How Much Advantage do Nanoscale Phenomena Afford for Pulmonary Delivery?

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ABSTRACT

The exact relationship between nanoparticle size and diffusivity through biological membranes is quantitatively unknown. It has been hypothesized that nanoscale effects, acting on particles <100nm in diameter, can offer enhanced diffusivity for transport through biological membranes such as mucus lining the lung. The first hurdle any pulmonary administered particles will encounter is the mucus barrier of the lungs, which has specifically evolved to trap and inhibit foreign particles and can be an insurmountable obstacle. Our results show that nanoparticles <100nm in diameter diffuse through mucus much faster than would be predicted from the Stokes-Einstein equation.

Keywords: Diffusion, Nanoparticles, Biological Barriers, Multiple Particle Tracking, Mean Squared Displacement.

1 INTRODUCTION

A wide range of nanoscale systems are being designed and developed as biomedical diagnostics and therapeutics. It has been hypothesized that the negligible effects of gravity and inertia on the nanoscale may provide advantages for biomedical applications [1], particularly in the realms of drug delivery and gene therapy where particle traversal of biological membranes has remained a bottleneck in progress [2]. Pulmonary administration of therapeutic particles is a preferred method to intravenous delivery which can cause discomfort and tissue damage.

Although it is generally suggested that the smaller the size of the particle the better the targeting and distribution effect, the exact relationship between size and biological effect is unknown. Diffusivity has traditionally been related to particle size via the Stoke-Einstein equation (Eqn. 1), which states that diffusion is inversely proportional to particle radius and sample viscosity [3].

$$D = \frac{kT}{6\pi\eta r} \tag{1}$$

We performed MPT experiments on 38nm, 55nm, 106nm and 226nm fluorescently-labeled, carboxylate-modified polystyrene nanoparticles through reconstituted mucus with the aim to understand and correlate diffusivity against nanoparticle size.

2 EXPERIMENTAL PROCEDURE

300µl of reconstituted CF mucus was placed into a vial and placed into the incubation chamber in which the microscope was contained to equilibrate to 37°C for approximately 15 minutes. 15µl of nanoparticle solution (~10^{10} particles/ml) was added to the vial of mucus and thoroughly stirred using a pipette. 200µl of the mucus/nanoparticle solution was then inserted into a µ-Slide 8 well sample holder (ibidi GMbH, Germany) and placed onto the microscope stage which was held within the pre-heated incubation chamber. The sample was left for approximately 15-30 minutes for the particles and mucus to reach equilibrium from the motion of the handling and transfer to the microscope stage. Using the microscope, areas within the mucus were chosen at random for observation. Roughly 5-10 particles could be seen per area, and therefore 10-15 areas were chosen at random. The frame rate chosen was the largest achievable by the microscope, software and camera (~19 frames/second).

A Zeiss Axiovert 200 inverted microscope, controlled by C-Imaging Simple-PCI acquisition software and a Hamamatsu EM-CCD camera was used to obtain 300 frames of particle trajectories through reconstituted CF mucus. The movie clips were analysed using Volocity (Version 4.0.1, Improvisation Ltd) to obtain the particle displacements of 50 particles for 2.6 seconds (50 frames) from which the Mean Squared Displacement (MSD) and diffusivity of the particle population was calculated.

The Diffusion Coefficient (D) was calculated by fitting the experimental MSD values to Eq. 2., where r is the particle radius, d is the dimension of space and α characterises the extent of impediment by random point obstacles (7).

$$\left\langle \Delta r^2(\tau) \right\rangle = 2dD\tau^{\alpha} \tag{2}$$

Reported Diameter (nm)	Measured Diameter (nm)	Theoretical Diffusion Coefficient Mucus (m^2s^{-1})	Experimental Diffusion Coefficient (m^2s^{-1})	Experimental Diffusion Coefficient Water (m^2s^{-1})
20	37.71	$1.77x10^{-16}$	$1.672x10^{-13}$	$8.66x10^{-12}$
40	54.75	$1.22x10^{-16}$	$8.837x10^{-14}$	$5.98x10^{-12}$
100	105.63	$6.34x10^{-17}$	$3.908x10^{-14}$	$3.10x10^{-12}$
200	226.00	$2.97x10^{-17}$	$3.852x10^{-14}$	$1.46x10^{-12}$

Table 1. Experimental data obtained from MPT experiments of 38nm, 55nm, 106nm and 226nm diameter particles. The manufacturer-reported and measured particle diameters are shown, along with theoretical predictions, calculated from the Stokes-Einstein equation, of the Diffusion Coefficients of these nanoparticle through both mucus (using the bulk viscosity value) and water.

3 RESULTS

The particle transport properties of 38nm, 55nm, 106nm and 226nm fluorescently-labeled, carboxylate-modified polystyrene nanoparticles through reconstituted mucus were determined using Multiple Particle Tracking. Particles were tracked for 2.6 seconds from which the ensemble Mean Squared Displacement and Diffusion Coefficient was calculated, the experimental Diffusion Coefficient values can be seen in Table 1.

The values in Table 1 show that the experimental Diffusion Coefficient obtained are much closer to the Diffusion Coefficients of nanoparticle diffusion in water, indicating that nanoparticles diffuse through mucus much faster than predicted by the Stokes-Einstein equation. The 38nm and 55nm nanoparticles also have much larger Diffusion Coefficient values than the larger 106nm and 226nm particle values, which indicate that the <100nm size of these nanoparticles is facilitating accelerated motion through the mucus.

4 DISCUSSION

Diffusion has traditionally been considered to be size dependent; with decreasing size resulting in increased diffusivity. Of the previous studies carried out of this nature, relatively few address sub-100nm nanoparticle transport properties. A study carried out by Lindman et al [4] investigated the effects of nanoparticle size on protein adsorption to particles. They examined the adsorption of Human Serum Albumin (HSA, 66kDa) to polymer particles ranging from 70nm – 700nm in diameter [4]. They found a lower degree of surface coverage for the 70nm than larger particles, suggesting that a higher degree of surface curvature, for smaller particles, interferes with binding. They hypothesise that there should be a size limit above which the binding properties are independent of curvature and approach those of a ”flat” surface [4]. From their experiments, they find this limit to occur for particles between 35nm – 60nm in radius. This range offers an explanation for the enhanced diffusion of the 38nm and 55nm nanoparticles compared to traditional models. Nanoparticles with a large curvature may experience a lower degree of surface curvature by mucus constituents, which impedes their motion less and has a lower effect on their overall size and change characteristics, whilst larger particles experience a larger extent of surface coverage. In addition, smaller frictional, inertial and gravitational forces acting on nanoparticles <100nm in diameter may allow faster diffusion through solutions than predicted by the Stokes-Einstein equation, adding further to the diffusive ability of the 38nm and 55nm nanoparticles in comparison to the larger 106nm and 226nm particles.

Our findings have shown that particle size has a more pronounced importance than hypothesised by the Stokes-Einstein equation and other previous models. Small decreases in particle size result in exponential increases in diffusivity, especially for particle sizes less than 100nm in diameter.

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