

Addressing the PEG Mucoadhesivity Paradox to Engineer Nanoparticles that “Slip” through the Human Mucus Barrier**

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Mucus linings serve as the body's first line of defense at exposed surfaces of the eye and respiratory, gastrointestinal, and cervicovaginal tracts. The high viscoelasticity and adhesivity of mucus traps and limits exposure to foreign pathogens,^[1,2] toxins,^[3] and environmental ultrafine particles,^[1,4] which are all typically removed by normal mucus clearance mechanisms. Numerous studies have demonstrated that human mucus also strongly immobilizes conventional synthetic nanoparticles,^[5–7] and therefore represents a hurdle for localized drug and gene delivery at mucosal surfaces, such as aerosol-based gene carriers for cystic fibrosis gene therapy.^[8] To increase the bioavailability of cargo therapeutics, it is important that carrier particles rapidly penetrate mucus to avoid being shed.

We recently discovered that nanoparticles coated with low molecular weight (MW) poly(ethylene glycol) (PEG) possess hydrophilic and near neutrally-charged surfaces that minimize mucoadhesion by reducing hydrophobic or electrostatic interactions.^[5] These coatings were inspired by viruses with similar surface properties that are capable of moving rapidly through human mucus.^[7,9] However, a vast amount of literature has shown that PEG can be strongly mucoadhesive, presumably by interpenetrating polymer network (IPN) effects between PEG chains and the mucus mesh^[10–12] and/or hydrogen bonding between ether oxygen atoms in PEG and sugars on glycosylated mucins.^[13] To reconcile the para-

doxical reports of PEG's interactions with mucus, we correlated the physicochemical properties of PEG-coated nanoparticles, specifically PEG MW and degree of surface coverage, to the dynamics of coated particles in fresh, undiluted human cervicovaginal mucus (CVM). The real-time transport rates of the particles were studied in mucus using multiple-particle tracking, a powerful technique that allows quantitative measurements of hundreds of individual particles.^[5,14] We show that low PEG MW and high (dense) PEG surface coverage are both required for rapid mucus penetration of coated particles, and that high MW PEG can increase mucoadhesion.

To determine the effect of PEG MW on the interactions of coated particles with mucus, we covalently conjugated a dense surface coverage of either 2 kDa (PS-PEG2k^{High}) or 10 kDa (PS-PEG10k^{High}) methoxy-PEG-amine onto 200 nm fluorescent carboxylated polystyrene (PS) nanoparticles. The MWs were chosen on the basis of our previous finding that coating particles with 2 kDa PEG provided mucus-penetrating transport properties^[5] and literature suggesting that adhesive IPN effects are likely enhanced at higher MWs.^[11] A dense surface coating was confirmed for each formulation by the near-neutral surface charge of coated particles, as well as fluorimetric assays that revealed 65–70 % of surface carboxy groups conjugated with PEG (Table 1). A fivefold reduction in PEG

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[**] This work was supported in part by the NIH 5U01AI066726 (R.C.), NIH R21HL089816 and R01EB003558 (J.H.), Cystic Fibrosis Foundation (HANES08G0), and fellowships from the NSF (Y.-Y.W.) and Croucher Foundation (S.K.L.). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Supporting information for this article is available on the WWW under <http://dx.doi.org/10.1002/anie.200803526>.

Table 1: Characterization of COOH- and PEG-modified PS nanoparticles and ratios of their ensemble average diffusion coefficients in mucus (D_m) compared to in water (D_w).

Particle Coating (PS-)	PEG MW [kDa]	Diameter [nm]	ζ -potential [mV]	PEG Coverage [%] ^[a]	D_m/D_w ^[b]
COOH ^[c]	N/A	217 ± 5	−59 ± 4	N/A	0.00042
PEG2k ^{High}	2	231 ± 6	−2 ± 4	69 ± 1	0.15
PEG10k ^{High}	10	229 ± 3	−1 ± 3	65 ± 1	0.00015
PEG2k ^{Low}	2	224 ± 6	−10 ± 3	42 ± 3	0.00022

[a] PEG coverage percentage was based on the number of COOH groups available for conjugation on the surface of PS-COOH nanoparticles (see Experimental Section). [b] Effective diffusivity values were calculated at a time scale of 1 s. D_w was calculated from the Stokes-Einstein equation.

[c] Data from previous study.^[5]

MW, from 10 kDa to 2 kDa, produced a 1000-fold increase in the mean-square-displacement (MSD) of coated particles in CVM at a time scale of 1 s (Figure 1a) and a near-uniform increase in individual particle speeds (Figure 1b). Importantly, PS-PEG2k^{High} nanoparticles penetrated CVM with effective speeds only sevenfold reduced compared to in water. The difference in the transport rates of particles coated with

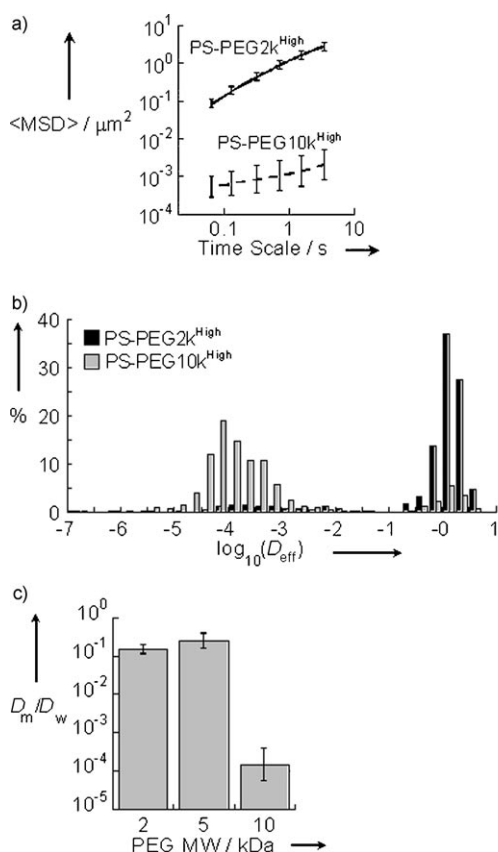


Figure 1. Transport rates of 200 nm PEG-modified PS particles with high surface coverage 2 kDa PEG (PS-PEG2k^{High}) or high surface coverage 10 kDa PEG (PS-PEG10k^{High}) in CVM. a) Ensemble-averaged geometric mean square displacements ($\langle \text{MSD} \rangle$) as a function of time scale. b) Distributions of the logarithms of individual particle effective diffusivities (D_{eff}) at a time scale of 1 s. Data represent five independent experiments with $n \geq 100$ particles per experiment (average $n = 141$ and 127 for PS-PEG2k^{High} and PS-PEG10k^{High}, respectively). c) Ratios of the ensemble average diffusion coefficients in mucus (D_m) compared to in water (D_w) for PS-PEG particles with PEG of varying MW. Particles synthesized with a similarly dense coating of 5 kDa methoxy-PEG-amine (PS-PEG5k^{High}) had an average size of (238 ± 4) nm and ζ -potential of (-6 ± 1) mV. Data represent three independent experiments with $n \geq 150$ particles per experiment for PS-PEG5k^{High}.

2 kDa and 10 kDa PEG was also reflected by the slope α of double logarithmic MSD versus time scale plots ($\alpha = 1$ represents unobstructed Brownian transport, whereas smaller α reflects increased obstruction to particle movement): average α was 0.83 for PS-PEG2k^{High} but only 0.35 for PS-PEG10k^{High}. We further tested particles densely coated with 5 kDa PEG and found that they retain rapid mucus-penetrating properties (Figure 1 c), indicating that a critical MW exists between 5 and 10 kDa where dense PEG coatings transition from being mucoinert to mucoadhesive.

We next tested whether the extent of PEG surface coverage may also help explain some of the conflicting reports of PEG interaction with mucus. We prepared particles with a lower surface coverage of 2 kDa PEG (PS-PEG2k^{Low}), which had a more negative surface charge and approximately 40% lower PEG coverage (Table 1). Surprisingly, this relatively small difference in the surface PEG coverage led to a

700-fold decrease in the average transport rate of PS-PEG2k^{Low} compared to PS-PEG2k^{High} at a time scale of 1 s (Figure 2 a), and uniformly slower speeds for PS-PEG2k^{Low}

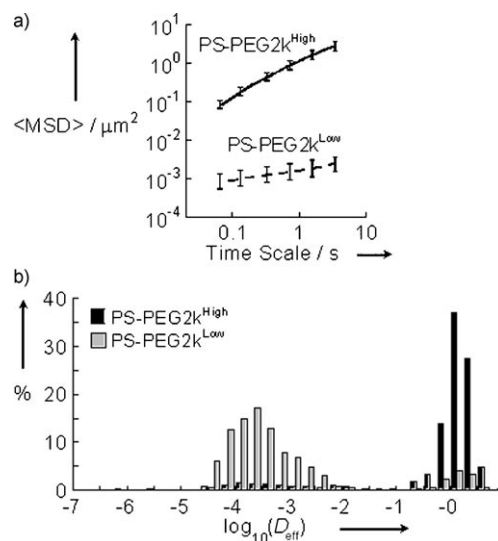


Figure 2. Transport rates of 200 nm PEG-modified PS particles with higher surface coverage 2 kDa PEG (PS-PEG2k^{High}) or lower surface coverage 2 kDa PEG (PS-PEG2k^{Low}) in CVM. a) Ensemble-averaged geometric mean square displacements ($\langle \text{MSD} \rangle$) as a function of time scale. b) Distributions of the logarithms of individual particle effective diffusivities (D_{eff}) at a time scale of 1 s. Data represent five independent experiments with $n \geq 90$ particles per experiment (average $n = 141$ and 132 for PS-PEG2k^{High} and PS-PEG2k^{Low}, respectively).

(Figure 2 b). Indeed, the transport dynamics of PS-PEG2k^{Low}, with an α of 0.27, were similar to PS-PEG10k^{High}. Taken together, our results indicate that PEG-coated mucus-penetrating particles must simultaneously satisfy the design requirements of 1) sufficiently low PEG MW and 2) sufficiently high density of PEG surface coverage.

We examined previous studies of PEG interaction with mucus to test the applicability of these principles to published observations. Unfortunately, many studies did not extensively characterize the physicochemical properties of PEG-coated particles, especially with respect to the density of surface PEG. A small number of studies reported surface charge, at best an indirect measurement of PEG surface coverage for core particles with non-neutral surface charge. Nevertheless, it is interesting to note that most previous studies of PEG-coated drug delivery systems, which were composed of hydrophobic, anionic core particles with diameters of 200–500 nm, reported surface charges more negative than -10 mV and significant mucoadhesion (Figure 3). An exception is our previous study, where particles well coated with low MW PEG, as reflected by surface charges between -2 and -6 mV, penetrated mucus at rates up to only fourfold reduced compared to those in pure water.^[5] In so far as surface charge can be used as an indicator of PEG coverage, that observation and the characterization of PS-PEG2k^{Low} particles in the present study suggest that a critical threshold for particle surface charge (measured under pH-neutral condi-

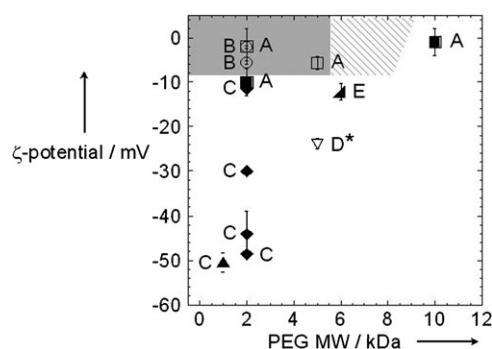


Figure 3. Phase diagram correlating mucoadhesive versus mucoadhesive particle behavior to surface charge and PEG MW for various PEG-coated nanoparticles (200–500 nm in size) reported herein and in the literature. PEG-coated nanoparticles reported to be non-mucoadhesive compared to control particles are indicated by open symbols, and those reported to be mucoadhesive are indicated by filled symbols. The shaded region represents the confirmed range of PEG MW and particle ζ -potential (i.e., PEG surface coverage), and the hatched region an additional predicted range that provides a mucoadhesive coating. A) Present study; B) PEG-coated PS nanoparticles;^[5] C) PEGylated poly(methyl vinyl ether-co-maleic anhydride) nanoparticles;^[16,17] D) poly(lactic acid)-PEG nanoparticles^[15] (*: mucoadhesion was not observed based on adhesion to an in vitro mucin-secreting cell line); E) PEG-coated poly(ethyl-2-cyanoacrylate) nanospheres (mucoadhesion was inferred from improved bioavailability compared to free drug).^[18]

tions), between -10 and -7 mV, may govern the mucoadhesive versus mucoadhesive properties of particles. The only paper we found that did not observe mucoadhesion, despite an apparently low PEG surface coverage, studied the association of particles to an in vitro mucin-secreting cell line.^[15] However, the cells used in that study are unlikely to produce mucus gels with the mesh structure and adhesivity of physiological human mucus, which contains a dynamic mixture of mucins produced by both goblet cells and mucin-secreting glands, as well as proteins, lipids, and ions.^[9,19] The critical threshold of PEG coverage may also depend on particle size, since a higher degree of curvature may require greater PEG coverage.^[5] While the design principles established here appear broadly applicable to particles of different sizes and core compositions, the exact threshold of PEG MW and surface coverage needed to achieve mucoadhesion may depend on the specific system of interest.

Particles that are sufficiently small and non-mucoadhesive can diffuse rapidly in the interstitial fluid between mucus mesh fibers, without experiencing the bulk viscosity of mucus, which is typically 2000-fold or higher than that of water at low shear rates.^[5] Since PS-PEG2k^{High} nanoparticles readily diffuse through mucus, the greatly slowed transport of both PS-PEG10k^{High} and PS-PEG2k^{Low} must be due to their strong adhesion to mucus. The highly adhesive nature of mucus is likely due to a high density of negatively charged glycans that contain both strong proton acceptor and donor groups and to hydrophobic naked protein domains that are further coated with lipids.^[9,20] Accordingly, each mucin fiber may form low-to moderate-affinity interactions with any hydrophobic, cationic, and/or hydrogen bonding surface. The 3D network structure of mucus and the high flexibility of individual mucin

fibers^[9] further ensure sufficient polyvalent interactions to tenaciously immobilize nearly all conventional particles. The mucoadhesion of uncoated PS particles is primarily due to polyvalent hydrophobic interactions.^[5] For particles with low coverage of 2 kDa PEG, the surface PEG is likely inadequate to prevent hydrophobic interactions between the PS core and mucins. A higher surface PEG coverage blocks hydrophobic adhesive interactions, since the entropic penalty of mucins displacing water and PEG in order to form hydrophobic anchors with the PS core becomes prohibitive. However, while the effective lengths of surface PEG molecules (Table S1 in Supporting Information) are short compared to the average mesh spacing of mucus (up to several hundred nanometers),^[5] higher MW (e.g., 10 kDa) PEG chains may be long enough to significantly entangle with mucins, as suggested previously by Peppas and co-workers,^[12,21] especially in regions of high mucin fiber density. The mucoadhesion observed with 10 kDa PEG coatings may also reflect a greater number of intermolecular interactions, such as hydrogen bonding, with mucins.

The development of mucoadhesive surfaces involves a fine balancing of interactions between particles and mucus. Coating particles with a dense layer of low MW PEG effectively reduces hydrophobic interactions, hydrogen bonding, and IPN effects to levels below the threshold required to slow and immobilize particles. This simple design principle may facilitate the widespread development of biodegradable drug- and gene- loaded mucus-penetrating particles for the treatment of various mucosal diseases,^[22] including cancer and inflammation in the respiratory, gastrointestinal, and female reproductive tracts.

Experimental Section

The general experimental methods were as follows (details are available in Supporting Information): PEG-coated nanoparticles were synthesized by covalent conjugation of different MW methoxy-PEG-amine to 200 nm fluorescent carboxylated PS particles.^[5] Particles were characterized for size, surface charge, and PEG surface coverage. The displacements of particles were tracked in fresh, undiluted human CVM using multiple-particle tracking.^[5,23]

Received: July 19, 2008

Published online: October 31, 2008

Keywords: drug delivery · mucosal tissues · nanomedicine · particle tracking · surface chemistry

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