Article

# Predicting First Traversal Times for Virions and Nanoparticles in Mucus with Slowed Diffusion

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ABSTRACT Particle-tracking experiments focusing on virions or nanoparticles in mucus have measured mean-square displacements and reported diffusion coefficients that are orders of magnitude smaller than the diffusion coefficients of such particles in water. Accurate description of this subdiffusion is important to properly estimate the likelihood of virions traversing the mucus boundary layer and infecting cells in the epithelium. However, there are several candidate models for diffusion that can fit experimental measurements of mean-square displacements. We show that these models yield very different estimates for the time taken for subdiffusive virions to traverse through a mucus layer. We explain why fits of subdiffusive mean-square displacements to standard diffusion models may be misleading. Relevant to human immunodeficiency virus infection, using computational methods for fractional subdiffusion, we show that subdiffusion in normal acidic mucus provides a more effective barrier against infection than previously thought. By contrast, the neutralization of the mucus by alkaline semen, after sexual intercourse, allows virions to cross the mucus layer and reach the epithelium in a short timeframe. The computed barrier protection from fractional subdiffusion is some orders of magnitude greater than that derived by fitting standard models of diffusion to subdiffusive data.

## INTRODUCTION

Biological hydrogels, such as mucus, are ubiquitous in the human body and they play a vital role in microscopically regulating particle transport (1). For example, specially prepared nanoparticles may pass through mucus, but in general their movement is obstructed (2–4). Virions of different infections have been shown to be trapped or to pass through mucus to varying degrees, partly in accordance with their size (2–9). In particular, normal, acidic cervicovaginal mucus greatly hinders the movement of virions of herpes simplex virus (HSV) (8) and human immunodeficiency virus (HIV) (10), whereas mucus that is neutralized by semen deposited during coitus or by bacterial vaginosis provides a much less effective barrier against the same virions (10,11). Many experiments focused on particle tracking in mucus, or in simulated biological hydrogels, show subdiffusive behavior (2,4,6,10,12,13) or greatly slowed diffusive behavior (14–17).

The efficacy of hydrogels in providing such a barrier against infection is an important area of study (1,16). Fundamental to this is an understanding of particle diffusion in these systems. Single- and multiple-particle tracking experiments are frequently used to analyze the behavior of particle diffusion through mucus and gels (2–4,6,10,12,14–16). Typical results are in the form of two-dimensional images (and, less commonly, three-dimensional images (12)), which can be used to find trajectories for individual particles. Single-particle tracking experiments make it possible to measure the mean-square displacement of the particles, and many studies infer diffusion coefficients from this (12,18). In standard diffusion, the mean-square displacement of diffusing particles scales as a linear function of time, but in more general models of diffusion such as time-scaled diffusion and fractional subdiffusion, the mean-square displacement scales sublinearly with time. In experimental observations, there is often a large amount of noise in measurements of mean-square displacements, leading to different possible interpretations. Moreover, the means are sometimes calculated as ensemble averages over many particle trajectories, sometimes as time averages over a single-particle trajectory, and sometimes as a combination of the two. The ensemble and time averages are equivalent in standard diffusion, but they are different in time-scaled diffusion and fractional subdiffusion (19). A better understanding of the methods to be applied in particle diffusion through gels is therefore needed.

In the Materials and Methods section, we describe different mathematical models for diffusion, namely, standard diffusion, time-scaled diffusion, and fractional subdiffusion. We present formulae for the first-traversal-time distribution and the associated survival probability for diffusing particles traversing a layer. The first-traversal-time distribution, $f(t)$, gives the probability of a particle arriving at time zero and completely traversing the layer

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by the later time $t$. The survival probability, $S(t)$, is the probability that the particle is still in the layer at time $t$. For an ensemble of noninteracting particles entering the layer at time $t = 0$, the survival probability represents the proportion of those particles that remain in the layer.

In the Results section, we consider the diffusion of HIV virions in human cervicovaginal mucus. Most of the models for the transport theory of HIV virions, including diffusion across layers, assume standard diffusion (20,21). We use data from experimental observations of mean-square displacements relevant to this problem for three different diffusion models, standard diffusion with an effective diffusion coefficient, time-scaled diffusion, and fractional subdiffusion. We calculate traversal-time statistics based on each of the models. There are large variations in the traversal-time statistics depending on which model is used. Traversal times calculated from a subdiffusive model reveal that the motion of virions is greatly hindered by the presence of acidic mucus. This calculation holds the promise that a thin layer of mucus is thus capable of providing an effective barrier against particle transport.

## MATERIALS AND METHODS

In this section, we are interested in calculations relating to the time it takes a particle governed by a particular diffusion process to traverse a layer. For each of the mathematical diffusion models—standard diffusion, time-scaled diffusion, and fractional subdiffusion—we determine the survival probability, $S(t)$, for particles initially localized at a boundary, $x = 0$, to be in the domain $0 \le x \le h$ at a later time $t$, given that $x = h$ is an absorbing boundary. Fig. 1 is a schematic representation of an initial innoculum of virions entering a mucus layer with absorption at the epithelium. The analysis in this article could be extended to multilayer models taking into account the explicit arrival times of particles and dependent on the different characteristics of the layers. An analagous process could be applied for an axisymmetric three-dimensional model representing the entire vaginal mucosa.

The survival probability is calculated using

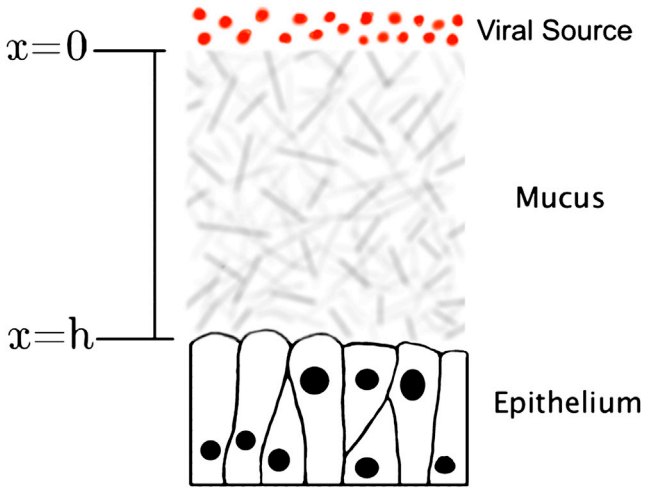


FIGURE 1 Schematic representation of the geometry of the model system for diffusive transport of virions across a mucus layer with an absorbing boundary at the epithelium. To see this figure in color, go online.

$$S(t) = \int_0^h \rho(x,t)dx,$$

where $\rho(x,t)$ is the probability distribution for the position of the particle in the domain at time $t$. The first-traversal-time distribution can then be found from

$$f(t) = -\frac{\partial S}{\partial t}.$$

### Standard diffusion

A mathematical description of the physical process that underlies standard diffusion is Brownian motion (Bm). This can be derived as the diffusion limit of a standard random walk. A standard random walk, in one space dimension, is the process where a walking particle, at each time step, will step to the left or right with equal probability. The diffusion limit is found by taking the length of the spatial and time steps simultaneously to zero (22). The standard diffusion equation provides a model for random motion of the particle in a spatially homogenous medium.

For a particle undergoing Bm, the evolution of the probability density, $\rho(x,t)$, is governed by a diffusion equation. In one dimension, this is given by

$$\frac{\partial \rho}{\partial t} = D\frac{\partial^2 \rho}{\partial x^2}, \tag{1}$$

where $D$ is the diffusion coefficient dependent on physical properties of the diffusing particle and the environment. In this Gaussian process, if two particles have the same diffusion coefficient, $D$, then all their transport properties are equivalent.

The fundamental solution of the diffusion equation is given by

$$\rho(x,t) = \frac{1}{\sqrt{4\pi D t}}\exp\left(-\frac{x^2}{4Dt}\right).$$

The mean-square displacement is then given by

$$\langle x^2 \rangle = 2Dt, \tag{2}$$

where the average is an ensemble average. The first-traversal-time statistics are found by solving the diffusion equation (Eq. 1) subject to a zero-flux boundary at $x = 0$,

$$\frac{\partial \rho}{\partial x}(0,t) = 0, \tag{3}$$

and an absorbing boundary at $x = h$,

$$\rho(h,t) = 0. \tag{4}$$

An infinite-series solution to this equation can be found using the standard method of separation of variables. Applying the boundary conditions above, we obtain the solution

$$\rho_{\mathrm{Bm}}(x,t) = \frac{2}{h}\sum_{n=0}^{\infty}\left[\exp\left(-\left(\frac{(2n+1)\pi}{2h}\right)^2 Dt\right) \times \cos\left(\frac{(2n+1)\pi}{2h}x\right)\right]. \tag{5}$$