TITLE

Mass and momentum transport in microcavities for diffusion-dominant cell culture applications

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ABSTRACT

For the informed design of microfluidic devices, it is important to understand transport phenomena at the microscale. This letter outlines an analytically-driven approach to the design of rectangular microcavities extending perpendicular to a perfusion microchannel for microfluidic cell culture devices. We present equations to estimate the spatial transition from advection- to diffusion-dominant transport inside cavities as a function of the geometry and flow conditions. We also estimate the time required for molecules, such as nutrients or drugs to travel from the microchannel to a given depth into the cavity. These analytical predictions can facilitate the rational design of microfluidic devices to optimize and maintain long-term, physiologically-based culture conditions with low fluid shear stress.

PAPER

Replenishing nutrients in traditional cell culture systems can induce significant fluid shear not seen *in vivo*, disrupt intercellular signaling and extracellular matrix binding, and alter proliferation and migration behavior [1,2]. With its inherently low Reynolds numbers, *Re*, microfluidics technology has become attractive for establishing appropriate culture conditions by enabling cell culture in microcavities [3,4], *in vitro* differentiation of shear sensitive cells [5-7] and the generation of stable spatiotemporal gradients to study chemotaxis [8-10], among other applications [11,12]. However, they often rely on guesswork or extensive simulations of different geometries and flow conditions to produce the desired microenvironment. In this letter, we derive equations describing the mass and momentum transport in a microcavity extending perpendicular to a perfusion channel, which is the simplest microfluidic geometry considered for creating a diffusion-dominant region in the vicinity of cell cultures with continuous replenishment of nutrients and removal of cellular waste.

Microcavities offer an ideal platform for low-shear diffusion-dominated cell types since they can easily (i) mimic *in vivo* microenvironments, (ii) do not necessarily require complex barriers or membranes, (iii) consume relatively small quantities of culture media and (iv) allow for the precise control of fluid behavior [13,14]. To understand how flow around complex geometries affect nearby cell cultures, we consider the case of flow past a rectangular cavity. Intuitively, a cavity extending perpendicular to the freestream flow will see diminishing advection velocities to a point where they become negligible relative to diffusion rates. We derive an equation for the minimum depth into a cavity where this occurs. While a very long cavity would mostly be diffusion-dominant, it may not be feasible since the time required for the transport of nutrients and waste through long cavities can be prohibitive. Thus, we also present a simple model to predict the time required for molecules in the freestream to reach a given cavity depth.

Fig.1 shows a schematic of the problem formulation, where cells seeded at the bottom of a rectangular microcavity are exposed to a velocity field that decays along the cavity depth, y. At a critical cavity depth, y^* advection velocities become negligible compared to diffusive mass transport. To formulate the problem analytically, we evaluate the local Peclet number, Pe, at the center of the cavity (maximum velocity for a given cross section). The Peclet number,

$$Pe = u \cdot a \cdot D^{-1} \qquad (1)$$

relates the time it takes a particle moving with a velocity, u in the bulk flow to travel a characteristic length, a with the time it takes for that particle to diffuse the same length, where D is the diffusion constant. The transition between advection-dominant and diffusion-dominant mass transport occurs at approximately $Pe = 1$ and is decisively diffusive at $Pe \leq 0.1$

As a representative example, we consider the diffusion of a small molecule ($D \approx 7\text{x}10^{-10}\text{m}^2/\text{s}$) traveling $a = 50$ μm, roughly two cell diameters. For a diffusion-dominant microenvironment, that is $Pe = 0.1$, Eq. (1) yields a critical velocity of $u^* \leq 1$ μm/s. Given this, we are interested in finding an explicit analytical equation relating the overall velocity field to the depth into a cavity, i.e. $u = f(y)$, to ultimately estimate the critical cavity depth, y^* necessary to meet the condition $u \leq u^*$.

Finding y^* requires knowledge of the velocity field, which we derive from cavity flow parameters. We simplified Weiss and Florsheim's solution [15] to the biharmonic equation that assumes low Reynolds number in the streamfunction-vorticity formulation of the Navier-Stokes equations. The model is two dimensional in x and y and assumes infinite thickness ($z = \infty$). In our solution, we: (i) consider only centerline velocities, which is maximum for a given cross section; (ii) eliminate oscillatory terms to isolate the decay profile; and (iii) set the coordinate system origin to the top of the cavity as depicted in Fig. 1a. We find that the velocity decay is given by,

$$u = u_D \cdot e^{-4.24 \cdot y/W} \qquad (2)$$

where u_D is the maximum velocity at the top of the cavity, W is the width of the cavity and y is the length into the cavity. Remarkably, the velocity decay constant only depends on the depth into the cavity and the width of the cavity. Setting $Pe^* = u^*a/D$, substituting u^* for u into Eq. (2) and rearranging yields the critical cavity depth,

$$y^* = \frac{-W}{4.24} \cdot \ln\left(\frac{Pe^* \cdot D}{u_D \cdot a}\right) \qquad (3)$$

We used finite element analysis to verify that with increasing cavity thickness, 3D centerline velocities converged to the 2D solution in Eq. (2). Thus, Eq. (2) represents a worse case in velocity decay as compared to the 3D simulations and therefore, Eq. (3) is a conservative estimate for y^*.

To experimentally validate Eq. (2), we fabricated a microfluidic device by sandwiching double-sided medical grade tape, AR8890 (Adhesives Research, Glen Rock) - with a perfusion channel and a microcavity cutout - between two standard glass microscope sides, per previously developed protocols [16]. The cavity dimensions were $W = 1$ mm, $L = 15$ mm, thickness $d = 200$ μm and main channel height $h = 500$ μm. With a flow rate of $Q = 500$ μL/hr, we measured a maximum velocity at the top of the cavity of $u_D = 1.2$ mm/s [24]. Velocities in Fig. 2a were examined under a fluorescence microscope, Zeiss Axiovert 200 (Zeiss, Gena) by measuring streak lengths of 10 μm-diameter latex particle standards

(Beckman Coulter, Pasadena) after 100 ms exposure at 450 nm excitation. Qualitative observations of the flow field (Fig. 2a) were similar to those published extensively in literature [17-20]. The experimental centerline velocity distribution in the cavity agrees well with the theoretical decay from Eq. 2 as shown in Fig. 2b. The resulting value for $y^* = 1.5$ mm is calculated via Eq. (3), which indicates the critical depth for diffusion-dominant flow.

In order to assess the dynamics of mass transport from the perfusion channel to the cellular microenvironment in the cavity, we propose a simplified model of the transport process. In our model, we assume that molecules travel first, along a streamline primarily by advection t_a and second, from that position to the bottom of the cavity mainly by diffusion, t_d, (Fig 3a). The advection time is estimated by considering a molecule traveling from the entrance of the cavity to the centerline at depth y as $t_a \approx (y^2+0.25W^2)/u(y)$, where substituting $u(y)$ with Eq. (2) yields,

$$t_a = u_D^{-1} \cdot e^{4.24 \cdot y/W} \left(y^2 + 0.25 \cdot W^2\right)^{1/2} \qquad (4)$$

The estimated time required for molecules to travel from y to the bottom of the cavity y^* by diffusion is,

$$t_d = 0.5\left(y^* - y\right)^2 D^{-1} \qquad (5)$$

Since there are as many possible trajectories for nutrient delivery as streamlines into the cavity, the minimum time, t_c required to reach a steady state concentration at y^* is given by the minimum time required to travel through any of the possible paths by advection and diffusion,

$$t_c = \min\left(t_a + t_d\right), \quad 0 \le y \le y^* \qquad (6)$$

Eq. (6) does not have an explicit analytical solution but can be solved using numerical methods, as depicted graphically in Fig. 3b for the transport of fluorescein inside a microcavity of depth of $y^* = 1.5$ mm.

We verified our model by experimentally quantifying the evolution of the concentration profile of fluorescein inside the cavity as it enters from the perfusion channel. Time-lapsed images were acquired with a fluorescence microscope and quantification was determined by measuring the average pixel intensity of a 0.5 mm wide by 0.05 mm tall region at the bottom of the cavity. The experimental steady state value of $t_c = 4.5$ min shown in Fig. 3c agrees well with the analytical model. Equation (6) can also be used to estimate the t.ime required for the delivery of a drug or for the time required to remove waste products secreted by cells.

Additional values for y^* and the corresponding t_c are tabulated in Table I for typical flow conditions and geometries used in microfluidics. To determine if conditions at these values of y^* are physiological for diffusion-dominated, interstitial flow in tissues, we estimate the shear stresses from Eq. (2) using the relation, $\tau = \mu(\partial u/\partial y)$. Resulting stresses are physiologically-relevant to stresses expected in interstitial flow [21,22]. The estimates in Table I provide a quick reference to develop microfluidic designs.

In summary, we derived an equation to predict the transition from advection- to diffusion-dominant regions in a microcavity, which can be used to design devices mimicking *in vivo* diffusion-dominant microenvironments for cell culture. We also derived the time needed to obtain a steady-state concentration of nutrients in the system. Shear stress approximations show that transport conditions in microcavities in the vicinity of cell cultures are similar to physiological behavior of the interstitial flows.

Both equations can be used for the rational design of microcavities for cell culture under diffusion-dominant conditions. Microcavities and similar structures are simple to fabricate, with potential applications in diffusion-dominant cell culture, protein crystallization and applications that require stagnant flow with continuous replenishment of soluble chemicals.

FIGURES

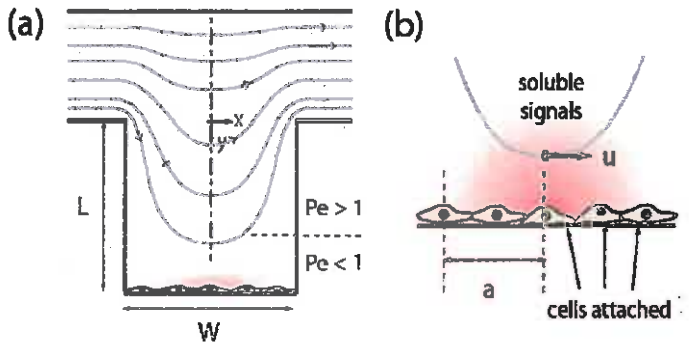

FIG. 1. Problem formulation: cells attached to the bottom of a rectangular microcavity that is perpendicular to the freestream flow in a microchannel. (a) Intuitively, velocity decays as fluid flow enters the microcavity. At $Pe = 1$ advection velocities match rates of diffusion. The physiological range of flow conditions for many cell types occur at $Pe < 0.1$, where mass transport is diffusion-dominant. (b) With the proper geometrical design of microcavities, velocities near the vicinity of cultures should be sufficiently small, as calculated by Pe to ensure that soluble signals are able to travel some characteristic distance, a and are not removed.

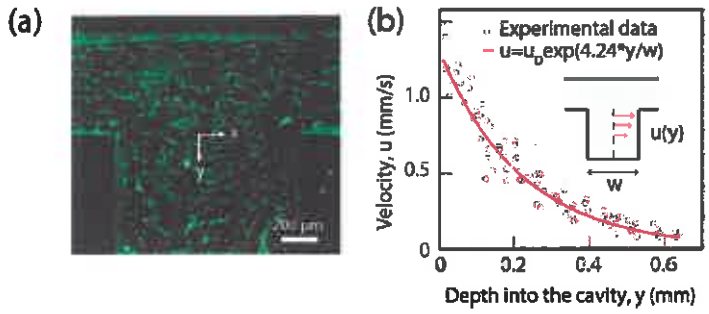

FIG. 2. Experiments were used to validate the analytical model derived for predicting the velocity decay in microcavities. Tracer particles were sufficiently small (>10 times smaller than the smallest cavity dimension) and followed streamlines in the flow. (a) Images of beads flowing at 500 μL/hr from a perfusion microchannel into a 1 mm wide cavity. The figure is a composite of nine independent pictures at 100 ms exposure. (b) Velocities in experiments were obtained by measuring streak lengths, where $n = 90$. Data points correlate well with the analytically-derived curve.

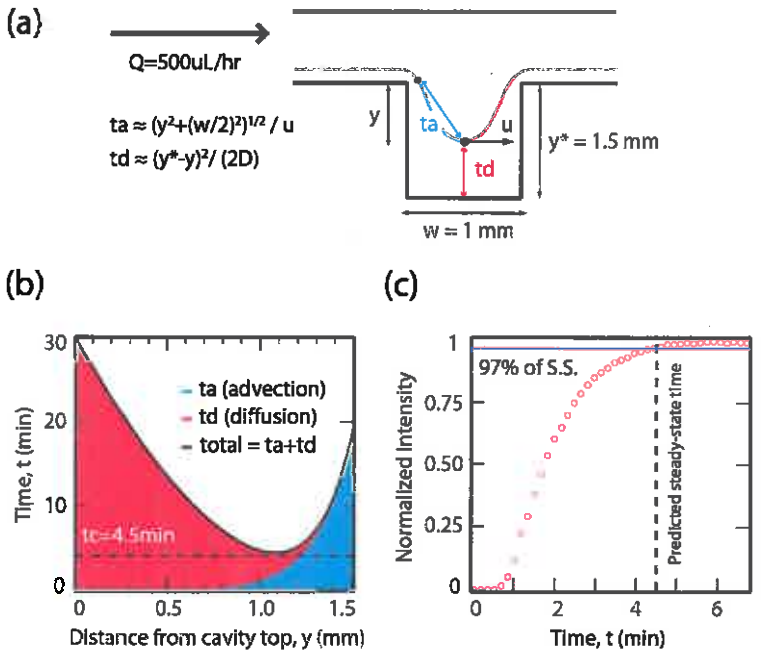

FIG. 3. Experiments of nutrient delivery in microcavities using fluorescein as a representative small molecule to validate Eq. (6). (a) Illustration of the model used to estimate the time required for small molecules to reach cells at y^* from the perfusion channel. The model assumes that first, molecules travel only by advection to the centerline of the cavity, and then only by diffusion to the bottom; the total time for the mass transport through any streamline trajectory can be calculated by adding both contributions. (b) The minimum time required for nutrients to migrate from the freestream to y^* through any possible path is $t_c = 4.5$min. At this minimum, nutrients would travel roughly 1.1 mm by advection and 0.4 mm by diffusion to reach y^* and would roughly reach steady state concentration. (c) For validation, fluorescence intensity at y^* was measured at 10s intervals with 860 ms exposure and 450 nm excitation. In agreement with our estimate from Eq. (6), the intensity reached 97% of the steady state value at 4.5 min.

TABLE I. Estimation of the cavity depth required to generate a diffusion dominant microenvironment for a given velocity at the top of the cavity, u_D and a cavity width, W using Eq. (2-3). The value of t_c estimates the time for the concentration of molecules at the bottom of the cavity to reach steady state. All the values where calculated for fluorescein, where $Pe^* = 0.1$, $D = 7\text{x}10^{-10}$ m^2/s and $a = 50\mu$m. Corresponding shear stresses, τ are physiological for cells exposed to low-shear, interstitial flow.

W(mm)	u_D(mm/s)	y^*(mm)	t_c(min)	τ(dyn/cm^2)
0.2	1	0.310	0.354	3.03×10^{-4}
0.5	1	0.775	1.546	1.20×10^{-4}
1.0	1	1.550	4.550	5.96×10^{-5}
0.2	10	0.419	0.410	3.00×10^{-4}
0.5	10	1.046	1.808	1.19×10^{-4}
1.0	10	2.092	5.361	5.96×10^{-5}

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