Microstructural Alterations of Sputum in Cystic Fibrosis Lung Disease

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Supplementary Figures

Figure S1

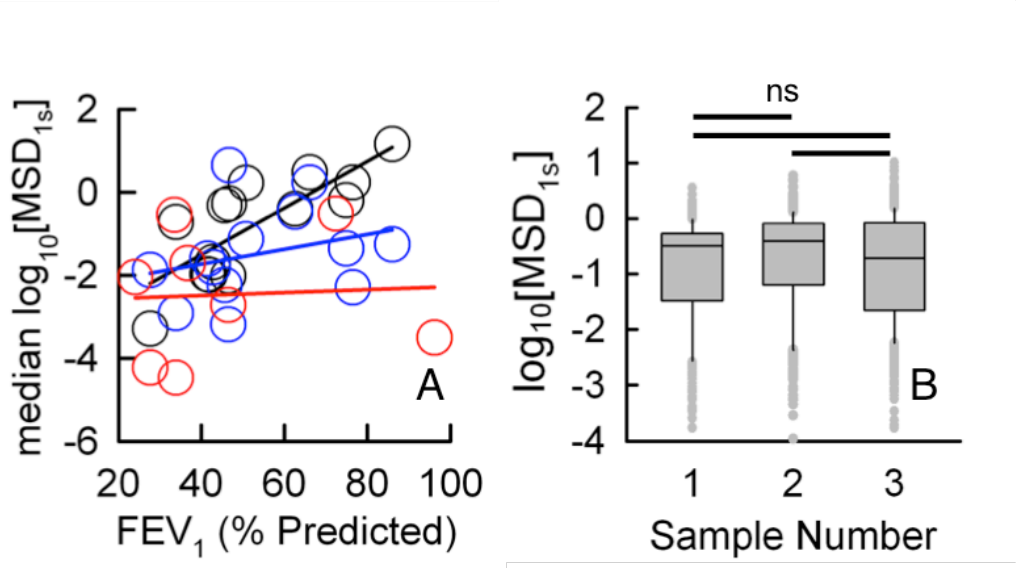

Figure S1. *100, 200, and 500 nm MIP transport in CF sputum versus patient clinical status and 100 nm MIP transport in multiple samples collected from the same patient.* **(A)** The correlations of median $\log_{10}[MSD_{1s}]$ measured with 100 (black, n = 15), 200 (blue, n = 14) and 500 nm (red, n = 8) MIP versus FEV_1. Results of the linear regression analysis were as follows: R^2 = 0.62, P < 0.001 for 100 nm MIP; R^2 = 0.08, P = 0.35 for 200 nm MIP; R^2 < 0.001, P = 0.89 for 500 nm MIP. **(B)** $\log_{10}[MSD_{1s}]$ of 100 nm MIP in 3 sputum samples collected from the same patient with ~10 minutes between expectorations. A Mann-Whitney test was used to compare between samples and were considered significant when P < 0.05.

Figure S2

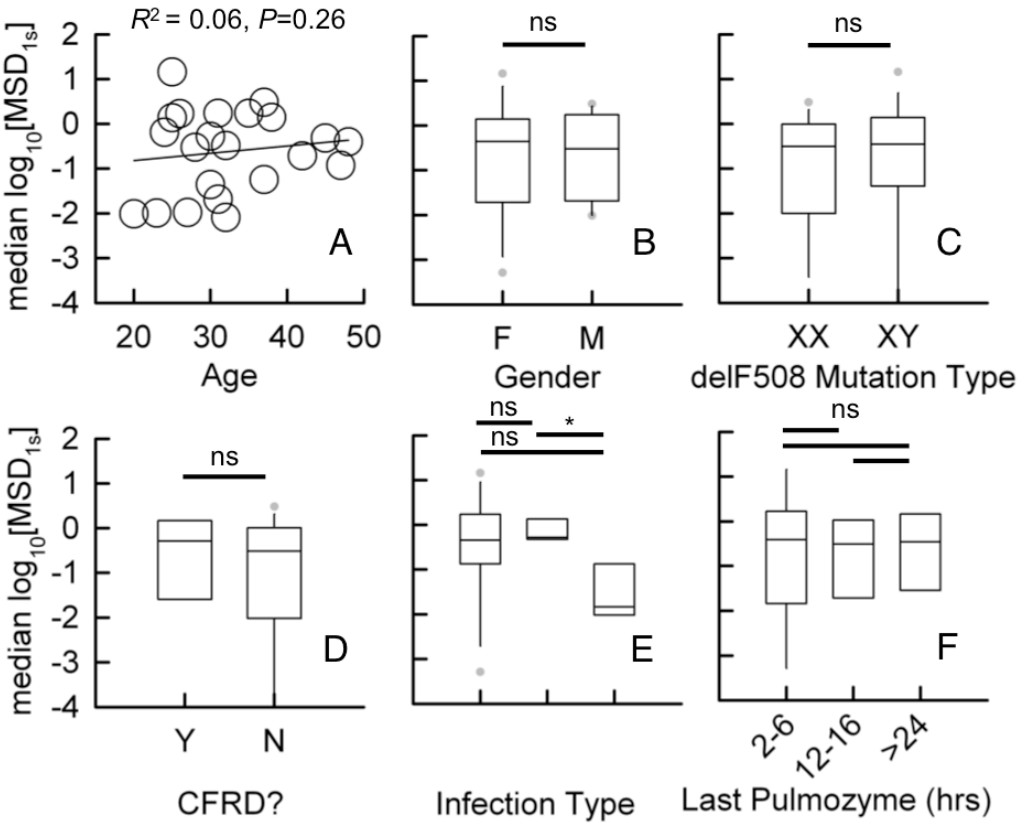

Figure S2. *Effect of age, gender, mutation type, CF-related diabetes (CFRD), infection type and time since the patients' last Pulmozyme (rhDNase) inhalation on CF sputum microstructure measured with 100 nm MIP.* **(A-F)** The correlations of median log_{10}[MSD_{1s}] versus **(A)** age, **(B)** gender, **(C)** delF508 mutation type (homozygous=XX; heterozygous=XY) **(D)** CF-related diabetes (CFRD) status, **(E)** infection types (*P. aeruginosa* = *PsA*; *Methicilin-resistant Staph. aureus* = *MRSA*) and **(F)** time since the last rhDNase (Pulmozyme) treatment. A student's t-test was used to compare between conditions in part **D** (ns = P > 0.05; *= *P*<0.05).

Figure S3

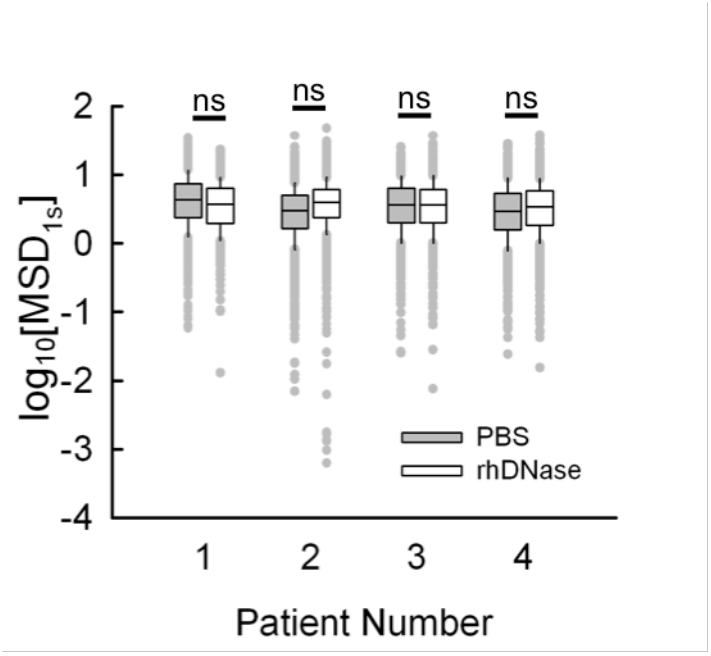

Figure S3. *100nm MIP transport in interstitial fluid from CF sputum before and after rhDNase treatment.* Box-and-whisker plots of measured MSD per μm^2 at time τ = 1 s (MSD_{1s}) of 100 nm muco-inert nanoparticles (MIP) in interstitial fluid from sputum collected from 4 CF patients before treatment (gray bars) and after treatment with rhDNase (white bars). Whole sputum samples were treated with 7 μg/mL rhDNase for 30 min at 37ºC. CF sputum interstitial fluid was characterized by centrifuging PBS and rhDNase-treated sputum samples at 21,000×*g* for 1 hr and measuring MIP diffusion in the supernatant by MPT. A Mann-Whitney test was used to compare between samples and were considered significant when $P < 0.05$.

Figure S4

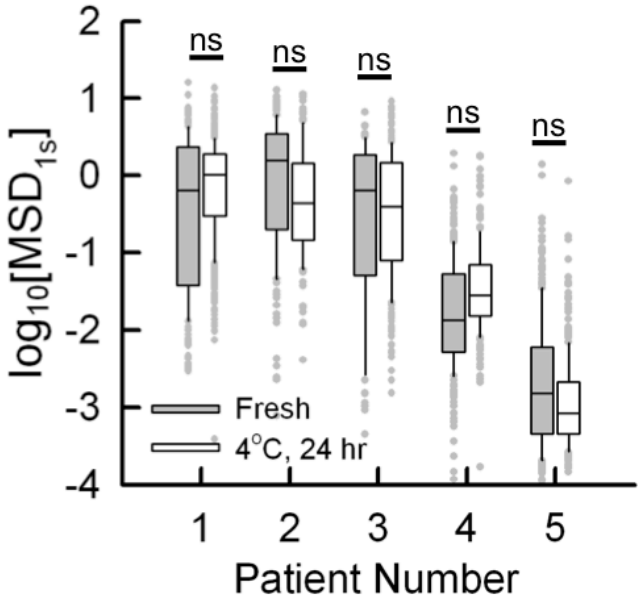

Figure S4. *100nm MIP transport in CF sputum immediately after collection* (*fresh) versus samples stored at 4ºC for 24 hr.* Box-and-whisker plots of measured MSD per μm^2 at time τ = 1 s (MSD_{1s}) of 100 nm muco-inert nanoparticles (MIP) in sputum collected from 5 CF patients immediately after collection (fresh; gray bars) and after refrigeration for 24 hours at 4ºC (4ºC, 24 hrs; white bars). A Mann-Whitney test was used to compare between samples and were considered significant when $P < 0.05$.

Figure S5

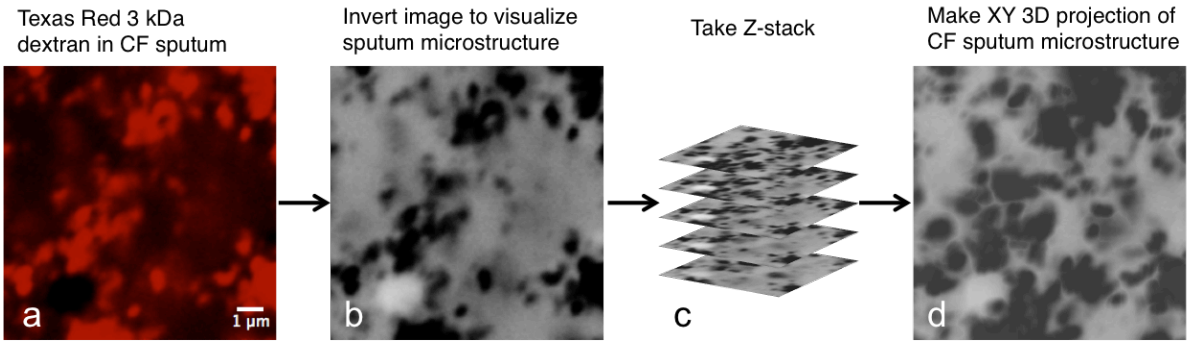

Figure S5. *Visualizing porous structure of CF sputum using low molecular weight Texas red-labeled dextran.* **(a)** Confocal imaging of 3 kDa Texas red-labeled dextran in CF sputum sample. **(b)** Inverted image of dextran in CF sputum shows solid matrix of CF sputum (white) and dextran-stained fluid filled pores (black). **(c)** Z stacking process of images to visualize 3D structure. **(d)** 3D reconstructed image of sputum generated by stacking 2D images.

Supplementary Tables

Table S1. Eigenvalues and percentage variation explained for each principal components determined after principal component analysis of measured CF sputum properties, including $\log_{10}[MSD_{1s}]$ (microstructure), mucin, DNA, cystine (disulfide bond), and total solids content for n=18 patients. Components considered significant when eigenvalues > 1 (labeled with *).

Principal Component	Eigenvalues	Variation Explained (%)
1*	3.51	65.2
2	0.84	15.6
3	0.48	9.0
4	0.30	5.6
5	0.25	4.6

Table S2. Principal Component 1 (PC1) Correlation Coefficients for $\log_{10}[MSD_{1s}]$ (microstructure), mucin, DNA, cystine (disulfide bond), and total solids content for n=18 patients. Correlation coefficients with an absolute value >0.4 were considered significant (labeled with *).

Parameter	PC1 Correlation Coefficient
$Log_{10}[MSD_{1s}]$*	-0.51
Mucin*	0.42
DNA*	0.49
Cystine	0.29
Percentage Solids*	0.48