Extravasation of Brownian Spheroidal Nanoparticles through Vascular Pores

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ABSTRACT In modern cancer treatment, there is significant interest in studying the use of drug molecules either directly injected into the bloodstream or delivered by nanoparticle (NP) carriers of various shapes and sizes. During treatment, these carriers may extravasate through pores in the tumor vasculature that form during angiogenesis. We provide an analytical, computational, and experimental examination of the extravasation of point particles (e.g., drug molecules) and finite-sized spheroidal particles. We study the advection-diffusion process in a model microvasculature, consisting of a shear flow over and a pressure-driven suction flow into a circular pore in a flat surface. For point particles, we provide an analytical formula

\[ \frac{S}{k} = \frac{1}{\kappa} + \frac{\pi}{4(1 + 0.395^\frac{1}{2})^{2/3}} + 2PQ \]

for the dimensionless Sherwood number \( S \), i.e., the extravasation rate, in terms of the pore entry resistance (Damköhler number \( \kappa \)), the shear rate (Péclet number \( P \)), and the suction flow rate (suction strength \( Q \)). Brownian dynamics (BD) simulations verify this result, and our simulations are then extended to include finite-sized NPs, in which no analytical solutions are available. BD simulations indicate that particles of different geometries have drastically different extravasation rates in different flow conditions. In general, extreme aspect ratio particles provide a greater flux through the pore because of favorable alignment with streamlines entering the pore and less hindered interaction with the pore. We validate the BD simulations by measuring the in vitro transport of both bacteriophage MS2 (a spherical NP) and free dye (a model drug molecule) across a porous membrane. Despite their vastly different sizes, BD predicts extrava-

\[ S_{\text{exp}} = 10.6 \times 10^{-4} \pm 1.75 \times 10^{-4} \text{ and } S_{\text{exp}} = 16.3 \times 10^{-4} \pm 3.09 \times 10^{-4}, \text{for MS2 and free dye, respectively, thus demonstrating the practical utility of our simulation framework.}

INTRODUCTION

Advancements in manufacturing techniques on the micro- and nanometer scale have permitted printing of nanoparticles (NPs) of a custom shape and size (1). NPs are able to evade biological/biophysical barriers because of their size and deliver payloads more efficiently compared to free molecules such as the more studied and well-understood peptides (2–4). Despite these advantages over conventional carriers, there is only a qualitative understanding of the physical mechanism of NP transport. Certainly, improving the still-low efficiency (5) of delivery to disease sites like cancer could lead to superior therapeutic response and imaging diagnostics. Reported rates of delivery are as low as a median of 0.7% for some NPs, and there is evidence that shape is a major factor (rates can be as high as 15% injected dose per gram tissue for NPs such as single-walled nanotubes (SWNTs)) (6).

There are a variety of ways to transport NPs, for example, via ingestion by circulating tumor-associated phagocytes (7,8); however, these methods may be specific to the tumor and may not be suitable for other tumors. Another promising mechanism for transporting NPs to solid tumors is via the process of extravasation, which we describe below. The abnormal and haphazard growth of the endothelial cell layer comprising the inner lining of tumor blood vessels leads to the formation of small pores (\( O(100) \) nm in size) between the endothelial cells (9). NPs may traverse these pores and reach the interstitial sites in tumors where they can then deliver the drug to cancer cells or enable imaging. Tumors and their vasculatures are quite heterogeneous (9), and thus, the success of this process depends on the tumor flow conditions, porosities, and vascular geometries. For example,
there may be pressure gradients (10,11) present across the pore, inducing secondary flows, which create suction.

Drug delivery via extravasation is a multistep process. The NP carriers are loaded with the cargo using appropriate mechanisms (12) and injected into the blood stream of the subject. The carriers then circulate through the network of blood vessels. In any blood vessel, one observes a shear-induced margination of particles smaller than red blood cells toward the periphery of the vessel (13,14). This migration is driven by collisions with the larger red blood cells. When the particles are hemodynamically trapped in the cell-free periphery, also known as the Fähræus-Lindqvist layer, they may interact with the leaky vasculature in tumors and extravasate through the pores. Once extravasated, they diffuse through the intercellular regions (12) to reach the cancerous cells and deliver the cargo. Thus, the extravasation process involves complex coupled fluid-particle transport on the microscale.

Transport via extravasation is greatly affected by particle size and interactions with the tumor microvasculature (15). In this work, we study the microscale physics of extravasation with respect to basic physical parameters and provide a fundamental understanding of the process of extravasation. Interactions of an NP with the complex flow and vascular geometry near the pore causes the extravasation ability of an NP to depend on its shape and size. This is confirmed by many studies (16), including Chauhan et al. (17) who found that rod-shaped and spherical NPs experienced significantly different uptake in E0771 mammary tumors in mice. Kersey et al. (18) discovered that thin and highly deformable particles were better adapted for extravasation through sieves of different grades. Smith et al. (6) demonstrated the difference in extravasation ability of quantum dots (spherical) and SWNTs, both in living mice and in an in vitro model. In a comparison case study, they showed that quantum dots had a greater uptake in LS174T colon tumor, whereas SWNTs showed increased extravasation in U87MG brain tumor in mice. As inferred from these studies, the dependence of extravasation rate on NP geometry is tumor specific. Optimizing NP geometry for the maximal extravasation rate is critical for the design of effective and targeted treatment. Although there have been previous studies on modeling the extravasation process, e.g., Podduturi et al. (19), Sefidgar et al. (20), and Sinek et al. (21), a detailed investigation of the effect of NP shape and size is lacking. In this study, we use Brownian dynamics (BD) simulations of spheroidal particles to model the rich microscale physics and show how our method may be used to predict uptake rates of NPs.

The remainder of this article is organized as follows. First, in Dimensionless Parameters of the Microvasculature, we introduce the relevant dimensionless parameters in our model of the microvasculature, and we identify the range of values they take in physiological systems. In Singular Perturbation Analysis, we briefly describe the mathematical theory of the transport of point particles. In BD for Point Particles and BD for Finite-Sized Particles, we describe the BD method of simulating both point particles to model the special case of the extravasation of drug molecules and finite-sized spheroidal particles (particles of nonzero size) to model the case of NP-based drug delivery. In Validation of BD Through In Vitro Experiments, we describe in vitro experiments on the transport of unpregylated spherical bacteriophage MS2 (an NP) and free dye (a molecule) across porous membranes. In Flux of Point Particles and Flux of Finite-Sized Particles, we present our results for the BD simulations of point and finite-sized particles, and in Comparison of BD Simulations and Experiments, we compare our experiments with the flux measured from BD. We conclude in the Conclusions with a discussion on how to interpret the results of BD for real applications.

**Materials and Methods**

### Dimensionless Parameters of the Microvasculature

The process of extravasation as described in the Introduction is illustrated in Fig. 1. We first introduce the analytical problem. All physical quantities introduced here, such as material properties, geometric scales, and fluid flow properties are dimensional, whereas all field quantities, such as concentration and position are dimensionless, unless otherwise noted. A schematic of the pore geometry is shown in Fig. 2 A, and we observe that the physics of extravasation occurs on the length scale of the pore radius \( a \). Let \( r \) denote the equatorial radius and \( t \) denote the polar radius along the axis of symmetry \( p \) for a spheroidal particle; a schematic of the particle geometries considered is shown in Fig. 3. We define the dimensionless equatorial and polar radii as follows:

\[
\alpha = \frac{r}{a}, \quad \beta = \frac{t}{a}. \tag{1}
\]

In the following, we introduce the three competing processes that affect the extravasation rate of the particles: imposed flows (i.e., convection), Brownian diffusion, and resistance to extravasation through the pore. We then introduce the important dimensionless numbers that describe the problem.

In our model, we investigate the effects of two physiologically relevant flows: shear and suction flows. We denote the shear rate by \( \dot{\gamma} \), and a dimensionless suction strength \( Q \) can then be defined as follows:

\[
Q = \frac{\text{volumetric fluid flux through pore}}{2\pi\dot{\gamma}a^3}. \tag{2}
\]

The volumetric flow rate is related to the pressure difference \( \Delta p \) across the hole by the relation \( 2\pi a^3 Q = (\Delta p)a^4 / 3\mu \) (22), where \( \mu \) is the fluid viscosity. In addition to the imposed flows, particles move around because of Brownian diffusion. To define the important nondimensional numbers, we use the orientation-averaged diffusivity \( D \), which will be discussed in more detail in BD for Finite-Sized Particles.

To model the mass transfer through the pore analytically, we model the resistance to extravasating through the pore using a mass transfer coefficient \( k \). Instead of modeling the cylindrical pore directly, although we do indeed do that in Comparison of BD Simulations and Experiments when we directly compare simulations with our experiments, this allows us to model the entirety of the pore as a boundary condition at the pore inlet. If the pore is of zero length, i.e., an infinitely thin aperture, particles that enter the pore would extravasate immediately; this case corresponds to an
Infinite $k$. If the pore is of some nonzero length, particles that enter the pore now have the following two options: extravasate or diffuse back into the domain. This case thus corresponds to a finite $k$, representing the fact that there is a probability less than one that particles entering the pore extravasate.

In addition to making the extravasation process analytically tractable, the use of a mass transfer coefficient also allows for the possibility of incorporating hydrodynamic interactions with the pore geometry. The mobility of particles is reduced because of the presence of nearby no-slip surfaces, which affects the diffusion process. In the literature, the reduced mobility inside the pore is often referred to as “hindered diffusion” (e.g., Dechadilok and Deen (23)). Directly accounting for hydrodynamic interactions is a complicated process, and although it certainly can be done, providing a mathematical parallel, in the remainder of the article, we refer colloquially rating hydrodynamic interactions with the pore geometry. The mobility of particles and the pore, there would be a nontrivial relationship between $k$ and the imposed flow, because the mobility of a particle in a pore generally depends on both orientation and position, which imposed flows would affect.

Mathematically, modeling the resistance to extravasating through the pore using a mass transfer coefficient $k$ is equivalent to modeling a first-order reaction or adsorption with rate $k$ at the pore inlet. As a result of this mathematical parallel, in the remainder of the article, we refer colloquially to the mass transport as a “reaction” or “adsorption.” It is important to note that, in reality, after extravasating through the thin pores, drug delivery NPs are still required to navigate the complex tumor interstitium.

We have now introduced the three competing processes (convection, diffusion, and reaction), and we now demonstrate how we compare their relative importance. There are three relevant timescales: the convection timescale $t_c = 1/\gamma$, the diffusion timescale $t_d = a^2/D$, and the reaction timescale $t_r = a/k$. Near the pore, reaction and diffusion are competing processes whereas diffusion and convection compete away from the pore in the bulk. Suction may be present, in which case convection and diffusion may be competing processes around the pore as well. The ratio of the diffusive timescale to the timescale for each of these competing physical processes gives rise to the following dimensionless numbers:

$$P = \frac{\gamma a^2}{D},$$

$$P_Q = \frac{QP}{a},$$

$$\kappa = \frac{ka}{D}.$$  \hfill (3c)

Here, $P$ is the shear-rate-based Péclet number (Eq. 3a). A diffusion dominated process corresponds to a small Péclet number, whereas a convection dominated process corresponds to a large Péclet number. Similarly, we define $P_Q$ as a suction-rate-based Péclet number. It should be noted that from Eqs. 2, 3a, and 3b, the suction Péclet number is independent of the shear rate. The Damköhler number $\kappa$ is the ratio of the diffusion to reaction timescales.

FIGURE 1 (A) Schematic of the extravasation process. NPs migrate to channel periphery and extravasate through pores near tumors. (B) A close-up of a single pore, i.e., the region in the gray circle in (A) is shown. To see this figure in color, go online.

FIGURE 2 Near the pore, one can have both shear and suction flows, which may combine to form rich fluid structures upstream. (A) A schematic of the physical model for mass transport near a pore is shown. The Cartesian coordinate system $(x, y, z)$ is shown, and we also use the polar coordinate system $(\rho, \theta, z)$ (data not shown) in our analysis. (B) A schematic of the capture tube phenomenon is shown. To see this figure in color, go online.
timescales. Small values of $\kappa$ correspond to a diffusion-dominated (or the so-called "reaction-limited") regime, whereas large values correspond to an adsorption-dominated (or "diffusion-limited") regime. Thus, small values of $\kappa$ correspond to large pore-entry resistance (indicative of a large adsorption timescale), and particles may diffuse away before adsorption. At large values of $\kappa$, particles enter the pore before they have time to diffuse away from the pore and into the bulk fluid domain.

In many tumors observed in tumor models, the pore size is $\sim 100-200$ nm in radius (24). Microcirculatory shear rates are $O(1000) s^{-1}$, and particle diffusivity is $O(10^{-11}) m^2/s$ (25). Thus, the Péclet number can be as large as $O(10)$, which implies that bulk diffusion and convection timescales are comparable and thus, they both influence extravasation. As per Shah et al. (25), we estimate that the adsorption coefficient at a physical pore is $\kappa = O(1)$, because of the fact that the pore length is on the order of the pore radius (26,27). The strength of suction flow is related to the pressure difference via the fluid viscosity, and for the values of oncotic pressures of the pore radius (26,27). The strength of suction flow is related to the pressure difference via the fluid viscosity, and for the values of oncotic pressures of the pore radius (26,27).

The Sherwood number $S$ is a dimensionless measure of the extravasation rate (flux) through the pore. As extravasation occurs at the pore, the NP flux is normalized using the pore radius as length scale, the corresponding diffusion timescale, and the bulk concentration of particles $\phi_a$, as

$$S = \frac{\text{flux} \times a}{D \phi_a}. \quad (4)$$

As discussed in the Introduction, in this study, we quantify the dependence of the Sherwood number on the shear-rate- and suction-rate-based Péclet numbers and the Damköhler number in the physiologically relevant regime of values. We first do this for point particles analytically and verify our result using BD simulations. Both the analytical theory and the BD simulations are improvements on, and a logical completion of, the work first developed by Shah et al. (25). The agreement between the analytical solution and point-particle BD simulations gives us the confidence to generalize our point-particle BD simulations to simulations of finite-sized particles, because no analytical solutions are available for that case.

**Singular perturbation analysis**

Consider a polar coordinate frame $(r, \theta, z)$ and a Cartesian system $(x, y, z)$ fixed at the center of the pore in Fig. 2 A. We pose the problem of extravasation of point particles as an advection-diffusion equation of the form (Eq. 5 a), where $\phi$ is the concentration scaled by the bulk concentration $\phi_a$, and all quantities are scaled using the diffusion timescale $t_d$ and the pore radius $a$ as the length scale. Because the equations are linear, we set $\phi_a = 1$.

$$\nabla^2 \phi = P U \cdot \nabla \phi, \quad (5a)$$

$$\nabla \cdot (P \phi - Pu \cdot U) \phi = \kappa \phi \quad \text{at} \quad z = 0, \rho \leq 1, \quad (5b)$$

where $\mathbf{n}$ is a unit vector that points out of the pore entrance. Equation 5 b is a Robin boundary condition describing the balance between the diffusion, reaction (adsorption), and convection at the pore entrance. Equation 5 b, together with the no-flux boundary condition (Eq. 5 c) on the wall, constitutes a mixed-boundary value problem. $P$ and $\kappa$ are the Péclet and Damköhler numbers defined in Dimensionless Parameters of the Microvasculature. The dimensionless bulk velocity field is $U = (U_x, U_y, U_z) = (U_r, U_\theta, U_z)$. Yan et al. (28) showed that this velocity field can be approximated very well by the superposition of shear and Sampson flow fields as shown in (Eq. 6 a).

The expression for the dimensionless Sampson velocity field is given by (Eq. 6 b):

$$U = z \hat{x} + U_z \hat{z}, \quad (6a)$$

$$U_z = U_z \hat{p} + U_z \hat{z}, \quad (6b)$$

where hat symbols denote unit vectors and where,

$$U_z = \frac{3}{4} Q \zeta (R_1 - R_2) \left( \frac{1}{R_1} - \frac{1}{R_2} \right), \quad (7a)$$

$$U_z = -\frac{3}{4} Q \zeta (R_1 - R_2) \left( \frac{\rho - 1}{R_1} - \frac{\rho + 1}{R_2} \right), \quad (7b)$$

$$R_1 = \left( z^2 + (\rho - 1)^2 \right)^{1/2}, \quad (7c)$$

$$R_2 = \left( z^2 + (\rho + 1)^2 \right)^{1/2}, \quad (7d)$$

$$\zeta = \left( 1 - \frac{1}{4} (R_2 - R_1)^2 \right)^{1/2}. \quad (7e)$$

We have the no-slip condition $U_z = 0$ at the wall $(z = 0, \rho \leq 1)$ and over the pore $(z = 0, \rho \leq 1)$, $U_z = (0, 0, -3Q(1 - \rho^4)^{1/2})$. When $Q = 0$, the flow field is simply $U = z \hat{x}$. The superposition of the shear and Sampson flows causes the streamlines to form a capture tube upstream. This is a surface that separates the streamlines that enter the pore from those that do not, as illustrated in Fig. 2 B. The concept of the capture tube will be useful in explaining some of the numerical results for finite-sized particles in later sections.

The Sherwood number is the flux through the pore, normalized with the characteristic length scale and diffusivity. Without loss of generality, we set $\alpha = 1$. The Sherwood number is then a function of $\kappa$, $P$, $Q$ and expressed as an area-averaged flux in the integral form,

$$S(\kappa, P, Q) = \frac{1}{\pi} \int_{\text{pore}} \left( \frac{\partial \phi}{\partial z} - P U_z \phi \right) \, dA. \quad (8)$$

We observe that there is a contribution to the flux from both diffusion and convection processes. Previous analytical (29) and computational studies (30) of the same problem assumed instant adsorption ($\kappa \gg 1$ and $Q = 0$). The analysis by Shah et al. (25) lifts this restriction as the Sherwood number was expressed in terms of the Péclet number to $O(P^{1/2})$, with
coefficients involving $\kappa$. Approximations using the method of resistances were developed to easily compute the Sherwood number from $\kappa$ and $P$. In this work, we extend the perturbation theory approach to include terms to $O(Q)$ and obtain the following result:

$$S_{opt}(\kappa, P, Q) \approx \left[ 1 + \frac{\pi}{k} \frac{4}{(1 + 0.3959 P^{1/2})^{2/3}} \right]^{-1} + 2PQ. \quad (9)$$

The algebraic details of the derivation are given in the Supporting Materials and Methods. In Flux of Point Particles and Flux of Finite-Sized Particles, we will see that Eq. (9) is surprisingly accurate for a wide range of $P$, $Q$, and $\kappa$, even though it is only formally valid when $P \ll 1$ and $PQ \ll 1$. The nature of Eq. (9) warrants a discussion here. A striking aspect of Eq. (9) is the suction term on the right-hand side, $2PQ = 2QP$: the suction strength $Q$ affects the Sherwood number linearly and independently of the Damköhler number. This is attributed to the fact that the capture tube transports all the particles and fluid inside it to the pore. The fluid flux, and thus the flux of particles caused by a flow field $PU = -3PQ(1 - \rho^2)^{1/2}$ over the pore ($\rho < 1$), is exactly $2QP$. In addition, we have an advection-diffusion process transporting particles from the bulk to the pore at a rate equal to the first term in Eq. (9). Suction-based transport and adsorption dynamics at the pore compete with each other. When suction is sufficiently strong, the flux is dominated by $PQ$ as seen in Eq. (9).

**BD for point particles**

We now describe the BD algorithm as a general simulation tool for extravasation processes. First, we describe the BD algorithm for point particles. This algorithm is a major improvement over the one in Shah et al. (25) because one can now easily simulate Robin boundary conditions, coupled with any flow condition. Then, we introduce the BD algorithm for a general spheroidal particle, which reduces to the point-particle BD in the limit of vanishing particle size.

As shown in Fig. 4, the simulation is set up in a large rectangular box ($60 \times 60 \times 30$) with the bottom face being a wall with a circular pore of unit radius. We introduce a large number of Brownian particles ($N = 400,000$) randomly distributed inside the box and apply the flow described by Eq. (6a). These Lagrangian particles are advanced by dimensionless Langevin equations of motion that incorporate the stochastic nature of Brownian motion,

$$d\mathbf{x} = P\mathbf{U}(\mathbf{x})dt + \sqrt{2}d\mathbf{W}. \quad (10)$$

Here, $d\mathbf{W}$ is a vector of Gaussian random variables with zero mean and variance $dt$. The time step is $dt$, the change in position vector is denoted by $d\mathbf{x}$, and $\mathbf{U}$ is the flow velocity (Eq. 6a). During the course of a simulation, a particle hitting the bottom wall is reflected elastically back into the domain. If it hits the circular pore, it is reflected elastically with a probability $(1 - \kappa \sqrt{\pi dU})$ or terminated (extravasated) otherwise. This rejection-sampling condition is equivalent to (Eq. 5b) (31). To ensure a constant number of particles in the domain, we impose periodic boundary conditions in the wall-parallel directions and reintroduce particles randomly if they are adsorbed. Any particle that moves above and beyond the top face is reintroduced in the domain at a random location near the top face. The Sherwood number is given by the rate of particles adsorbing at the pore normalized by the far-field concentration estimated from the distribution of particles in the domain.

**BD for finite-sized particles**

An advantage of the BD approach to understanding particle motion is the ability to easily model additional physics such as external force fields and electrostatic interactions or more realistic scenarios such as particles having a finite size and shape. Here, we generalize the point-particle BD algorithm from the previous subsection to model the motion of general spheroids. Each spheroidal particle has a center of mass vector $\mathbf{x}$ and is associated with an orientation vector $\mathbf{p}$ that points in the direction of the axis of symmetry as seen in Fig. 3, except for a sphere, which has no orientation. For a spheroid of equatorial radius $r$ and polar radius $t$ along the axis of symmetry, one can define an aspect ratio $e = \min[r, t]/\max[r, t]$ and an eccentricity $\epsilon = \sqrt{1 - e^2}$. By varying $e$ or $e$, our BD algorithm models a wide variety of axisymmetric shapes ranging from flat disks to thin rod-like particles.

Spherical particles have anisotropic diffusivity that depends on $(e, e)$. Both prolate and oblate spheroids have a greater tendency to diffuse along their longer dimension as compared to perpendicular to it. The anisotropic diffusivity of general spheroids can be decomposed into a parallel diffusivity $D_\parallel$ in the direction of the axis of symmetry and a perpendicular diffusivity $D_\perp$ in the cross-sectional plane perpendicular to the axis of symmetry. For a spheroid,

$$D = D_\perp (1 - pp) + D_\parallel pp, \quad (11)$$

where

$$D_\perp = \frac{k_BT}{6\pi \mu a^2 \beta_0 Y^3}, \quad (12a)$$

$$D_\parallel = \frac{k_BT}{6\pi \mu a^3 \beta_0 X^3}, \quad (12b)$$

where $k_B$ is the Boltzmann constant, $\mu$ is the fluid viscosity, and $T$ is the fluid temperature. We define the ratio of the maximal dimension of the spheroid to the pore diameter as $\beta_0 = \max[r, t]/a$. For the remainder of this article, we refer to particles with the same maximal dimension colloquially as having the same size. Geometric form factors $X^3$ and $Y^3$ can be found in Kim and Karrila (32), and their functional dependence on $(e, e)$ have been tabulated in Table S1. For spherical particles, $X^3 = Y^3 = 1$, one recovers the Stokes-Einstein isotropic diffusivity. For prolate spheroids, in the limit as $e \to 0$, we recover the slender body diffusivity (33) for rod-shaped fibers:

$$D_\perp = k_BT \ln[1/e]/8\pi \mu a. \quad D_\parallel = k_BT \ln[1/e]/4\pi \mu a.$$ 

The extravasation process occurs on the diffusion timescale based on translational diffusivity, and as mentioned before, the Péclet number $P$ is defined by Eq. 3a using an orientation-averaged translational diffusivity $D = D_\parallel/3 + 2D_\perp/3$. The ratio of this mean diffusivity to the translational diffusivity along $\mathbf{p}$ is denoted by $f$, i.e.,

$$D = fD_\parallel, \quad (13)$$
The orientation vector undergoes a rotational diffusion, i.e., diffusion in the orientation space, which is the surface of the unit sphere in $\mathbb{R}^3$. The rotational diffusivity is given by

$$d_r = \frac{k_B T}{8\pi \mu a^3 \tilde{\rho}_0 Y_C},$$

(14)

where $Y_C$ is another geometric factor tabulated in Table S1. One can define a rotational diffusion-based Péclet number $P_r$, and its associated suction Péclet number:

$$P_r = \frac{\dot{\gamma}}{d_r}, \quad P_{0r} = QP_r.$$

(15)

The dimensionless equations of motion are obtained by inverting the equations for drag force and torque on a general spheroid translating and rotating about its center of mass through a local background flow $U$, strain rate $\mathbf{E}$, and vorticity $\mathbf{\Omega}$ (32).

$$\mathbf{d}x = P \mathbf{U} dt + \sqrt{\frac{2}{f} (\mathbf{p} + \sqrt{\mathbf{R}_3} (1 - \mathbf{p}))} \cdot d\mathbf{W}$$

and

$$d\mathbf{p} = P (\mathbf{\Omega} \times \mathbf{p} + \mathbf{R}_3 (1 - \mathbf{p}) - \mathbf{E} \cdot \mathbf{p}) dt + \frac{1}{\tilde{\rho}_0} \sqrt{\mathbf{R}_2} \left( \sqrt{2} (1 - \mathbf{p}) \cdot d\mathbf{W} - 2 \rho dt \right).$$

(16a)

(16b)

The factors $\mathbf{R}_{1,2,3}$ (Table S1) are geometric factors that arise after rescaling physical quantities using the timescale based on orientation-averaged diffusivity and the length scale equal to the pore radius. The center of mass $\mathbf{x}$ of the spheroid is evolved according to Eq. 16a. Anisotropic translational diffusion of $\mathbf{x}$ is accounted for by the anisotropic diffusion terms in Eq. 16a, where $d\mathbf{W}$ is a vector of random normal variables with zero mean and variance $dr$. The orientation $\mathbf{p}$ of the spheroid is evolved according to Eq. 16b. We recognize the drift in Eq. 16b from Jeffery’s equation, whereas the diffusion term accounts for the diffusion of $\mathbf{p}$ on the surface of a unit sphere. $(1 - \mathbf{p}) \cdot d\mathbf{W}$ is the Brownian process on the plane tangent to the surface of the unit sphere in $\mathbb{R}^3$ and is simulated by a discrete random walk of variance $dt$. The $-2pdp$ term ensures that $\mathbf{p}$ remains on the unit sphere. We note that simulating the orientation dynamics is only relevant for nonspherical spheroids.

The adsorption/reaction dynamics for finite-sized particles is identical to that of point particles. In other words, the probability of exiting the pore upon contact is also $\kappa \sqrt{\pi dt}$. However, in the case of finite-sized particles, the pore is treated as a cylindrical tube of length that is half the maximal dimension of the particle considered, and particles are allowed to exit at the bottom end of this tube. Physically, this corresponds to the case in which at least half the volume of the particle is considered; this allows us to consistently compare the Sherwood number for the different particles. It is assumed that steric effects dominate the lubrication interactions between the cylindrical pore and the finite-sized particles, and excluded volume effects between particles and the surface are modeled as elastic collisions. To simulate cases with $Q > 0$, the flow within the tube is set to $U = (0, 0, -3Q(1 - I_{\rho}^{1/2})$ to match the Sampson flow solution at the pore inlet ($z = 0, \rho \leq 1$). We note that this is an approximation to the real velocity field, because the Sampson flow solution is only formally valid for flow through an infinitely thin aperture.

We run an ensemble of at least 10 simulations for 4E5 particles with a dimensionless time step of 1E–3 to 1E–4 for point particles and 4E–4 for finite-sized particles, for a total duration of 100 time units. The SD in the Sherwood number in our simulation is usually less than 5% from the mean and is controlled by the ensemble size, time step, and number of particles. The chosen parameter values and number of simulations in an ensemble allow us to achieve high-accuracy results in a reasonable amount of computer runtime.

**Validation of BD through in vitro experiments**

To demonstrate the practical utility of our simulation framework, we also conduct experiments to quantify the flux of both unpegylated spherical bacteriophage MS2 and free dye across a porous membrane. These are of drastically different sizes, where the free dye represents the point-particle limit. We note that related experiments have been performed by Smith et al. (6), but these experimental results have not been compared to simulations.

Bacteriophage MS2 has been shown to be a highly promising candidate as a drug delivery and imaging NP (34,35). We seek to measure the rate of transport of MS2, which is approximately a spherical particle with $r = 13.5$ nm, across a porous track-etched membrane, and for comparison, we also repeat the experiments with free dye. The MS2 were manufactured by the Francis Lab at the University of California, Berkeley (the fabrication procedure is described in Elsöhl et al. (35), and the MS2 is labeled with Alexa Fluor 680 Maleimide, and Alexa Fluor 594 was used as the free dye (Fisher Scientific, Hampton, NH). We fabricate devices to conduct these experiments. Fig. 5A shows a schematic of the device used in the experiments: two slabs of acrylic, each with a chamber of volume $V_{in} = V_{out} = 196 \mu L$ cut out, are separated by a track-etched porous membrane. Fig. 5B shows a picture of the experimental device: the slabs are secured together tightly using bolts and C-clamps to ensure that fluid does not leak around the membrane and that the two chambers are aligned with each other. The membrane we employ here is a Whatman Nuclepore track-etched polycarbonate membrane (General Electric Healthcare Companies, Chicago, IL), and the area of exposed membrane is $A \approx 19.63 \text{ mm}^2$. The thickness, pore density, and pore size of the membrane is determined through scanning electron microscopy (SEM) imaging. The thickness of the membrane was measured through Ga ion beam milling at 30 kV by a focused ion beam (Helios Nanolab 600i; FEI Company, Hillsboro, OR), and we determine that the membrane thickness is $L_z \approx 9.6 \mu m$. Both plan-view (Fig. 5C) and cross section view (Fig. 5D) images were taken using a focused ion beam (Helios Nanolab 600i) with a 2-kV electron beam. We determine that the pore radius is $a \approx 30.01 \text{ nm}$ (with an SD of 0.94 nm), and the pore density is $\rho \approx 6.7 \times 10^6 \text{ pores/cm}^2$. The pores of the membrane are well defined and nearly circular, as seen in Fig. 5C, although occasional overlapping pores are observed, as seen in Fig. 5E.

The experimental procedure is as follows. At time $t = 0$, one chamber is loaded with a known concentration $C_0$ of fluorescently dye-conjugated MS2 or free dye, and the other chamber is filled with blank phosphate-buffered saline (PBS) solution (pH 7.4). We use $\phi_m = 600 \text{ mM}$ for the MS2, whereas we use $\phi_m = 30 \text{ mM}$ for the free dye. Each chamber has two small tubes connected to it (see Fig. 5, A and B). After the two chambers are sealed together with the membrane inside, the fluids are introduced into each chamber through these tubes using a syringe. The PBS is first introduced into the “out” chamber, and then the MS2 or free dye is introduced into the “in” chamber, to minimize mixing through the membrane. In a previous iteration of the experiment, we observed that no undesired mixing occurred between the two chambers during the introduction of fluid, because we measured negligible fluorescence intensity at $t = 5$ min in the chamber with PBS. The device now sits in the dark, so as to not damage the fluorescent dye. After a known amount of time (approximately 1 day for the MS2 and 1 h for the free dye; tabulated in Table 1), the PBS that was formerly free of MS2 or dye is extracted from the device using a syringe through the same tube through which the fluid was introduced. The second tube connected to each chamber is present to allow air to escape/enter while the chamber fluid is being introduced/extracted.

The fluorescence intensity of the extracted solution is measured (Horiba FluoroLog-3; Horiba Instruments, Edison, NJ) in a quartz cuvette ($3 \times 3 \text{ mm}, 45 \mu L$; Hellma Analytics, Müllheim, Germany). This intensity
is mapped to a concentration $f_{out}$ using a calibration curve constructed from the measured fluorescence intensities of known concentrations, and we carefully take into account the small amount of free dye that is present in the MS2 solution by filtering the MS2 through centrifugation (Amicon Ultra-0.5 mL Centrifugal Filter; EMD Millipore, Burlington, MA) and measuring the fluorescence intensity of the MS2-free solution. This mapped concentration and the known elapsed time is then used to determine the flux of particles. At the end of the experiment, the device is then disassembled and cleaned with deionized water and methanol. The used membrane is discarded, and a fresh membrane is used for every experiment. To ensure the accuracy of our experiments with MS2, we conduct two additional tests. First, using dynamic light scattering (Zetasizer Nano ZS90; Malvern Instruments, Malvern, United Kingdom), we confirm that the MS2 is not aggregating over the course of an experiment (see Fig. S2). Second, we confirm that an insignificant amount of MS2 sticks to the walls of the device by checking that the sum of concentrations in the “in” and “out” solutions remains the same before and after the experiment. In doing this, we observe that our calibration procedure results in approximately a 5% error. More details on these tests are described in the Supporting Materials and Methods, Section 3.

### TABLE 1: Experimental Data for MS2 and Free Dye Diffusion Experiments

<table>
<thead>
<tr>
<th></th>
<th>$\Delta t$ [h]</th>
<th>$f_{out}$</th>
<th>$S_{exp}/S_{in}$ [ms$^{-1}$]</th>
<th>$S_{exp}$</th>
<th>$S$ (BD)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MS2 experiment 1</strong></td>
<td>22.33</td>
<td>39.9 nM</td>
<td>4.61 E$-7$</td>
<td>8.71 E$-4$</td>
<td></td>
</tr>
<tr>
<td><strong>MS2 experiment 2</strong></td>
<td>22.15</td>
<td>48.4 nM</td>
<td>5.52 E$-7$</td>
<td>10.4 E$-4$</td>
<td></td>
</tr>
<tr>
<td><strong>MS2 experiment 3</strong></td>
<td>21.67</td>
<td>46.0 nM</td>
<td>5.36 E$-7$</td>
<td>10.1 E$-4$</td>
<td>8.53 E$-4$</td>
</tr>
<tr>
<td><strong>MS2 experiment 4</strong></td>
<td>21.50</td>
<td>57.4 nM</td>
<td>6.84 E$-7$</td>
<td>12.9 E$-4$</td>
<td></td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td>NA</td>
<td>NA</td>
<td>5.58 E$-7$ ± 9.27 E$-8$</td>
<td>10.6 E$-4$± 1.75 E$-4$</td>
<td>[7.74 E$-4$, 9.74 E$-4$]</td>
</tr>
<tr>
<td><strong>Dye experiment 1</strong></td>
<td>1.17</td>
<td>3.24 $\mu$M</td>
<td>1.38 E$-5$</td>
<td>14.8 E$-4$</td>
<td></td>
</tr>
<tr>
<td><strong>Dye experiment 2</strong></td>
<td>1.00</td>
<td>3.91 $\mu$M</td>
<td>1.95 E$-5$</td>
<td>20.9 E$-4$</td>
<td></td>
</tr>
<tr>
<td><strong>Dye experiment 3</strong></td>
<td>1.08</td>
<td>2.85 $\mu$M</td>
<td>1.31 E$-5$</td>
<td>14.0 E$-4$</td>
<td>27.6 E$-4$</td>
</tr>
<tr>
<td><strong>Dye experiment 4</strong></td>
<td>1.00</td>
<td>2.92 $\mu$M</td>
<td>1.45 E$-5$</td>
<td>15.6 E$-4$</td>
<td></td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td>NA</td>
<td>NA</td>
<td>1.52 E$-5$ ± 2.89 E$-6$</td>
<td>16.3 E$-4$± 3.09 E$-4$</td>
<td></td>
</tr>
</tbody>
</table>

Initially, the inlet concentration for MS2 is $f_{in} = 600$ nM and for the free dye (Alexa Fluor 594) is $f_{in} = 30$ $\mu$M. The outlet concentrations shown are measured after the experiments. The results from BD simulations are also shown in the last column. For the MS2, the Sherwood numbers measured using BD simulations are also reported for slightly larger and slightly smaller pores, corresponding to ± one SD of the membrane pore radius as measured by SEM; these results are shown in square brackets.
RESULTS AND DISCUSSION

Flux of point particles

The three dimensionless numbers $P$, $\kappa$, and $Q$ completely determine the extravasation rate. In this section, we compare the extravasation rates from BD to those predicted by the analytical result (Eq. 9).

The effect of Péclet number $P$ and Damköhler number $\kappa$

In Fig. 6A, the Sherwood number $S(k, P, Q = 0)$ is plotted. The analytical result (Eq. 9) with $Q = 0$ was validated using accurate boundary element simulations by Shah and Shaqfeh (36) as well as the singular perturbation theory of Shah et al. (25). We note that the BD simulations are in agreement with (Eq. 9) even when $P = O(10)$, but the two must deviate from each other eventually because of periodic boundary conditions on a finite-sized box, and because Eq. 9 is valid only when $P \ll 1$. At $P \ll 1$, diffusion dominates the bulk physics, and the growth in extravasation rate with the Péclet number is quite slow. When the Péclet number is $P = O(10)$, the Sherwood number is $O(P^{1/2})$. At very large Péclet numbers ($P = O(100)$), the mass transport boundary layer over the pore is fully developed, and the Sherwood number has $P^{1/3}$ power law dependence associated with the Graetz Lévêque boundary layer (also see Shah and Shaqfeh (36)).

As explained in Shah et al. (25), in the reaction-limited regime of the Damköhler number ($\kappa \ll 1$), the reaction (adsorption) process dominates any other physical process near the pore and the Sherwood number is given by the rate of reaction (adsorption), $S(k \ll 1, P, 0) = \kappa$. In the diffusion-limited regime ($\kappa \gg 1$), the Sherwood number asymptotically approaches an upper bound $(4/\pi)(1 + 0.3959P^{2/3})^{2/3}$ that depends only on the Péclet number. The BD simulation predicts the Sherwood number correctly in the regime that encompasses the physiologically relevant values of $\kappa$ and $P$.

The effect of suction flow strength $Q$

The boundary element simulations of Shah and Shaqfeh (36) and the BD simulations of Shah et al. (25) both do not account for the case $Q > 0$. We perform point-particle BD simulations and compare the effect of this additional pressure gradient-driven flow field with theory Eq. 9. The comparison is shown in Fig. 6, B and C for suction strengths $Q = 1$ and $Q = 5$, respectively, representing physiologically moderate and large values of $Q$. The Sherwood number from BD and from Eq. 9 agree rather well when the Damköhler number is large, and the flux profile is indeed linear with $PQ$. At small values of $PQ$, we expect Eq. 9 to be incorrect as suction and adsorption dynamics balance each other in this regime. This explains the mismatch around $P = 1$ in the predictions of Eq. 9 and BD in Fig. 6, B and C.

BD simulations thus capture the entire range of physics and are in agreement with Eq. 9 except in the case of small suction strength and Damköhler number. The theoretical result Eq. 9 is therefore not universally valid but undoubtedly useful in understanding the qualitative trends and effects of the various dimensionless parameters. The results of this section alone are valuable in understanding molecular drug transport in the microvasculature.

Flux of finite-sized particles

We obtain simulation results for the Sherwood number for the following three representative particle geometries: 1) spherical particles ($\epsilon = 1$), 2) slender rod-shaped particles (prolate spheroid with $\epsilon = 0.1$), and 3) flat disk-shaped particles (oblate spheroid with $\epsilon = 0.1$). We will refer to these geometries colloquially as spheres, rods, and disks, respectively. We fix the Damköhler number to $\kappa = 300$ because the trends will be qualitatively similar for other values of $\kappa$. We discuss the influence of size, shape (aspect ratio), and suction strength on the Sherwood number. In the limit of vanishing size, the Sherwood number predicted by finite-sized particle BD is identical to that predicted by point-particle BD, as confirmed in the Supporting Materials and Methods. In the following sections, we refer to $\beta_0 = 0.0001$ as the point-particle limit of a spheroid. The largest spheres and disks we simulate

![Figure 6](image-url) Simulation of the point-particle flux through a pore with varying suction. The Sherwood number is plotted as a function of the Péclet number for $\kappa = (0.1, 1, 10, 300)$. The continuous line is the approximate perturbation result (Eq. 9), and the filled circles with error bars are the point-particle BD results. (A) shows $Q = 0$, (B) shows $Q = 1$, and (C) shows $Q = 5$. To see this figure in color, go online.
are $\beta_0 = 0.7$, and the longest rod-shaped particles we simulate are $\beta_0 = 1.5$.

The effect of particle geometry and Péclet number $P$

Although all three particles demonstrate qualitatively similar trends, there are important differences in the extravasation ability of the different NPs. These differences arise from the different aspect ratios for each geometry. Spherical particles can clog the pore with an increase in size. Rod-shaped particles are slender and have greater flux compared to spheres of the same size. Compared to spheres of the same size, disks are flatter and thus see an effectively larger pore cross section, but they are not as effective as rod-shaped particles. The effect of particle geometry is more and more pronounced as the size increases, as seen in Fig. 7 A. Elongated particles appear to have the best extravasation ability, with all other dimensionless parameters equal.

In general, finite-sized particles diffuse through an effectively smaller cross-sectional area of the pore, and thus their flux is smaller compared to the point limit ($\beta_0 = 0.0001$), as seen in Fig. 7 A. Steric hindrance at the pore entrance dominates the effect of the shear flow on the finite-sized particle flux as compared to the point-particle limit. For particles much smaller than the pore, shear flow mildly enhances the particle flux. For large particles, diffusion and excluded volume interaction are the dominant processes at physiologically relevant values of $P$. As size is increased (e.g., $\beta_0 = 0.7$), spherical particles experience a drop in flux with increasing shear rate. Unlike spherical particles, this trend of flux reducing with shear rate is evident in rod-shaped particles only at larger sizes. For spheres and disks larger than the pore, the flux is simply zero. In contrast, for rods larger than pore diameter (e.g., $\beta_0 = 1.5$ in Fig. 7 A), the flux is nonzero but drops quickly with increasing Péclet number. The reduction in flux with increasing Péclet number is due to increased steric hindrance at the pore. It is also noteworthy that the flux of rod-shaped particles can be as large as four times that of spherical particles and twice that of disk particles of the same size (for example, $\beta_0 = 0.7$ in Fig. 7 A).

The effect of particle geometry and suction flow strength $Q$

With suction flow, we notice that the flux of all particles increases linearly at large values of the Péclet number and thus the suction Péclet number $P_Q$; Fig. 7 B shows the case in which $Q = 1$. This is quite similar to the phenomenon observed for point particles and indeed reflected in the plot for $\beta_0 = 0.0001$ for each geometry in Fig. 7 B. The linear growth of flux can be explained readily by the capture tube phenomenon. The fluid capture tube essentially traps and transports particles upstream directly into the pore under sufficiently strong flow conditions. For spheres and disks of size comparable to the capture tube dimension and pore size, steric interaction with the pore increases the possibility of particles escaping the capture tube. Thus, as compared to rods, the flux of these particles is smaller. Rod-shaped particles that are entangled with the capture tube align with the streamlines and thus align with the pore, almost irrespective of their sizes and thus result in the maximal flux among the three geometries we consider. Consequently, any steric issues with rod-shaped particles do not appear to have an effect until the particle size becomes very large. Under extreme suction conditions ($P_Q \gg 1$), steric hindrance is

![FIGURE 7](image-url) The Sherwood number predicted by BD simulation with $\kappa = 300$ is plotted as a function of the Péclet number for various dimensionless sizes ($\beta_0$) of spheres, rods, and disks. The cases of (A) pure shear flow ($Q = 0$) and (B) shear with suction flow ($Q = 1$) are shown. To see this figure in color, go online.
overcome, and the flux for any particle grows with the same linear profile.

Imposing a suction flow of strength $Q = 1$ highlights the mechanics of elongated particle transport, and it deserves a more detailed discussion. In Fig. 7B, we see that as the suction Péclet number increases, rods with size comparable to but smaller than the pore diameter are eventually driven through the pore at approximately the same rate ($S = 39.2 \pm 0.22$ at $P = 20$ for $\beta_0 = 0.7$) as much shorter particles ($S = 39.5 \pm 0.25$ at $P = 20$ for $\beta_0 = 0.3$). In contrast, longer rods ($\beta_0 = 1.5$) have a smaller flux ($S = 33.9 \pm 0.29$ at $P = 20$) because the rotation and suction-based Péclet number $P_{Qr}$ is $\sim O(1)$ (recall Eq. 15). This leads to increased interaction between the rods and the pore entrance caused by rotational diffusion, contributing to higher steric hindrance. As the suction flow becomes stronger, $P_{Qr}$ grows larger, and suction overcomes rotational diffusion, leading to the linear growth in flux observed at a large $P_Q$.

### Comparison of BD simulations and experiments

In this subsection, we discuss how to use the BD framework to simulate the experiments described in Validation of BD Through In Vitro Experiments, and we introduce an appropriate and consistent nondimensionalization procedure for the experimental results. To allow for a meaningful comparison between the experimental and BD fluxes, we first define a Sherwood number for the experimental data. We define a dimensional experimental Sherwood number as follows:

$$ S^*_{\text{exp}} = \frac{\phi_{\text{out}} V_{\text{out}}}{(\rho \pi a^2 A) (\Delta t)} \left[ \frac{\text{mol}}{\text{m}^2 \text{s}} \right], \quad (17) $$

where $\Delta t$ is the amount of time the MS2 or free dye is left to diffuse, $A$ is the exposed membrane area in the diffusion device, $\phi_{\text{out}}$ is the concentration of the MS2 or free dye measured at the end of the experiment in volume $V_{\text{out}}$ of PBS, $\rho$ is the pore density, and $a$ is the pore radius. Defined in this way, $S^*_{\text{exp}}$ gives the number of particles extravasated per exposed pore area per time. We note that the quantity $S^*_{\text{exp}} / \phi_m$ is also useful, because it gives a measure of the percent of particles extravasating. To allow for a consistent comparison with the BD simulations, $S^*_{\text{exp}}$ is nondimensionalized so that:

$$ S_{\text{exp}} = \frac{S^*_{\text{exp}} a}{D \phi_m}, \quad (18) $$

where $D$ is the diffusion coefficient of MS2 or free dye. For the MS2, the Stokes-Einstein diffusion coefficient is used ($D = 15.9 \mu m^2/s$), and for the free dye, a previously reported value is used ($D = 280 \mu m^2/s$) (37). We note that this nondimensionalization for the experimental results is analogous to the Sherwood number defined in Eq. 4. In Table 1, we show the results of four separate experimental trials, and we determine that $S_{\text{exp}} = 10.6 E-4 \pm 1.75 E-4$ for the MS2 and $S_{\text{exp}} = 16.3 E-4 \pm 3.09 E-4$ for the free dye.

In BD for Finite-Sized Particles, we described our BD algorithm for finite-sized particles. In particular, we described a domain in which the pore is treated as a cylindrical tube of length that is half of the maximal dimension of the particle considered, and the resistance to entry into the pore is described through a Damköhler number $\kappa$. This allowed us to compare different particles in a consistent manner. For the purpose of comparing the BD algorithm to the experiments conducted here, we modify the simulation slightly to accurately represent the experimental setup. Instead of the rectangular box as shown in Fig. 4, we simulate particles in two rectangular boxes connected by a long cylindrical pore of length $L = L^* / \alpha$; see Fig. 8A for a schematic. At time $t = 0$, 10E6 particles are randomly initialized in one box only, with none in the other box. This way, there is no need to determine the exact value of $\kappa$ that exactly corresponds to the experimental membrane. The Sherwood number is directly calculated through the rate of particles that pass through the center $z = -L/2$ of the long pore. The length of the experimental membrane pores considered in this article is nondimensionally $L = 320$, which is very long and would thus require a large computational cost to attain a steady-state result. Thus, we note that with a membrane of this thickness, the large resistance to extravasation is governed by transport through the long pore, i.e., the corresponding value of $\kappa$ is very small and the process is reaction limited. In the long pore, the transport is dominated by Fick’s law, and thus the Sherwood number will be inversely proportional to the pore length $L$ when $L \gg 1$ as we have here. As a result, we conduct the simulation for a pore length of $L = 20$ and divide the calculated Sherwood number by a factor of $320/20 = 16$.

For the spherical bacteriophage MS2, we have $\epsilon = 1$ and $\beta_0 = 0.45$, and we find through our BD simulation that

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**FIGURE 8** (A) Schematic of the simulation domain used to model the experimental setup. (B) The mean dimensionless concentration profile $\phi(z)$ measured in BD simulations within the pore for both MS2 and free dye is shown. The profiles are approximately linear verifying the Fick’s law approximation. To see this figure in color, go online.
\[ S = 8.53 \times 10^{-4}. \] We note that this result corresponds to the mean membrane pore radius as measured by a SEM of \( a = 30.01 \text{ nm} \). For \( \beta_0 = 0.465 \) and \( \beta_0 = 0.436 \), corresponding to minus and plus one SD of the pore radius SEM measurement, respectively, we obtain Sherwood numbers of \( S = 7.74 \times 10^{-4} \) and \( S = 9.74 \times 10^{-4} \), which indicates the sensitivity of the flux to the pore size. For the free dye, we have \( \epsilon = 1 \) and \( \beta_0 = 0.0001 \), i.e., the point-particle limit, and we find through our BD simulation that \( S = 27.6 \times 10^{-4} \); because the dye is essentially a point-particle relative to the pore radius, the Sherwood number is insensitive to changes in the pore radius. In Fig. 8 B, the mean dimensionless concentration profiles \( \phi(z) \) in the pore for both cases, measured from the simulations, are plotted, where \( \phi = 1 \) corresponds to the initial concentration in the box loaded with particles. The profiles are approximately linear, verifying our Fick’s law assumption.

The in vitro experiments and the BD simulation are in favorable agreement with each other. We note that the dimensional Sherwood numbers scaled by \( \phi_0^s \) for the MS2 and free dye are \( S_{exp}/\phi_0^s = 5.58 \times 10^{-7} \pm 9.27 \times 10^{-8} \text{ m/s} \) and \( S_{exp}/\phi_0^s = 1.52 \times 10^{-5} \pm 2.89 \times 10^{-6} \text{ m/s} \), respectively; even though \( S_{exp}/\phi_0^s \) for free dye is nearly 30 times that of the MS2, the straightforward nondimensionalization (Eq. 18) permits a meaningful comparison with our simulation. This demonstrates the ability of our simulation method to predict extravasation rates of drugs of vastly different sizes such as a drug delivery NP of size on the order of the pore diameter and a molecule that is of essentially infinitesimal size compared to the pore. There are a number of possible sources of error that would have to be taken into account to achieve better quantitative agreement in the nondimensional Sherwood number: a few membrane pores overlap one another creating a much larger effective pore, which would change \( \beta_0 \) significantly (see Fig. 5 E for some of the overlapping pores imaged through SEM), the membrane pores are certainly not perfectly cylindrical, and the simulation does not take into account any electrostatic effects (which may be important for the diffusion of a molecule). The setup of our BD simulation framework certainly permits the consideration of additional physical forces and effects such as electrostatic effects; modeling of these effects is topics of future investigation, but we believe that here we have demonstrated the ability of our simulation to predict the Sherwood number of an experimental system and highlighted the potential usefulness of our simulation in understanding the transport of drug delivery NPs and molecules.

CONCLUSIONS

We have introduced a fast BD simulation algorithm that predicts the extravasation ability of point particles as well as particles of finite size. These simulations are consistent with our analytical result for point particles, i.e., Eq. 9, specifically in the physiologically relevant range of parameters, and capture the extravasation of drug molecules. For NPs, we do not account for the particle-wall lubrication interactions under the reasonable assumption that the flux of NPs is affected mostly by steric interactions at the pore. The finite-sized particle BD simulations provide a realistic understanding of the interactions between the flow and the pore with the particle geometry, and our experiments with bacteriophage MS2 and free dye demonstrate how our simulation may be used to accurately simulate a realistic drug delivery system. Even though we compare our BD simulation only to experiments with spherical MS2 and free dye, the high degree of agreement between the various aspects of this study (perturbation theory, point-particle BD, finite-sized particle BD, and diffusion experiments) provides strong evidence for the validity of our finite-sized particle BD simulations for nonspherical particles.

To highlight how our BD simulations may be used in practice, we describe a hypothetical situation in which a researcher has to choose between a spherical NP of size \( \beta_0 = 0.7 \) (e.g., quantum dots) and a rod-shaped NP (e.g., SWNT) of size \( \beta_0 = 0.7 \) to treat or image a tumor. Note that for the hypothetical particles under consideration, \( \beta_0 \) may be calculated using particle size and tumor pore-size distribution studies (24). In this example, we aim to show how to compare the physical dimensional extravasation rates of the two particles. Recall that the physical extravasation rate is a dimensional flux that is related to the Sherwood number via Eq. 4. Given the same tumor and flow conditions, the physical extravasation rates for NPs of the same size but different shapes can be quite different. To demonstrate this, we plot in Fig. 9 the ratio of the dimensional fluxes for the two particle shapes of rods to spheres. This is simply equal to the ratio of the respective Sherwood numbers multiplied by the ratio of the orientation-averaged diffusivities (using (Eq. 4)) (i.e., \( D_{rod}S_{rod}/D_{sph}S_{sph} \)).

From Fig. 9 A, we see that in the absence of a pressure gradient (\( Q = 0 \)), the rod-shaped NP is consistently better suited for extravasation. This is accentuated in the case of \( \beta_0 = 0.7 \) in which long rod-shaped particles are more successful at extravasation as compared to large spherical particles. The ratio of dimensional fluxes increases with shear rate, because the dimensionless fluxes of the rods and spheres increase and decrease, respectively, with increasing shear rate, as observed previously in Fig. 7 A for \( \beta_0 = 0.7 \). As noted previously, the trend of dimensionless flux reducing with shear rate occurs for rods only at larger sizes (e.g., \( \beta_0 = 1.5 \)).

In the presence of a pressure gradient across the pore (\( Q = 1 \)), Fig. 7 B showed that under strong flow conditions (\( PQ \gg 1 \)), a spherical particle has a dimensionless flux approaching that of the rod-shaped particle, especially for larger sizes. Based on the BD results for each particle, dimensionless fluxes approach 2\( PQ \), and thus the ratio of the dimensional fluxes should asymptotically approach the
ratio of the diffusivities at large shear rates; this is observed in the ratio of dimensional fluxes with \( Q = 1 \) in Fig. 9 B.

For particular applications, the amount of molecules (drugs or imaging dye) that may be loaded on an NP may depend, for example, on the total surface area available. The true efficiency of the NP in this case is the total amount of molecules transported. By scaling the y axes of Fig. 9, A and B by the ratio of surface areas of rods to spheres (which is 0.0789 for \( \varepsilon = 0.1 \)), we would see that a simple comparison of the dimensional NP fluxes can be quite different from a comparison of the actual amount of molecules extravasated. In this manner, one can incorporate application-specific scaling factors related to processes outside of the physical transport mechanism and arrive at suitable rules for choosing an optimal NP. We have thus demonstrated how our BD simulations may be used to guide the choice of particle shape.

In general, there may be a preferential uptake of particles of certain geometries, depending on the tumor microvascular geometry and flow conditions. BD simulations of finite-sized particles in a simplified model of the tumor microvasculature, such as the work presented in this article, can drive in silico design to guide experiments and perhaps even clinical strategies. Certainly, our BD algorithm (a Lagrangian particle solver) may be easily integrated with large-scale fluid simulations and model even more complex situations.

**SUPPORTING MATERIAL**

Supporting Materials and Methods, two figures, and one table are available at http://www.biophysj.org/biophysj/supplemental/S0006-3495(18)30933-0.

**AUTHOR CONTRIBUTIONS**

P.N.S. contributed to analytical and computational aspects of the work and wrote the manuscript. T.Y.L. contributed to experimental and computational aspects of the work and wrote the manuscript. I.L.A. and S.H.K. contributed to experimental aspects of the work. B.R.S. and E.S.G.S. directed and supervised research.

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