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Supplementary Materials for

Nanoparticles that do not adhere to mucus provide uniform and long-lasting drug delivery to airways following inhalation

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:
(available at advances.sciencemag.org/cgi/content/full/3/4/e1601556/DC1)

- movie S1 (.avi format). Diffusion of PLGA NP in freshly collected CF sputum.
- movie S2 (.avi format). Diffusion of PLGA-PEG NP in freshly collected CF sputum.
- movie S3 (.avi format). Diffusion of PLGA/F68 NP in freshly collected CF sputum.
- movie S4 (.avi format). Diffusion of PLGA/F127 NP in freshly collected CF sputum.
- movie S5 (.avi format). Diffusion of 60-nm NP on freshly excised mouse tracheas.
- movie S6 (.avi format). Diffusion of 300-nm NP on freshly excised mouse tracheas.

Supplementary Materials

Materials and Methods

PEGylation of PS nanoparticles

Fluorescently labeled carboxyl-modified polystyrene (PS) nanoparticles (NP) (40 to 1000 nm) (Life Sciences, Frederick, MD) were covalently coated with poly(ethylene glycol) (PEG) by carbodiimide chemistry, as previously described [*67*]. Briefly, PS NP in 200 mM borate buffer (pH 8.0) were mixed with methoxy-PEG-amine (mPEG-NH2, MW 5 kDa; Creative PEGworks, Chapel Hill, NC), N-hydroxysulfosuccinimide (sulfo-NHS; Sigma Aldrich, St. Louis, MO), and 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC; Sigma Aldrich). The reaction was allowed to proceed overnight at 25°C on an orbital shaker. NP were washed via ultracentrifugation through a 100k MWCO membrane (Amicon Ultra-0.5 mL; Millipore, Temecula, CA), resuspended in ultrapure water to the initial particle volume and stored at 4°C until use.

Fluorescent labeling of biodegradable PLGA and PLGA-PEG polymers

Poly(lactide-co-glycolide) (PLGA, 15 kDa; SurModics Pharmaceuticals, Birmingham, AL) and PLGA45k-PEG5k (PLGA-PEG; Jinan Daigang Biomaterial Co., Jinan, China) were activated by reaction with p-nitrophenyl chloroformate (PCNF) and subsequently reacted with an amine fluorescent dye (Alexa Fluor® 555 (AF555) cadaverine or Alexa Fluor® 647 (AF647) cadaverine; Life Technologies, Frederick, MD). Briefly, the polymers (50 mg/mL) were dissolved in dichloromethane (DCM), along with PCNF (2 equivalents) and pyridine (4 equivalents). The reaction was allowed to proceed overnight at room temperature under continuous stirring. The polymers were precipitated in cold ethyl ether, collected via filtration, and dried overnight in a vacuum oven. Activated polymers (50 mg/mL) were dissolved in anhydrous dimethylformamide (DMF), along with dye (AF555 cadaverine or AF647 cadaverine, 0.1 equivalents) and triethylammine (4 equivalents). The reaction was allowed to proceed overnight at room temperature under continuous stirring. The fluorescently labeled polymers were then precipitated in cold ethyl ether, dissolved in DCM, precipitated in cold ethyl ether again, collected via filtration, and dried overnight in a vacuum oven. Fluorescently labeled polymers were stored at –20°C until use.

Multiple particle tracking of biodegradable NP in CF mucus

NP transport rates were measured via multiple-particle tracking (MPT) by analyzing trajectories of fluorescently labeled NP recorded using an EM-CCD camera (Evolve 512; Photometrics, Tuscan, AZ) mounted on a Zeiss Axio Observer D1 inverted epifluorescent microscope (Zeiss, Oberkochen, Germany). AF555-labeled PLGA or PLGA-PEG NP were added to 30 μl of cystic fibrosis (CF) sputum at a final concentration of 3% v/v in custom made chambers. Samples were allowed to equilibrate for 1 h at room temperature before imaging. Movies were captured using Metamorph software (Molecular Devices, Sunnyvale, CA) at a temporal resolution of 66.7 ms/frame for 20 s [*68*]. Movies were analyzed using a custom-written automated particle-tracking program in MATLAB (Mathworks, Natick, MA), based on the algorithm of Crocker and Grier [*69*], as previously described [*68*]. Briefly, the time-averaged mean squared displacement (MSD) as a function of time scale (τ) was calculated from the coordinates of individual NP centroids, using a equation: $\langle \Delta r^2(\tau) \rangle = \langle [x(t + \tau) - x(t)]^2 + [y(t + \tau) - y(t)]^2 \rangle$ where $\langle \Delta r^2(\tau) \rangle$ = MSD. Median MSD values were determined based on the measured MSD for individual NP and not ensemble averaged over all NP given the inherently heterogeneous, non-Gaussian distributions of NP transport in CF sputum. Only data from NP tracked for at least 25 frames was included in the analysis. At least 150 particles were tracked per sample, with n = 4 CF mucus samples per particle condition. Sample movies are provided in movies S1–S4.

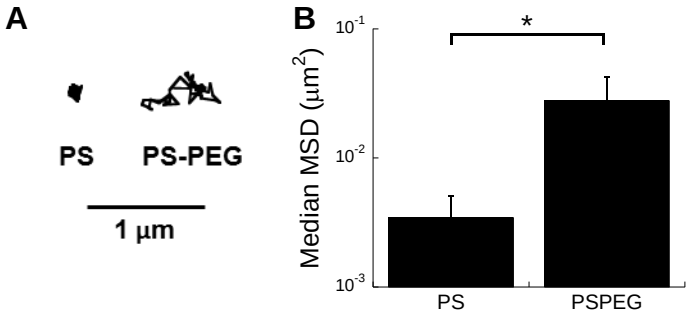

fig. S1. Diffusion of 300-nm PS and PS-PEG in human CF sputum. MPT was performed on NP administered *ex vivo* in freshly expectorated CF sputum. (**A**) Representative trajectories of NP. (**B**) Median MSD at a time scale of 1 s (n = 4). The data represent the mean ± standard error of the mean (SEM). *$p < 0.05$

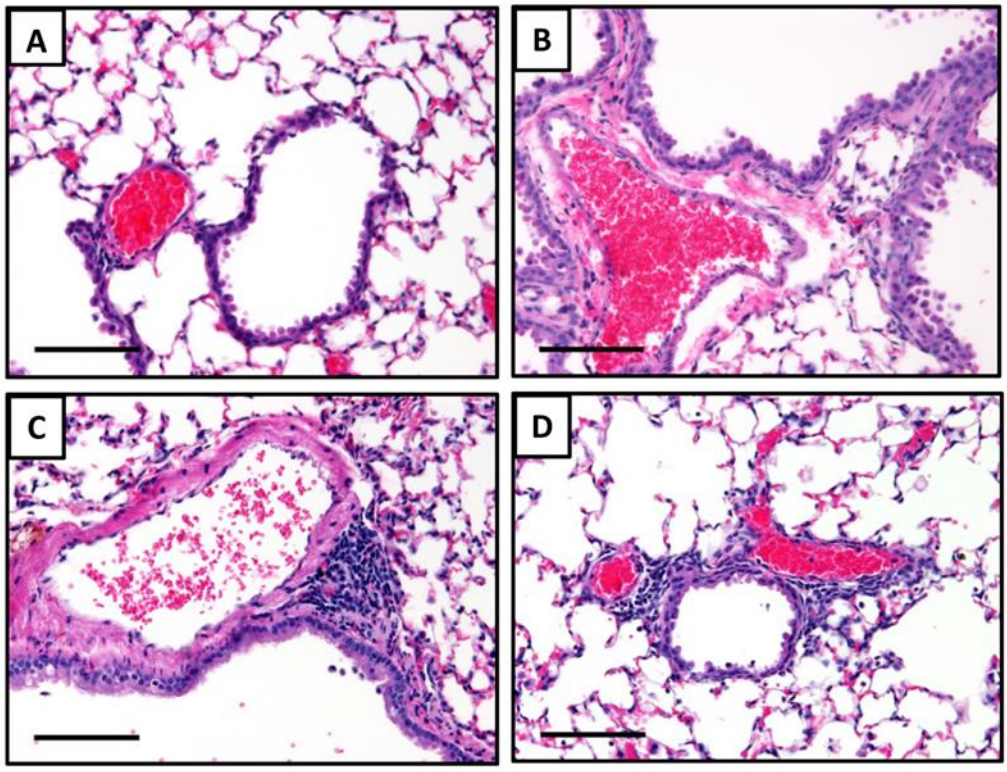

fig. S2. Lung histology at 24 hours after administration. Representative images of airways and pulmonary vessels from BALB/c mice treated with (**A**) saline, (**B**) 1 mg PLGA/F127, (**C**) 1 mg PLGA/F68, or (**D**) 1 mg PLGA/PF NP. Scale bars = 100 μm.

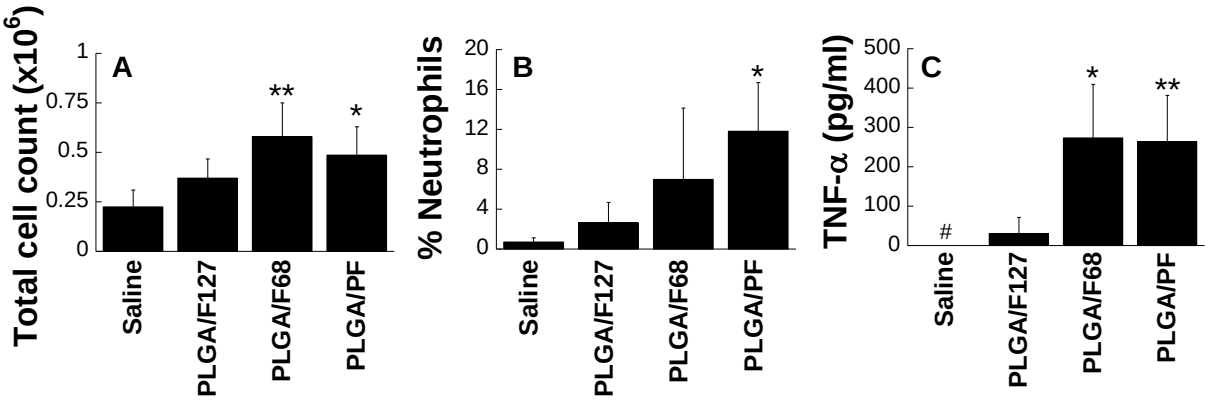

fig. S3. Safety profile of GRAS-based biodegradable NP. BALF analysis of mice (n = 7) locally treated with 1 mg of NP at 24 h post-administration: (**A**) total inflammatory cell counts, (**B**) percentage of neutrophils, and (**C**) concentration of TNF-α. The data represent the mean ± standard deviation. *denotes statistically significant differences (*$p < 0.05$, **$p < 0.01$) compared to saline control for (A) and (B), and compared to PLGA/F127 NP.