Microscopic Viscoelasticity of CF Sputum Determined by High-Resolution Nanoparticle Tracking

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Introduction. Therapeutic delivery of DNA-carrying nanoparticles to the lungs of patients with cystic fibrosis (CF) is limited by slow transport through viscoelastic airway mucus, which forms a continuous barrier over the epithelial cells in the upper airways. Using real-time multiple nanoparticle tracking, we quantified the motion of hundreds of individual particles in human CF sputum and gained significant insight into the properties of the mucus network and the effects of particle physiochemical properties on their transport rates in CF sputum. These results have guided us in the design of drug and gene vectors that diffuse more rapidly through mucus barriers in the lungs and gastrointestinal tract.

Experimental. Images of fluorescently-labeled carboxylated polystyrene microspheres with highly uniform diameters of 100, 200, 500, or 1000 nm embedded in mucus were acquired using a SIT camera mounted on an inverted epifluorescence microscope. These images were analyzed using a custom subroutine incorporated in Metamorph software. The displacements of the centroids of individual microspheres were simultaneously tracked in the focal plane of the microscope for 20 s at a rate of 30 Hz, as many times as necessary to monitor a total of ~100 particles for each tested specimen.

From the trajectories of the microspheres centroids, individual time-lag-averaged mean squared displacements (MSD) were computed, from which time-lag-dependent MSD distributions are generated. These distributions were normalized by the time-lag-averaged, ensemble-averaged MSD and subsequently analyzed by computing median, standard deviation, and skewness, statistical parameters that describe the heterogeneity of transport through the samples.

In addition, the frequency-dependent viscous (G”(w)) and elastic (G’(w)) moduli were determined from the unilateral Laplace transform of the MSD. Assuming that mucus is an incompressible, isotropic fluid and that inertial effects are negligible, the Stokes-Einstein equation was used to determine the complex modulus, which was projected in the Fourier domain and separated into the real (G”(w)) and imaginary (G’(w)) components.

Results and Discussion. Quantitative statistical analysis of the individual particle transport rates in CF sputum has led to the discovery of three fundamental transport regimes that govern the heterogeneities and mode of transport in mucus: (i) the microscopic domain, in which particles diffuse freely in the interstitial fluid present in mucus pores; (ii) the mesoscopic domain, in which particle transport is heterogeneous with the majority of particles moving sub-diffusively; and (iii) the macroscopic domain, in which particle transport is homogeneously sub-diffusive with particles uniformly trapped in mucus. By elucidating these three fundamental transport regimes with more quantitative statistical and numerical analysis of data recorded using multiple particle tracking, we have characterized the interstitial viscosity of mucus pores, the heterogeneities in particle transport rates as a function of the physiochemical particle properties, and the bulk-fluid viscoelasticity. This technique is powerful for characterizing gene

and drug carrier transport through mucus barriers and offers an accurate way to determine the rheological properties of small volumes of mucus (< 10 ul) from human patients or small animals.