Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary Information

Microfluidic platform for rapid measurement of transepithelial water transport

Byung-Ju Jin and A.S. Verkman

Departments of Medicine and Physiology, University of California San Francisco, San Francisco, CA 94143, USA

Computation of osmotic water permeability

The transepithelial osmotic water permeability coefficient (P_f, in cm/s) was deduced from *F(x)* for experiments involving a perfused volume marker dye and a transepithelial osmotic gradient. The total mass of injected dye is conserved along the channel is constant,

$$C(x)\,Q(x) = C_o\,Q_o \tag{1}$$

where C is dye concentration and Q is flow rate, and C_o and Q_o are dye concentration and flow rate at the channel inlet. The osmotic volume flux from the channel to the Snapwell compartment (J_v) is,

$$J_v = P_f\,v_w\,(W\,dx)\,\Delta Osm = -dQ \tag{2}$$

where dQ is the change in flow rat in the channel, v_w is the water molar volume ratio (18 cm^3/mol), W is the channel width, dx is the length of the small channel segment, and ΔOsm is the osmotic gradient across the cell monolayer. Equation (2) can be rewritten as,

$$dQ(x)/dx = -P_f\,v_w\,W\,\Delta Osm \tag{3}$$

Differentiating equation (1), $Q(x)\,dC(x)/dx + C(x)\,dQ(x)/dx = 0$, and combining with equation (3),

$$Q(x)\,dC(x)/dx - C(x)\,P_f\,v_w\,W\,\Delta Osm = 0 \tag{4}$$

giving,

$$P_f = Q(x)/C(x)\,/(v_w\,W\,\Delta Osm)\,dC(x)/dx \tag{5}$$

where C(x) is determined from *F(x)*. P_f was calculated from the initial slope of F(x) at x = 0, $(dF/dx)_o$,

$$P_f = Q_o/(v_w\,W\,\Delta Osm)\,(dF/dx)_o \tag{6}$$

Computation of diffusional water permeability

The transepithelial diffusional water permeability coefficient (P_d, in cm/s) was deduced from *F(x)* for experiments involving a perfused D_2O-sensitive dye and a D_2O-containing solution in the Snapwell compartment. The diffusional water flux into the microfluidic channel (J_d) is the same as the D_2O concentration change in the microfluidic channel under isosmolar conditions (in the absence of transepithelial volume flux),

$$J_d = P_d\ (W\ dx)\ \{C_{well} - C(x)\} = Q_o\ dC(x) \tag{7}$$

Equation (7) can be rewritten as,

$$d\{C_{well} - C(x)\}/\{C_{well} - C(x)\} = -\ P_d\ (W/Q_o)\ dx \tag{8}$$

where W is channel width, dx is length of the small segment, Q_o is perfusate flow rate, C is the D_2O concentration in the microfluidic channel, and C_{well} is the (constant) D_2O concentration in the Snapwell.

Integrating equation (8),

$$\ln\ [\{C_{well} - C(x)\}/\{C_{well} - C_o\}] = -\ P_d\ (W/Q_o)\ x \tag{9}$$

which can be rewritten as,

$$\{C_{well} - C(x)\}/\{C_{well} - C_o\}\ =\ \exp\{-\ P_d\ (W/Q_o)\ x\} \tag{10}$$

where C_o is the D_2O concentration in the microfluidic channel inlet, which is zero because of the H_2O_{PBS} perfusate.

The final D_2O concentration profile is thus,

$$C(x)/C_{well} = 1 - \exp\{-\ P_d\ (W/Q_o)\ x\} \tag{11}$$

The D_2O concentration (C(x)) was deduced from measured *F(x)* using the quadratic equation: $F(x) = (0.387\ C(x)^2 + 0.300\ C(x) + 0.307)/0.307$, as reported.[1] P_d was deduced by exponential regression of data to equation (11).

Computational modeling of solute profiles

Finite-element simulations were done using COMSOL Multiphysics (version 3.4). The spatial concentration profiles in the microfluidic channel, $C_A(x,z)$ and $C_B(x,z)$, are determined by diffusion and advection. There was no variation of the spatial concentration profiles in y-direction, (see Supplementary Fig. S3). The model is specified by a diffusion equation describing solute advection-diffusion coupled with the Navier-Stokes equation describing the fluid flow field,

$$\frac{\partial C_i}{\partial t} = \underbrace{-\mathbf{V}\cdot\nabla C_i}_{convection} + \underbrace{D\nabla^2 C_i}_{diffusion} \tag{12}$$

The velocity field, V, in the advection term was computed from the Navier-Stokes equation for an incompressible fluid and the continuity equation,

$$\rho\frac{\partial \mathbf{V}}{\partial t} + \rho(\mathbf{V}\cdot\nabla)\mathbf{V} = -\nabla P + \eta\nabla^2\mathbf{V} \tag{13}$$

$$\nabla\cdot\mathbf{V} = 0 \tag{14}$$

where ρ is fluid density, P is pressure and η is dynamic viscosity.

Solute concentrations are constant at the microfluidic channel inlet, and a constant velocity boundary condition was imposed for Navier-Stokes computations,

$$C_A = C_A^o,\ C_B = C_B^o \tag{15}$$

$$V = Q_o/(a\cdot b) = \text{constant} \tag{16}$$

where C_A is solute concentration and C_B is the fluorescent dye concentration, V is velocity, Q_o is the perfusate flow rate, and a and b are channel width and height, respectively. An advective flux boundary condition was imposed at the microfluidic channel outlet, which allows advective flux to exit the domain,

$$\nabla C_B = 0,\ \nabla C_A = 0 \tag{17}$$

A volume flux boundary condition was imposed at the interface between the microfluidic channel and the Snapwell insert,

$$J_v(x) = P_f v_w S\, \Delta Osm(x) \tag{18}$$

A no-slip boundary condition was imposed at other microfluidic channel walls. The spatial concentration profile was not sensitive to mesh density, with 200,000~400,000 mesh elements used for Fig. 3A.

Hydrostatic pressure-driven water transport

Transepithelial water transport is the sum of osmotic and hydrostatic pressure-driven fluxes, $J_v = L_p (RT \cdot \Delta Osm - \Delta P)$, where J_v is transepithelial water flux, L_p is the hydraulic conductance, P_f is the osmotic water permeability coefficient (related by $P_f = (RT/v_w)L_p$), v_w is the partial molar volume of water, R is the universal gas constant (62.4 mmHg·L/(mol·K)), T is the absolute temperature, ΔP is the pressure difference and ΔOsm is the osmolality difference between the microfluidic channel and the Snapwell. The hydrostatic pressure gradient across the porous filter, ΔP, can be estimated theoretically, as reported.[2] The pressure drop along the microfluidic channel is,

$$\Delta P_{channel} = a\, \mu\, Q_o\, L/(W\, H^3) \tag{19}$$

$$a = 12[1-192H/(\pi^5 W)\tanh(\pi W/(2H))]^{-1} \tag{20}$$

where $\Delta P_{channel}$ is the pressure drop along the microfluidic channel, L is channel length, W is channel width, H is channel height, and a is a dimensionless parameter that depends on aspect ratio, W/H. The pressure drop range is ~1.4 - 28 mmHg for perfusion with 0.5-10 μl/h range in flow rates. The ratio of the hydrostatic pressure-driven flux to the osmotic-driven flux, $\Delta P/(RT \cdot \Delta Osm)$, is << 1 (~0.00027-0.00054 for Q_o = 0.5-10 μl/h, ΔOsm = 300 mM), indicating that pressure-driven water flux is negligible.

Supplementary References

1. M. Kuwahara and A. S. Verkman, *Biophys J.*, 1988, **54**, 587-93.
2. M.J. Fuerstman, A. Lai, M.E. Thurlow, S.S. Shevkoplyas, H.A. Stone and G.M. Whitesides, *Lab Chip*, 2007, **7**, 1479-89.

Supplementary Figure and Video Legends

Supplementary Figure S1. Alternative methods to make tight water contact between the microchannel and the various types of porous membranes (polycarbonate and polytetrafluoroethylene). **A**. Convex microfluidic channel method. The first layer of the microfluidic channel was fabricated by pouring a 10:1 mixture of PDMS base and curing agent on the silicon master, and a second layer was fabricated by placing the first layer on the curved surface of convex optical lens (radius of curvature 5.4 cm). After baking the microfluidic channel, the Snapwell insert was mounted on the curved microfluidic channel with pressure to make tight contact. **B**. Filling of the convex microchannel during continuous perfusion with PBS containing fluorescein dextran (70 kDa) at 20-µL/h flow rate at indicated times. **C**. PDMS glue method. A highly viscous PDMS base (Sylgard 186A, 66700 mPa·s, Dow Corning) was used as a glue to contact the microfluidic channel with the porous membrane. Briefly, the viscous PDMS base solution was painted or spin-coated on a slide glass to make a very thin film upon which the microfluidic channel was placed so that the very thin glue layer adhered to the non-channel area (gaps between channels). **D**. Filling of the PDMS glue microchannel during perfusion with PBS containing fluorescein dextran at 10-µL/h flow rate at indicated times.

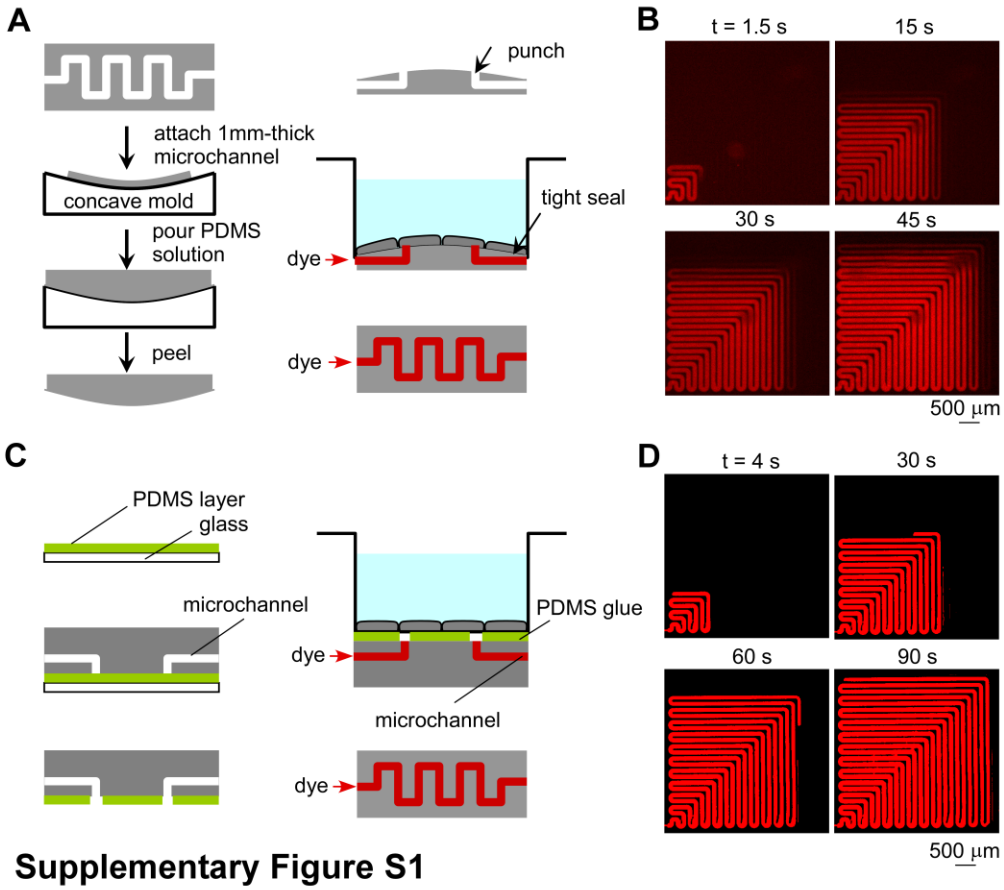
Supplementary Figure S2. Image analysis procedures. **A**. Original fluorescence image. **B**. Image following threshold analysis to visualize only the microfluidic channel. **C**. Eroded image into a skeleton image (channel centerline) used to compute distance from the origin. **D**. Integration method for fluorescence profile computation. *F(x)* was computed using a total of 60 fluorescence intensity points along the 10-cm length channel by area-integration. Additionally, each profile was normalized to its control profile measured prior to replacement of insert PBS with an anisomolar solution.

Supplementary Figure S3. Comparison of 3D and 2D computations. **A**. Schematic with 3D geometry with inlet boundary conditions (C_A = 300 mM, C_B = 10 µM, Q = 0.5 µl/h), advective outlet boundary conditions ($\nabla C_A = \nabla C_B = 0$), and volume flux boundary condition through the 5-mm-long channel bounded by the porous membrane. **B**. Pseudocolor images of three x-z cross-sections at y = 0, 50 and 100 µm for solute A (top) and dye B (bottom) in the microchannel for P_f = 0.001 cm/s, perfusate flow = 0.5 µL/h, ΔOsm = 300 mM, and diffusion coefficients $D_A = 10^{-9}$ m^2/s and $D_B = 5\times10^{-10}$ m^2/s. **C**. Pseudocolor images of three y-z cross-sections at x = 0, 2.5 and 5 mm for solute A (top) and dye B (bottom) for conditions as in Fig. S3B. No variation in fluorescence was seen in the y-direction. **D**. Comparison of 3D and 2D computations shown for dye B. Parameters: P_f = 0.001 cm/s, perfusate flow = 0.5 µL/h, ΔOsm = 300 mM, $D_A = 10^{-9}$ m^2/s and $D_B = 5\times10^{-10}$ m^2/s.

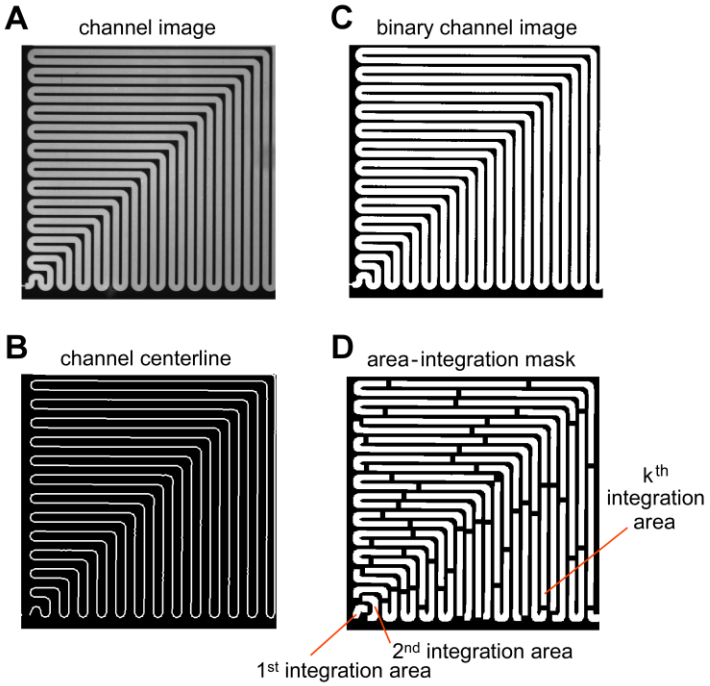
Supplementary Video 1. Filling of the microchannel during continuous infusion of PBS containing fluorescein dextran (500 kDa) at 10 µL/h perfusate flow rate over 2 min 30 s.

Supplementary Video 2. Effect of transepithelial osmotic water transport on particle velocity along the channel in FRT-AQP4 cells (left) and (non-transfected) FRT cells (right). The length of particle streak lines (200-ms exposure) is greatly reduced in FRT-AQP4 cells. The osmotic gradient was 700 mOsm and perfusate flow rate was 2 µL/h.

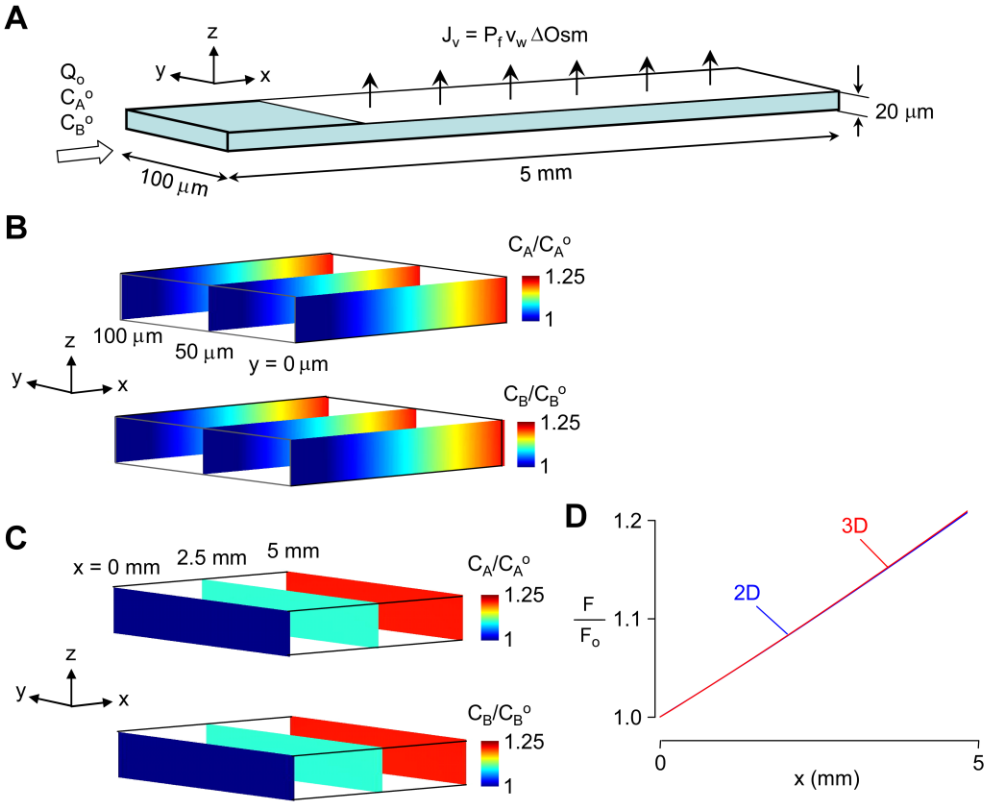
Supplementary Video 3. Filling of a multiplexed microchannel during continuous infusion with PBS containing fluorescein dextran at 10-µL/h perfusate flow rate.



Supplementary Figure S1



Supplementary Figure S2



Supplementary Figure S3