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The penetration of fresh undiluted sputum expectorated by cystic fibrosis patients by non-adhesive polymer nanoparticles

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Abstract

Highly viscoelastic and adhesive sputum has precluded efficient nanoparticle-based drug and gene delivery to the lungs of patients with cystic fibrosis (CF). We sought to determine whether nanoparticles coated with non-mucoadhesive polymers could penetrate CF sputum, and to use these “muco-inert particles” (MIPs) as non-destructive nanoprobes to characterize the sputum microstructure. Particles as large as 200 nm in diameter that were densely coated with low MW polyethylene glycol (PEG) moved through undiluted CF sputum with average speeds up to 90-fold faster than similarly-sized uncoated particles. On the other hand, the transport of both coated and uncoated 500 nm particles was strongly hindered. The local viscosity of sputum, encountered by the fastest 10% of 200 nm MIPs, was only 5-fold higher than that of water, whereas the bulk viscosity was 10,000-fold higher at low shear rates. Using measured transport rates of various sized MIPs combined with an obstruction-scaling model, we determined that the average 3D mesh spacing of CF sputum is $\sim 140 \pm 50$ nm (range: 60–300 nm). Taken together, these results demonstrate that nanoparticles up to 200 nm in diameter that do not adhere to CF sputum can move rapidly through this critical barrier by accessing pores that are filled with a low viscosity fluid. The results also offer hope that desperately needed sputum-penetrating drug- and gene-carrier nanoparticles can be developed for CF.

Keywords

Lung disease; Nanoparticle therapeutics; Mucus; Mucus penetrating particles; Particle tracking; Rheology

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1. Introduction

Imbalanced ion transport in the lung airways of cystic fibrosis (CF) patients leads to a decrease in the airway surface liquid volume, marked increase in mucus viscoelasticity, obstructed mucociliary clearance and accumulation of purulent sputum [1]. The respiratory tract represents a primary target of CF treatments due to the high mortality and morbidity stemming from pulmonary complications [2,3]. Therapeutics topically administered to the lungs, either on the market or under clinical development, include: mucolytics [4], antibiotics [5], the cystic fibrosis transmembrane conductance regulator (CFTR) gene [6], CFTR modulators [7], and other ion transport agents [8,9]. To improve therapeutic efficacy, a promising and increasingly tested strategy is to encapsulate drugs and genes within polymeric or liposomal nanoparticles. However, this approach has been strongly discouraged by the poor penetration of a variety of conventional particle systems through human mucus barriers. For example, polymeric particles with diameters 59-1000 nm were found to be firmly immobilized in human cervical mucus (diffusivity equal to zero) [10] and, therefore, subject to rapid clearance mechanisms. We recently showed that the movement of standard polymeric particles, 100-500 nm in diameter, is extremely slow in CF sputum [11], in agreement with previous diffusion chamber and fluorescence recovery after photobleaching (FRAP) studies by Sanders and coworkers [12, 13]. CF sputum as a critical barrier is also evident by the poor gene transfer with lipid-based and adenovirus vectors in sputum-covered tissues compared to sputum-depleted ones [14]. Inadequate sputum penetration has been suggested as an Achilles' heel to CF gene therapy [15-17]. Engineering particles that penetrate this formidable barrier is a critical challenge in the development of improved therapeutics for CF.

We recently discovered that rendering particle surfaces mucoinert, via attachment of low molecular weight (MW) poly(ethylene glycol) (PEG), allowed particles as large as 500 nm to rapidly penetrate fresh, undiluted human cervicovaginal mucus [18]. On the other hand, uncoated particles were almost completely immobilized [18]. This finding was attributed in part to the surprisingly large mesh spacing inherent to cervicovaginal mucus. In CF sputum, significantly higher concentrations of mucins, DNA, and actin are expected to markedly reduce the average size and size distribution of mesh spacings, and thus strongly hinder nanoparticle transport [11,13]. Neither the average size of the mesh spacings of physiological CF sputum, nor rapid particle penetration therein, has been established previously. Thus, we tested whether mucus-resistant coatings can facilitate rapid nanoparticle transport across CF sputum. We also investigated the architecture of CF sputum by fitting the transport rates of different sized coated nanoparticles to an obstruction-scaling model to determine the average size of the mesh spacings present in minimally perturbed sputum freshly obtained from human volunteers.

2. Materials and methods

2.1. CF sputum collection and rheological characterization

Sputum spontaneously expectorated from male and female CF patients ages 22-53, was collected at the Johns Hopkins Adult Cystic Fibrosis Program. The procedures conformed to ethical standards of the Johns Hopkins Medicine Institutional Review Board. Three to five samples were acquired from the weekly CF outpatient clinic, placed on ice upon collection and during transport, pooled together to minimize patient-to-patient variation, and studied on the same day. The total number of individual samples used for the present studies is 15. Samples for rheological characterization were stored at -20 °C until cone-and-plate rheometry (Rheometrics HADV-III; Brookfield, Middleboro, MA) was performed as previously described [11]. Briefly, the rheometer was set to steady mode and specimens warmed to 37 °C were subjected to shear deformation at controlled shear rates to measure the shear viscosity of CF sputum. The steady viscosity is the ratio of the measured stress induced within the specimen and the shear rate.

2.2. Nanoparticle preparation and characterization

Fluorescent carboxyl-modified polystyrene nanoparticles (COOH-PS) sized 100, 200, and 500 nm (Molecular Probes, Eugene, OR) were covalently modified with 3.4 kDa diamine polyethylene glycol (PEG) (NH₂-PEG-NH₂; Nektar Therapeutics, San Carlos, CA) at a 3:1 PEG:COOH ratio, as described previously [18]. Fluorescent amine-modified particles (Molecular Probes) were used as provided. Size and ζ -potential (surface charge) were determined by ZS90 Zetasizer (Malvern Instruments, Southborough, MA).

2.3. Multiple particle tracking in CF sputum

Nanoparticles were added to ~350 μ l of CF sputum (3% dilution; final concentration 0.008% wt/vol), transferred to 8-well glass chambers (LabTek, Campbell, CA), and equilibrated for 2 h at 37 °C prior to microscopy. The dynamics of particles were quantified using multiple particle tracking (MPT). Briefly, 20 s movies at 67 ms temporal resolution were acquired via a silicon-intensified target camera (VE-1000, Dage-MTI, Michigan, IN) on an inverted epifluorescence microscope (Axiovert, Zeiss, Thornwood, NY) with 100 \times /1.4 NA objective. Movies were analyzed with Meta-morph software (Universal Imaging, Glendale, WI) to extract x , y positional data over time. Time-averaged mean square displacement (MSD) and effective diffusivity (D_{eff}) for each particle were calculated as a function of time scale (τ) [19-21]. CF sputum was assumed to be locally isotropic but not necessarily homogeneous; thus, 2D diffusivity is equal to 3D diffusivity [19]. Bulk transport properties were calculated by geometric ensemble-averaging of individual transport rates. The tracking resolution was 10 nm, determined by tracking displacement of particles immobilized with a strong adhesive [22]. Particle transport mechanism (immobile, hindered, and diffusive) was classified as discussed previously [18,21].

2.4. Determination of the mesh spacing of CF sputum

The mesh spacing of CF sputum was estimated based on fitting an obstruction-scaling model to the measured particle diffusion rates using the maximum likelihood estimation [23]. This model was previously adapted to characterize mucus mesh spacing based on virus and DNA diffusion [10,24]. The model is valid in cases where there is negligible interaction between particles and the mesh, and where fluids between the mesh elements exhibit viscous drag (microviscosity) of water. Unlike healthy human mucus, fluids between the CF sputum mesh have markedly higher concentrations of free biopolymers, including DNA and actin fragments [12], rendering the microviscosity significantly higher. Thus, we adapted this model by replacing the viscosity of water with the viscosity of fluids within the pores of CF sputum, which was previously estimated, based on the diffusion of dextran in CF sputum, to be roughly 2.9-fold higher than water [12].

3. Results

3.1. Transport of uncoated particles in CF sputum

As a control, we confirmed that the movements of uncoated, amine-modified 200 nm polystyrene particles (NH₂-PS) are strongly hindered in CF sputum, as evident by their highly constrained non-Brownian time-lapse traces (Fig. 1A). The slow transport of NH₂-PS nanoparticles is also reflected in their geometric ensemble mean square displacement ($\langle \text{MSD} \rangle$) versus time scale (τ) relationship (Fig. 1B). At $\tau = 1$ s, the $\langle \text{MSD} \rangle$ of 200 nm NH₂-PS was $1.4 \times 10^{-3} \mu\text{m}^2$, which corresponds to a 5600-fold reduced transport rate compared to the same particles in water (Table 1). To evaluate the extent of the impediment to particle transport, the τ -dependent $\langle \text{MSD} \rangle$ was fitted to the equation $\langle \text{MSD} \rangle = 4D_0\tau^\alpha$, where D_0 is the τ -independent diffusivity and α is the anomalous diffusion exponent that reflects the extent of impediment ($\alpha = 1$ for pure unobstructed Brownian diffusion, such as particles in water; α

becomes smaller as obstruction to particle diffusion increases). The α value for NH₂-PS particles was 0.36, indicative of strongly obstructed transport. The hindered transport is corroborated by the uniformly low effective diffusivity (D_{eff}) values by nearly all uncoated particles (Fig. 1C).

3.2. Transport of PEG-coated particles in CF sputum

We covalently conjugated low MW (3.4 kDa) diamine polyethylene glycol (PEG) to polystyrene particles (PEG-PS). The dense PEG coating was confirmed by the near neutral surface charge of PEG-PS (Table 1); uncoated particles are highly negatively charged [18]. A fluorimetric assay revealed that all PEG-PS preparations exceeded 1 molecule of PEG per square nanometer of surface (assuming smooth particle surfaces; data not shown). Particles coated with low MW PEG exhibited greatly improved transport rates in CF sputum, as evident by the Brownian nature of typical 200 nm PEG-PS trajectories (Fig. 1D). Compared to 200 nm NH₂-PS, similar sized PEG-PS exhibited ~90-fold higher $\langle \text{MSD} \rangle$ ($\tau = 1$ s) (Fig. 1E). The differences in transport rates between 200 nm NH₂-PS and 200 nm PEG-PS particles in the same sputum samples were all statistically significant ($p < 0.0001$). Two hundred nanometer PEG-PS moved faster than 100 and 500 nm PEG-PS across all time scales. At $\tau = 1$ s, $\langle \text{MSD} \rangle$ of 200 nm PEG-PS was ~7- and ~61-fold higher than 100 and 500 nm PEG-PS, respectively (Fig. 1E). Overall, the transport of 100, 200, and 500 nm PEG-PS in CF sputum was slowed by 830-, 65-, and 1600-fold as compared to their theoretical speeds in water, respectively (Table 1).

Fast moving “outlier nanoparticles” represent a subpopulation of interest, as they are more likely to penetrate sputum and reach the airway epithelium. Therefore, we sorted D_{eff} of individual particles from fastest to slowest and classified them into 10 subgroups (Fig. 1F). The fastest 10% of 100 and 200 nm PEG-PS were slowed only 7- and 5-fold, respectively, in CF sputum compared to their speeds in water (at $\tau = 1$ s). The ~1.5-fold greater D_{eff} for the fastest 10% of 100 nm PEG-PS compared to the fastest 10% for 200 nm PEG-PS is in adequate agreement with the 2-fold difference predicted by the Stokes-Einstein equation for Brownian diffusion in a viscous fluid. However, the remaining 90% of 100 nm PEG-PS and all 500 nm PEG-PS possessed significantly lower D_{eff} than the corresponding fractions of 200 nm particles.

We next estimated the fraction of particles that may traverse a CF sputum layer as a function of time, based on the time scale dependent D_{eff} and Fick's second law of diffusion. The thickness of the airway surface liquid (ASL) layer is ~10 μm in cultured human airway epithelia obtained from freshly excised bronchial specimens of CF patients [25-27]. For the purpose of this rough estimation, the time scale dependent D_{eff} for individual particles was obtained by projecting the measured $\langle \text{MSD} \rangle$ versus τ relationship to sufficiently long time scale using measured α values at short time scales. We predict that substantial fractions (up to 35%) of PEG-coated nanoparticles may be capable of diffusing across a 10 μm CF sputum layer within 20 min (Fig. 2A). In contrast, much less than 1% of 200 nm NH₂-PS are expected to traverse the same sputum thickness even after 6 h (Fig. 2A and Table 1). Since the thickness of the sputum layer may vary substantially [28], we further estimated the fraction of 200 nm PEG-PS that may cross a range of CF sputum thicknesses. Fig. 2B shows that a significant fraction of 200 nm PEG-PS may penetrate a CF sputum layer as thick as 100 μm within 6 h.

We further analyzed the particle transport mechanisms by assigning particles to three non-overlapping transport modes; immobile (I), hindered (H), and diffusive (D) (where rates of movement: $D > H > I$). Classification was based on time scale-dependent effective diffusion coefficients [18,21]. Roughly 30% of uncoated 200 nm NH₂-PS were immobile, with an MSD below the resolution of the microscope, and over 95% of the particles experienced significant restriction (immobile plus hindered diffusion) (Fig. 3A). In contrast, less than 2% of coated

200 nm PEG-PS were immobile, and roughly 35% of 200 nm PEG-PS underwent diffusive motions with $\langle D_{\text{eff}} \rangle$ only 13-fold lower than that for the same particles in water (Fig. 3B). Interestingly, while only a small fraction of 100 nm PEG-PS was immobile, nearly 80% of them underwent hindered diffusion, in good agreement with the percentile distribution analysis (Fig. 2F). The larger 500 nm PEG-PS particles were immobilized and hindered nearly to the same extent as the uncoated 200 nm NH₂-PS.

3.3. Local and bulk viscosity of CF sputum

We measured the shear-dependent bulk viscosity of CF sputum by subjecting the specimens to steady state deformations at controlled shear rates using a sensitive cone-and-plate rheometer (Fig. 4). At a shear rate of 0.2 s^{-1} , CF sputum displayed an average bulk viscosity of nearly $3 \times 10^4 \text{ cP}$ (30,000 times the viscosity of water). At shear rates as high as 20 s^{-1} , the bulk viscosity of CF sputum remains nearly 300-fold more viscous than that of water. The overall shear-dependent viscosity of CF sputum used in this study is similar to that reported previously [11]. On the other hand, the local nanoscale viscosity of the interstitial fluids of sputum, as probed by the fastest 10% of 200 nm PEG-PS, was only 5-fold higher than the viscosity of water.

3.4. Microstructure of CF sputum

The barrier properties of CF sputum at length scales relevant to viruses and synthetic nanoparticles are intrinsically related to the structural arrangement of the CF sputum mesh. However, the 3D microstructure of hydrated, physiological CF sputum has not been reliably determined, as dehydration and other fixation procedures used in previous electron microscopy preparations may introduce artifacts or disrupt mucus microstructure. In contrast, the dynamics of mucus-resistant particles in fresh, minimally perturbed CF sputum may provide a more accurate estimation of the effective mesh spacings. We fitted the transport rates of various sized PEG-PS particles to an obstruction-scaling model, which was initially developed to model the diffusion of non-interacting solutes in hydrogels [23], but has been shown equally applicable to entangled and cross-linked gels such as mucus [10]. The diffusion data of 100 nm PEG-PS was excluded in the analysis as their slower transport than 200 nm PEG-PS indicates that they are partially adhesive to CF sputum, consistent with greater difficulty in achieving muco-inert coatings as particle size diminishes [18]. Using maximum likelihood estimation, the average mesh spacing of physiological human CF sputum was estimated to be $140 \pm 50 \text{ nm}$ (Fig. 5). Estimates using least square fitting yielded similar results ($170 \pm 50 \text{ nm}$).

4. Discussion

We show that polymer-based particles at least as large as 200 nm in diameter can be engineered to rapidly penetrate CF sputum. CF sputum-penetrating particles require muco-resistant surfaces, which can be achieved via a dense covalent coating of low MW polyethylene glycol (PEG), in agreement with our recent findings in fresh, undiluted cervicovaginal mucus [18]. The diffusive population of 200 nm PEG-PS penetrated CF sputum at an average rate only 13-fold slower than they would move through pure water. This finding suggests that the CF sputum possesses a mesh structure containing openings between structural elements that are filled with a low viscosity fluid, through which properly engineered muco-inert particles (MIPs) can penetrate and approach the epithelium. The ability to create nanoparticle-based carriers that penetrate the formidable CF sputum barrier may lead to new generations of nanomedicines for CF [29]. While the CFTR gene was discovered in 1989, no cure has been achieved to date, a challenge attributed in part to the ineffective transport of gene vectors in CF sputum [14-17, 30,31]. The development of mucus-penetrating gene carriers may significantly enhance the delivery of CFTR genes to airway epithelia.

We expect the sputum-penetrating particles described here may also rapidly penetrate mucus secretions present in other pulmonary diseases. Despite etiological differences, COPD and asthma share key pathological complications with CF, including: (i) markedly increased viscoelasticity of airway mucus due to hyper-secretion of mucins, (ii) compromised mucociliary clearance, and (iii) elevated inflammatory response [32,33]. Airway mucus in both COPD and asthma exhibits similar bulk rheological properties compared to CF sputum [34]. Sanders and coworkers also observed that the movements of standard (uncoated) nanoparticles are retarded to a similar extent in COPD and CF sputum [13].

We found that uncoated NH₂-PS particles adhere extensively to CF sputum. This is most likely attributed to polyvalent adhesive interactions between the hydrophobic core of polystyrene particles and hydrophobic domains along mucin fibers, and possibly partly due to adhesion to other CF constituents such as DNA and filamentous actins. By coating particles with PEG at high surface density to effectively shield the hydrophobic core of polystyrene, while maintaining a MW sufficiently low to avoid extensive mucoadhesive interaction via interpenetration [35,36] and hydrogen bonding [37,38], we engineered MIPs with greatly reduced adhesion to CF mucus constituents. It is important to note that the MW of PEG tested here is nearly 70% higher than the PEG used in our earlier work with cervicovaginal mucus from healthy volunteers [18], suggesting that PEG MW as high as 3.4 kDa can facilitate rapid mucus penetration [42].

The relationship between particle size and transport in CF sputum was not obvious *a priori*. Previous investigations, with uncoated particles, have documented faster transport with 100 nm particles compared to 200 and 500 nm particles [11,13]. More recently, we found mucus-resistant particles to exhibit higher $D_{\text{eff}}^{\text{mucus}}/D_{\text{eff}}^{\text{water}}$ ratios with increasing diameters up to 500 nm in human cervicovaginal mucus, attributed perhaps to less efficient PEG coatings of smaller particles due to the higher degree of curvature. Here, we found that 100 nm PEG-PS particles were more hindered by CF sputum than 200 nm PEG-PS, but less so than 500 nm PEG-PS. The rapid transport observed in CF sputum with larger 200 nm PEG-PS and small dextran molecules (MW 167 kDa; ~15 nm) [12] suggests 100 nm PEG-PS particles are likely slowed due to insufficient PEG coating. Nevertheless, the transport rates achieved with the 100 nm particles here are likely fast enough to allow more significant and reliable gene transfer in CF lungs. The rapid transport of 200 nm sputum-penetrating particles is also expected to enable numerous therapeutic applications for CF, since larger particles allow higher drug encapsulation efficiency and sustained release of a wider array of drugs.

Understanding the microstructure and effective mesh spacings of CF sputum has important implications to the development of nanoparticle therapeutics, as well as pathogen infection in CF patients. The mesh structure of CF sputum dried on a mica surface was previously characterized by atomic force microscopy (AFM), which suggested pore sizes in the range of 160-1440 nm [39]. CF sputum has also been studied by scanning electron microscopy (SEM), leading to pore size estimates of 100-400 nm [13]. Both techniques, however, possess notable limitations. For example, AFM involves scanning the surface of mucus, thereby characterizing only the 2D surface geometry of deposited sample rather than the complicated 3D mucus mesh network throughout the entire mucus volume. Sputum dehydration on a hydrophobic (mica) surface also likely affects AFM estimates. SEM requires fixation and dehydration steps during sample preparation, which can alter the biophysical properties of sputum samples [40]. This is exemplified by electron micrographs of human cervical mucus, which suggested mesh sizes in the range of 10-200 nm [10]; these dimensions are now disputed by the rapid transport of 500 nm MIPs in human cervicovaginal mucus, which implies the presence of effective mesh spacings in excess of 500 nm [18].

By studying the real-time dynamics of various sized minimally mucoadhesive particles in fresh, undiluted CF sputum, we were able to probe the 3D mesh spacing of physiological human CF sputum with minimal perturbation. The average mesh spacing estimate reported for undiluted, fresh, minimally perturbed CF sputum (140 ± 50 nm) suggests that the slow movement of 500 nm PEG-PS nanoparticles is most likely attributed to extensive steric obstruction imposed by the small mesh spacing of CF sputum. The difference in the average mesh spacing of CF sputum compared to human cervicovaginal mucus obtained from healthy volunteers is likely a direct consequence of the greater mucin, DNA and actin concentrations in CF sputum [39]. The smaller average mesh spacing, together with the ~3-fold greater microviscosity (viscosity of the fluids between the mesh elements), underscores distinct differences in barrier properties between CF sputum and healthy human mucus [43,44].

5. Conclusion

We found that by engineering a dense surface coating of non-mucoadhesive PEG polymers, polymeric particles as large as 200 nm can penetrate CF sputum. The design of polymeric particles with improved sputum penetration should strongly encourage the commercial development of new generations of nanoparticle-based drug delivery systems for CF and other pulmonary diseases, including COPD, asthma, and emphysema.

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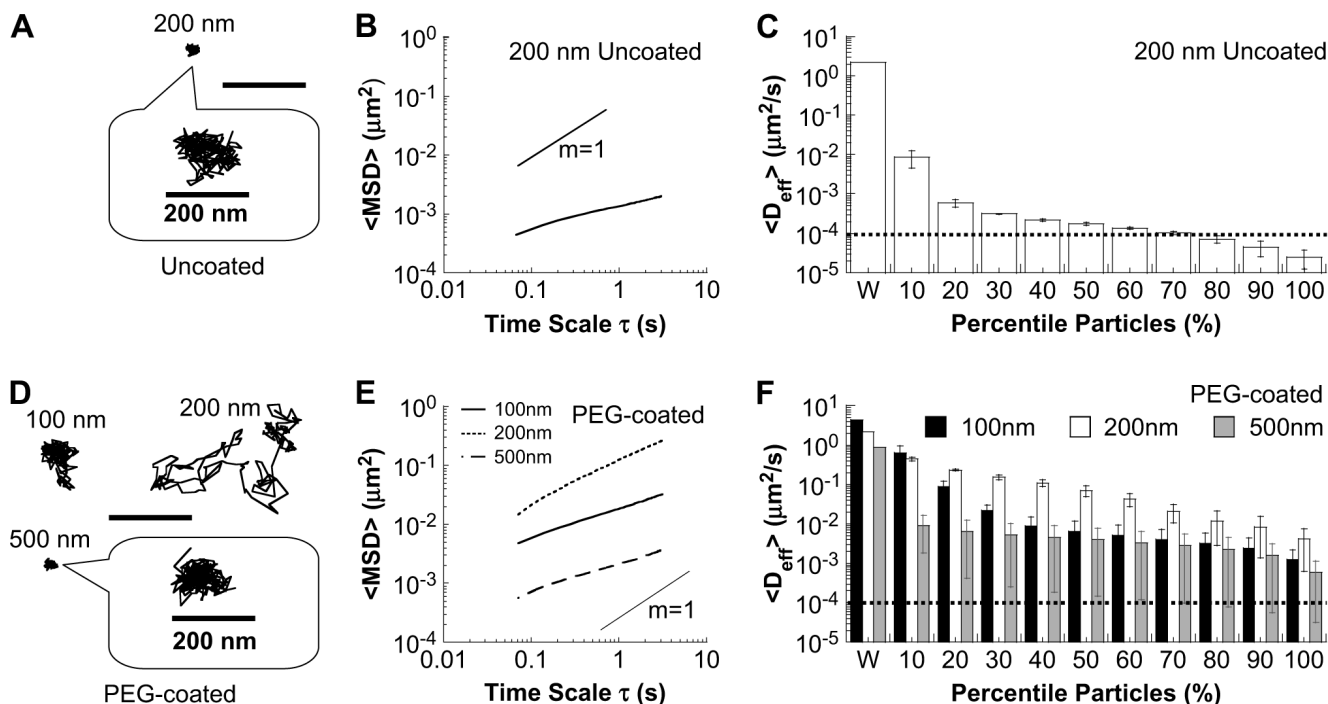


Fig. 1. Transport rates of uncoated ($\text{NH}_2\text{-PS}$) and PEG-coated (PEG-PS) polystyrene nanoparticles in fresh, undiluted CF sputum. Representative trajectories of (A) 200 nm $\text{NH}_2\text{-PS}$ and (D) 100-500 nm PEG-PS. Scale bars represent $1\ \mu\text{m}$ unless otherwise specified. Ensemble-averaged geometric mean square displacement ($\langle \text{MSD} \rangle$) of (B) 200 nm $\text{NH}_2\text{-PS}$ and (E) 100-500 nm PEG-PS particles as a function of time scale (τ). The slope of $m = 1$ corresponds to unobstructed diffusive behavior. Effective diffusivities ($\langle D_{\text{eff}} \rangle$) of every 10th percentile of (C) 200 nm $\text{NH}_2\text{-PS}$ and (F) 100-500 nm PEG-PS particles at $\tau = 1\ \text{s}$, from the fastest 10% of particles (10th percentile) to the slowest 10% of particles (100th percentile). Theoretical D_{eff} for the same sized particles in water is shown as W. The dotted line signifies the microscope's resolution ($D_{\text{eff}} = 0.0001\ \mu\text{m}^2/\text{s}$). Data represents 3 independent experiments, with an average of $n > 200$ particles per experiment. Error bars represent standard error of the mean.

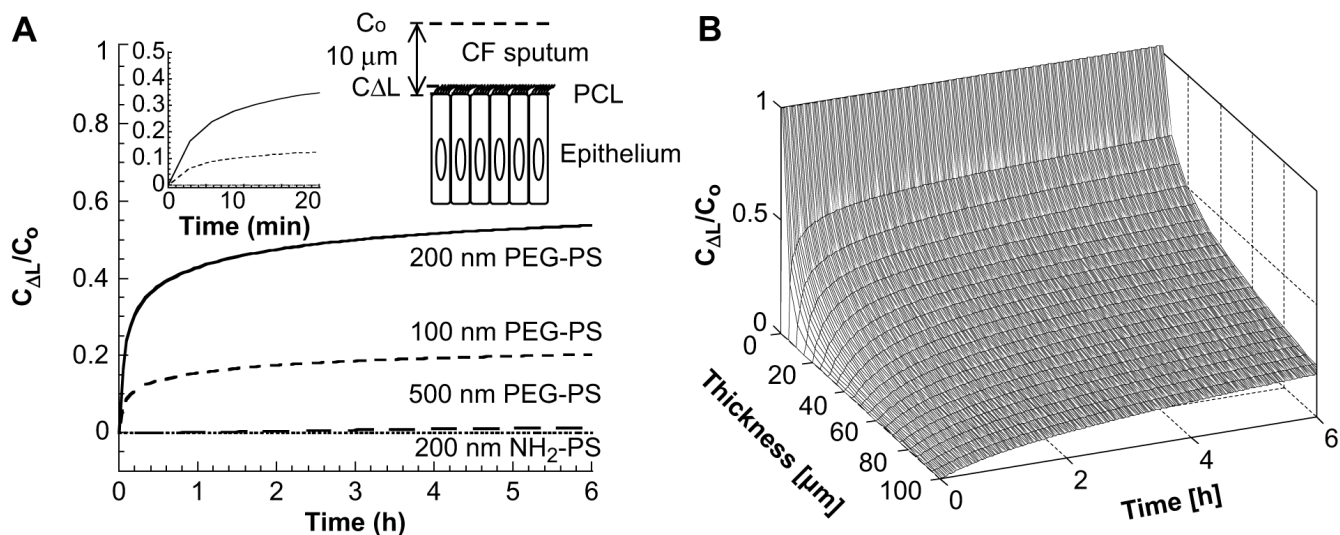


Fig. 2. Fraction of particles expected to penetrate CF sputum layers of a particular thickness over time. (A) Ratio of particle concentration near the apical surface of the airway epithelium ($C_{\Delta L}$) to initial luminal particle concentration (C_0) over time, assuming that the CF sputum layer covering the airway epithelium is 10 μm (ΔL) thick [25-27]. $C_{\Delta L}/C_0$ curves for 200 nm NH_2 -PS and 500 nm PEG-PS are not distinguishable - they both remain approximately zero even after 6 h. Left inset displays the change in $C_{\Delta L}/C_0$ at early time points. Right inset shows the schematic of the CF airway epithelium covered by highly viscoelastic sputum layer. Periciliary layer (PCL) in CF airways is collapsed and significantly thinner than that of normal airways due to CF-induced dehydration. The 70-100 nm thick glycocalyx covering epithelial cells is not depicted [41]. (B) The estimated penetrable fraction of 200 nm PEG-PS particles as a function of sputum layer thickness and time post-administration.

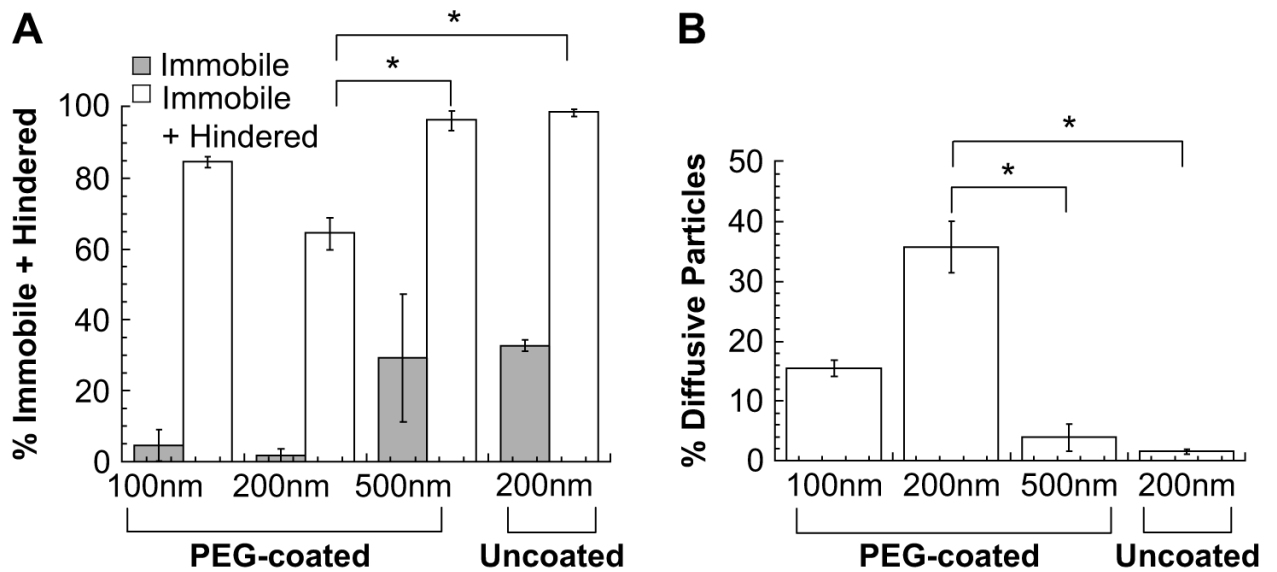


Fig. 3. Transport mechanism distributions of various sized particles in CF sputum, with or without PEG coating ($n = 3$ experiments): (A) immobile and immobile + hindered, and (B) diffusive particles. Data represents mean \pm SEM of 3 experiments, with $n > 200$ particles for each experiment. Immobile particles have an MSD below the microscope detection limit (10 nm). Differences in percentages of immobile, immobile + hindered and diffusive particles are statistically significant (*) for 200 nm PEG-coated particles as compared to either 200 nm uncoated particles or 500 nm PEG-coated particles ($p < 0.01$).

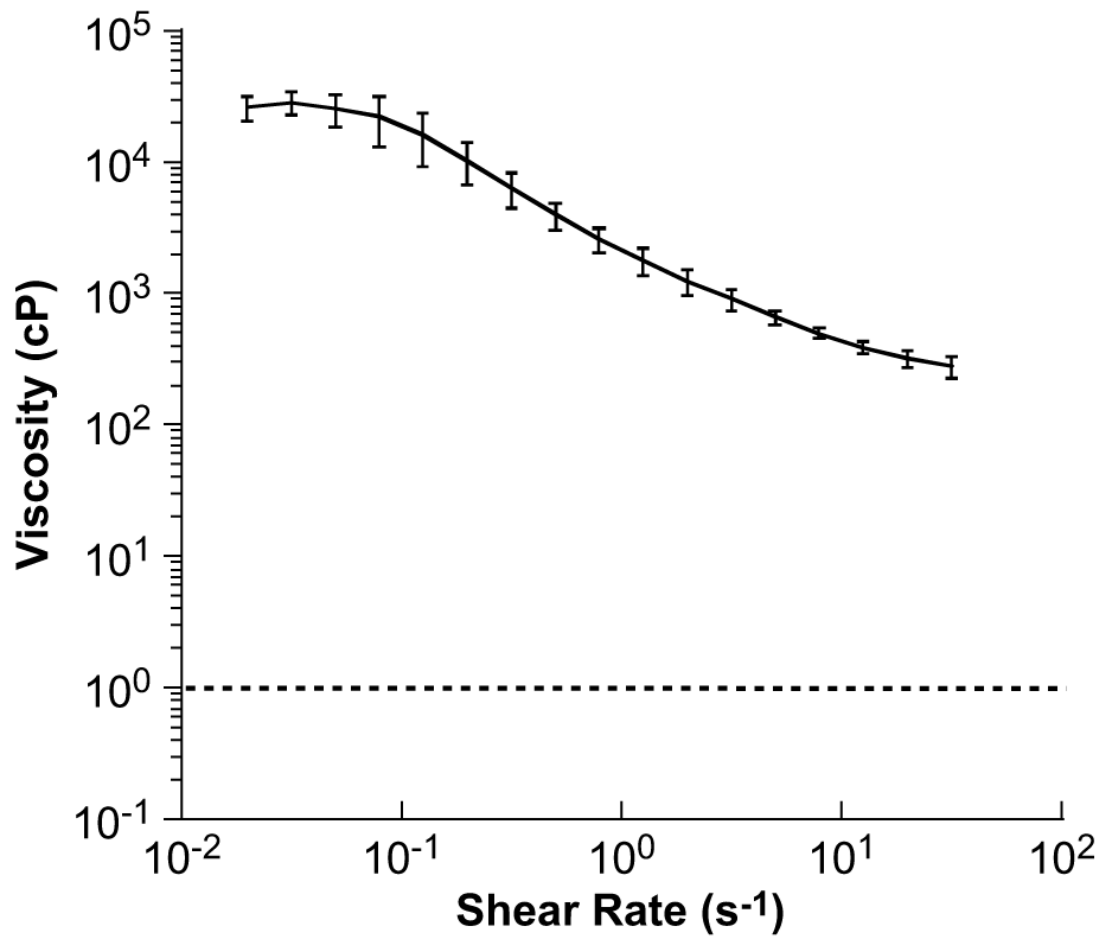


Fig. 4. Viscosity of fresh, undiluted CF sputum samples measured as a function of shear rate in a steady shear mode. Dotted line represents the viscosity of water. Error bars represent standard error of the mean for $n = 4$ pooled CF sputum samples.

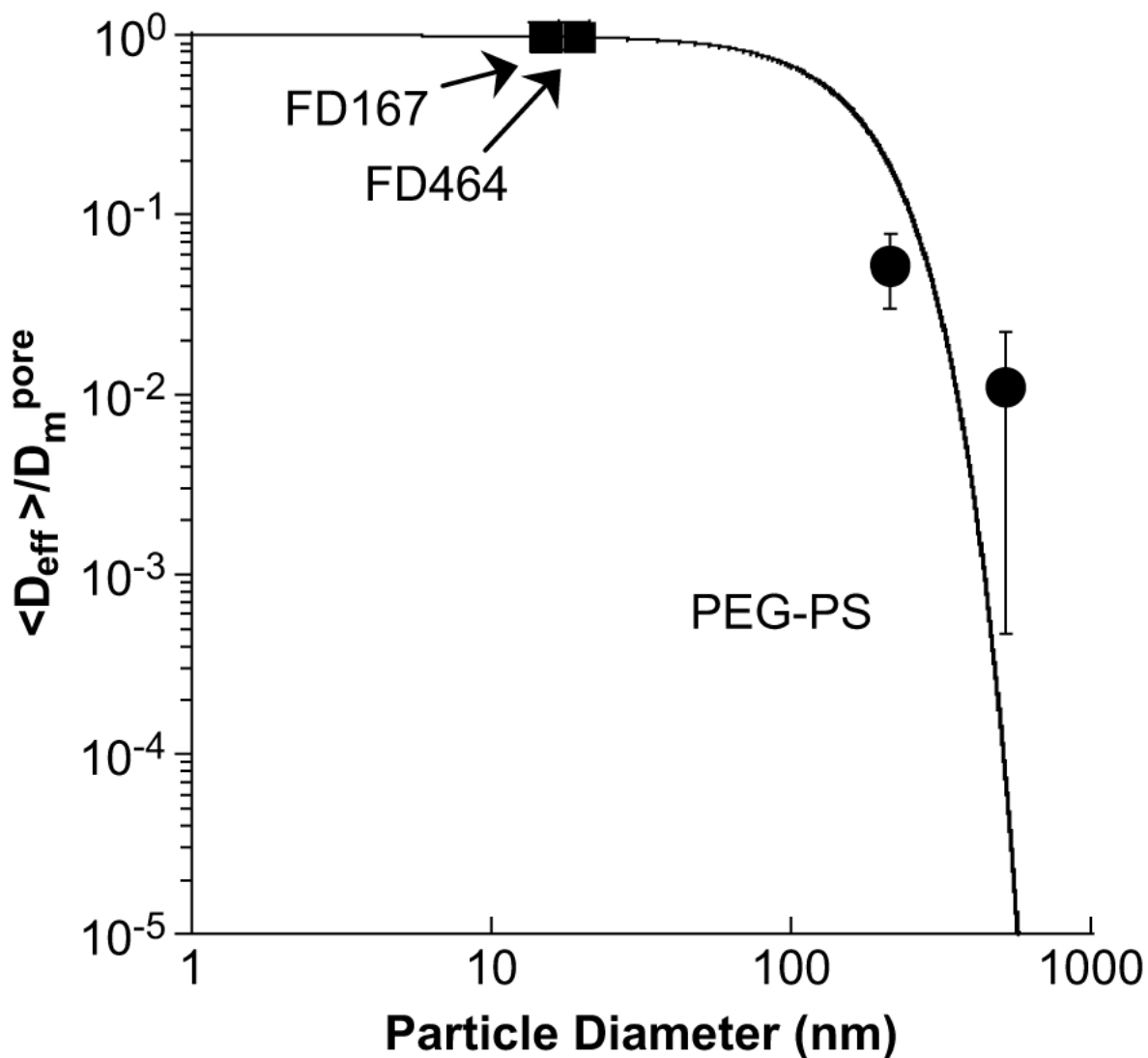


Fig. 5. Normalized ensemble-averaged diffusivities $\langle D_{\text{eff}} \rangle / D_{\text{m}}^{\text{pore}}$ for different sized (167 and 464 kDa) FITC-dextran (FD, solid square), and various sized (200 and 500 nm) PEG-coated PS particles (PEG-PS, solid circles) at $\tau = 1$ s, where $D_{\text{m}}^{\text{pore}}$ is the theoretical diffusivities of non-mucoadhesive particles in the aqueous pores of CF sputum. The solid line represents the theoretical $\langle D_{\text{eff}} \rangle / D_{\text{m}}^{\text{pore}}$ ratios for various sized 200 and 500 nm particles predicted by the obstruction-scaling model. A mucin fiber radius of 3.5 nm is assumed [10], leading to an estimated mesh fiber spacing in CF sputum of 140 ± 50 nm, obtained by maximum likelihood estimation fitting to experimental diffusivity ratios.

Table 1

Characterization of PEG-PS and NH₂-PS nanoparticles.

Size ^d (nm)	Surface chemistry	Diameter ^b (nm)	ζ-potential ^c (mV)	$D_w/\langle D_{eff} \rangle^d$	α^e	Penetrable percentage ^f (%)
100	PEG	114 ± 4	-2.5 ± 1.2	830	0.49	20
200	PEG	213 ± 6	-1.9 ± 3.7	65	0.72	54
500	PEG	515 ± 16	-4.0 ± 1.3	1600	0.46	1
200	NH ₂	227 ± 2	-5.6 ± 0.1	5600	0.36	<1

^a Provided by manufacturer.

^b Measured by dynamic light scattering. Error values represent standard error of the mean.

^c Measured at pH 7.0. Error values represent standard error of the mean.

^d D_w is the theoretical diffusivity of particles in water calculated from the Stokes-Einstein equation and $\langle D_{eff} \rangle$ is measured at time scale of 1 s. The $D_w/\langle D_{eff} \rangle$ ratio indicates by what multiple the average particle movement rate in fresh CF sputum is slower than in pure water.

^e Calculated by fitting data in Fig. 1B and E to $\langle MSD \rangle = 4D_0t^\alpha$, where D_0 is diffusivity and α is anomalous exponent equal to or less than 1. The larger the negative deviation from 1, the higher the degree of hindrance to particle motion.

^f Signifies the percentage of nanoparticles that are expected to penetrate 10 μm CF sputum layer in 6 h, which corresponds to $C_{\Delta L}/C_0$ at 6 h in Fig. 2A.