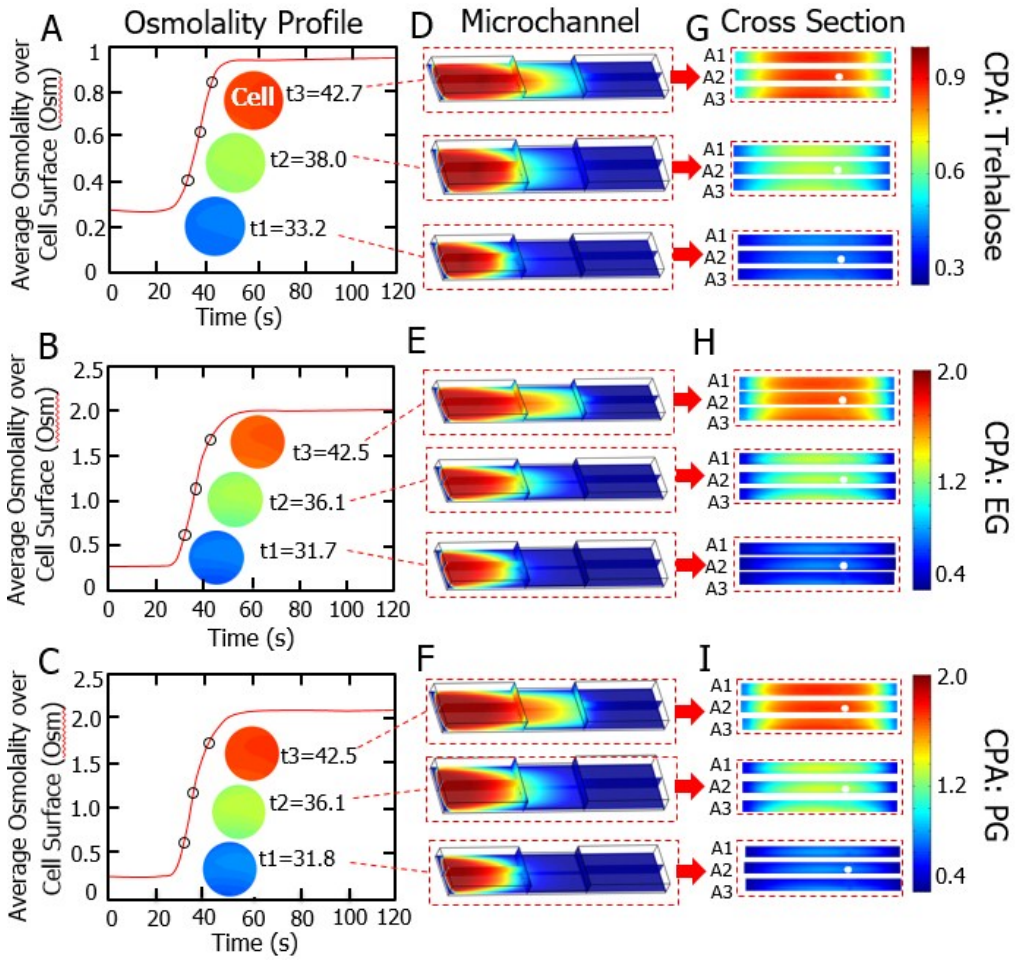

Figure S2. Predicted osmolality over the surface of a cell located at P2 and in the solutions in the microchannel after introducing the hypertonic solution made of cell culture medium with 0.56 M trehalose (A), 1.5 M EG (B), and 1.5 M PG (C). (A-C) average osmolality over cell surface *versus* time; (D-F) transient osmolality distributions in the microchannel at three typical times t1-t3 to achieve 20, 50 and 80% of osmotic shift over the cell surface; and (G-I) osmolality distributions of the characteristic cross sections (A1-A3, see inset ii in Figure 1B) at the typical times t1-t3. The solution in the microchannel was isotonic (0.3 Osm) initially. CPA: cryoprotective agent

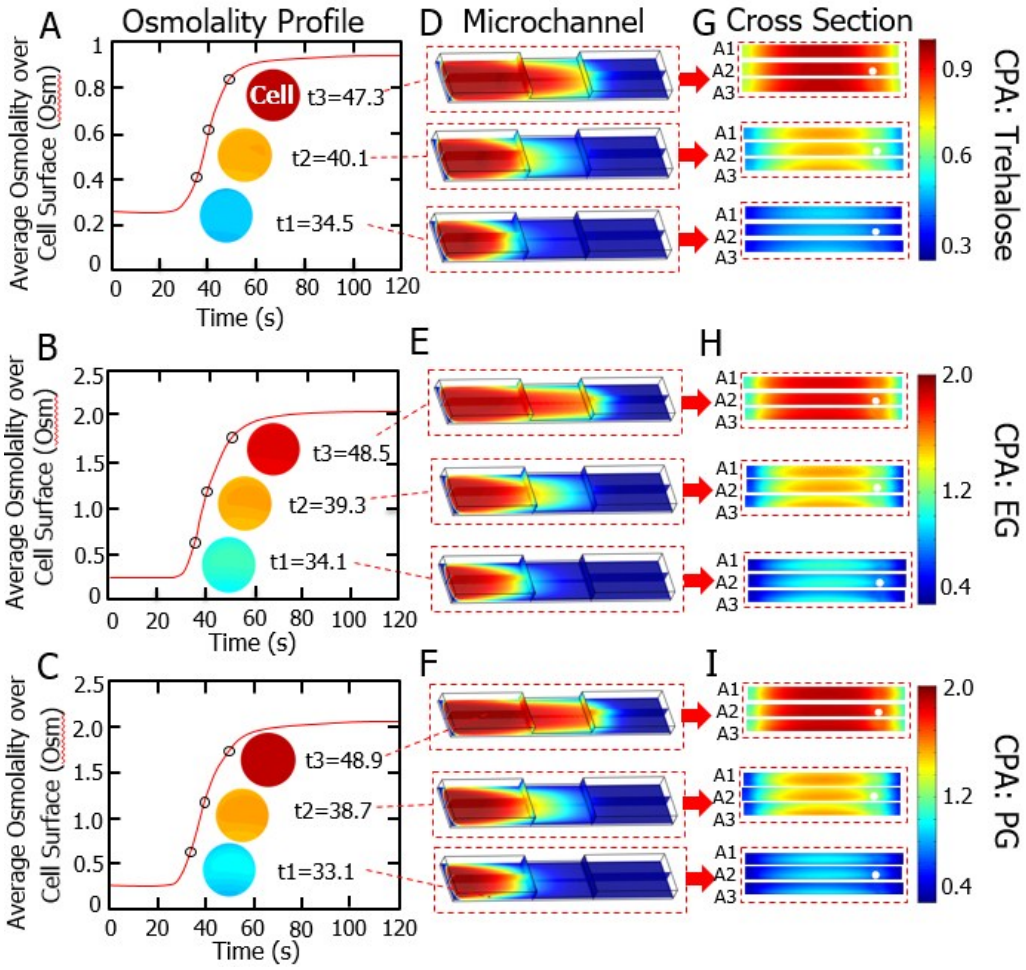

Figure S3. Predicted osmolality over the surface of a cell located at P3 and in the solutions in the microchannel after introducing the hypertonic solution made of cell culture medium with 0.56 M trehalose (A), 1.5 M EG (B), and 1.5 M PG (C). (A-C) average osmolality over cell surface *versus* time; (D-F) transient osmolality distributions in the microchannel at three typical times t1-t3 to achieve 20, 50 and 80% of osmotic shift over the cell surface; and (G-I) osmolality distributions of the characteristic cross sections (A1-A3, see inset ii in Figure 1B) at the typical times t1-t3. The solution in the microchannel was isotonic (0.3 Osm) initially. CPA: cryoprotective agent

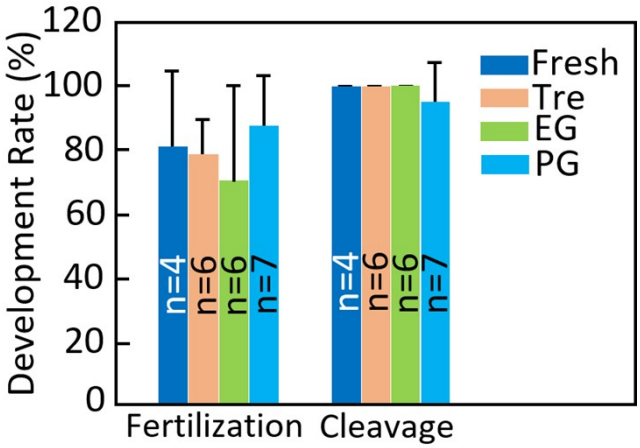

Figure S4. Fertilization and cleavage rates for the fresh and perfused oocytes. No statistically significant difference was observed in either the fertilization or the cleavage rate between the two groups (student's *t*-test, two-tail, $p>0.05$).

Supplementary Tables

Table S1. Parameters used in the numerical model

Parameters	*Units*	*Values*	*Description*
η_{EG}	Pa·s	1.480×10^{-3} [11]	viscosity of 1.5 M EG solution
η_{PG}	Pa·s	1.434×10^{-3} [12]	viscosity of 1.5 M PG solution
η_{Tre}	Pa·s	0.498×10^{-3} [13]	viscosity of 0.56 M Tre solution
D_{EG}	$m^2\cdot s^{-1}$	5.230×10^{-10} *	diffusion coefficient of 1.5 M EG solution
D_{PG}	$m^2\cdot s^{-1}$	5.398×10^{-10} *	diffusion coefficient of 1.5 M PG solution
D_{Tre}	$m^2\cdot s^{-1}$	1.554×10^{-9} *	diffusion coefficient of 0.56 M Tre solution
k	$m^2\cdot kg\cdot s^{-2}\cdot K^{-1}$	1.38×10^{-23}	Boltzman constant
T	K	298.15	Temperature
a_0	m	0.282×10^{-9} [14]	apparent hydrodynamic radius of a water molecule
ρ_{EG}	$kg\cdot m^{-3}$	995.2 #	density of 1.5 M EG solution
ρ_{EG}	$kg\cdot m^{-3}$	1000.6 #	density of 1.5 M PG solution
ρ_{Tre}	$kg\cdot m^{-3}$	1009.5 #	density of 0.56 M Tre solution
M_0	$mOsm/(kg_{H2O})$	268 #	osmolality of the isotonic solution (IS)&
M_{Tre}	$mOsm/(kg_{H2O})$	981 #	osmolality of 0.56M Tre in IS
M_{EG}	$mOsm/(kg_{H2O})$	2147 #	osmolality of 1.5M EG in IS
M_{PG}	$mOsm/(kg_{H2O})$	2017 #	osmolality of 1.5M PG in IS

*Calculated using Stokes-Einstein equation.[15]

#Measured by this study (Table S2).

&Quinn's Advantage medium with HEPES and 10% v/v serum substitute supplement.

Table S2. Experimentally measured osmolality values of various solutions. Unit: mOsmol/(kg_{H2O}).

Samples	Values					Mean ± SD
	n=1	n=2	n=3	n=4	n=5	
Isotonic solution (IS)*	267	268	268	268	268	268 ± 0
0.56 M Tre in IS	982	980	981	983	981	981 ± 1
1.5 M PG in IS	2070	2070	2072	2074	2070	2017 ± 2
1.5 M EG in IS	2149	2153	2152	2140	2142	2147 ± 5
CS 100[&]	103	103	102	103	103	103 ± 0
CS 290[&]	289	289	290	288	290	289 ± 1
CS 500[&]	498	500	501	499	498	499 ± 1
CS 900[&]	902	902	902	902	901	902 ± 0
CS 1500[&]	1500	1500	1501	1502	1499	1500 ± 1
CS 2000[&]	1998	2000	1998	2005	2006	2001 ± 3
CS 3000[&]	2990	3000	2996	2999	3002	2997 ± 4

*The isotonic solution was made by supplementing the Quinn's Advantage medium with HEPES and 10% v/v serum substitute supplement.

[#]DI water, deionized water.

[&]CS 100, Calibration Standard Solution of 100 mOsmol/(kg_{H2O}); and so on.

Table S3. Temperature-dependent cell membrane permeability coefficients for *in-vitro* matured human oocytes

No.	CPA/Salt/Sugar (concentration, mol/L)	L_p (μm/atm/min)	P_s (μm/s)	T (°C)	n	Refs.
1	Trehalose (0.56)	0.18±0.01	-	4	7	Present
2	EG (1.5)	0.14±0.07	0.03±0.02		6	
3	PG (1.5)	0.13±0.01	0.02±0.02		7	
4	Trehalose (0.56)	0.29±0.13	-	15	7	
5	EG (1.5)	0.30±0.25	0.07±0.02		8	
6	PG (1.5)	0.49±0.31	0.13±0.12		8	
7	Trehalose (0.56)	0.55±0.23	-	25	8	
8	EG (1.5)	0.55±0.27	0.17±0.12		9	
9	PG (1.5)	0.67±0.16	0.29±0.13		10	
10	DMSO (1.5)	1.65±0.15	0.79±0.10	30	9	16
11		0.70±0.06	0.25±0.04	24	12	
12		0.28±0.04	0.06±0.01	10	6	
13	Galactose (0.9)	0.24±0.05	-	8	5	17
14	EG (1.5)	0.32+0.08	0.06±0.03		8	
15	Galactose (0.9)	1.01±0.21	-	22	5	
16	EG (1.5)	0.77±0.21	0.20±0.10		8	
17	PG (1.5)	1.14±0.60	0.36±0.12		9	
18	DMSO (1.5)	0.63±0.18	0.26±0.06		9	
19	Galactose (0.9)	1.25±0.38	-	30	5	
20	EG (1.5)	1.39+0.45	0.48±0.03		8	
21	Propane-1,2-diol (1.5)	1.92±0.68	1.08±0.32	30	9	18
22		0.53±0.11	0.28±0.06	24	9	
23		0.41±0.23	0.13±0.04	10	6	
24	DMSO (1.5)	0.78	0.32	22	8	19
25	NaCl (0.5)	0.65±0.43	-	21	5	20
26	EG (1)	0.15	0.03	6.7	1	21
27		0.17	0.5	35.7	1	
28	NaCl (0.5)	0.55±0.37	-	37	10	22
29		0.55±0.38	-	30	11	
30		0.40±0.12	-	20	10	
31		0.40±0.20	-	10	14	
32	NaCl (0.5)	0.82±0.48	-	37	9	23
33		0.73±0.41	-	30	9	
34		0.47±0.34	-	20	9	
35		0.23±0.16	-	10	9	

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