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Mucus Penetrating Nanoparticles: Biophysical Tool and Method of Drug and Gene Delivery

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Abstract

A method that could provide more uniform and longer-lasting drug and gene delivery to mucosal surfaces holds the potential to greatly improve the effectiveness of prophylactic and therapeutic approaches for numerous diseases and conditions, including sexually transmitted infections, cystic fibrosis, chronic rhinosinusitis, inflammatory bowel disease, and glaucoma to name a few. However, the body's natural defenses, including adhesive, rapidly cleared mucus linings coating nearly all entry points to the body not covered by skin, has limited the effectiveness of drug and gene delivery by nanoscale delivery systems. This article discusses the recent development of the “mucus-penetrating particle” or “MPP” nanotechnology, and how it has been used to both enhance understanding of the nanoscale barrier properties of human mucus secretions, and to achieve more uniform and longer-lasting drug delivery to mucosal tissues following topical administration. Drug loaded MPPs possess non-adhesive coatings that allow them to rapidly penetrate mucus layers through openings in the mucus mesh at rates nearly as fast as they would penetrate pure water. Critically, MPPs allow enhanced drug and gene delivery to mucosal tissues without diminishing the protective function of mucus. Recent progress in the development of MPPs as a biophysical tool to probe the length-scale dependent rheological properties of mucosal secretions and as a method for drug and gene delivery is highlighted.

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Keywords

Mucosal drug delivery; Gene therapy; Nanoparticles; Nanotechnology; Mucus microstructure

1. Introduction

Mucus is a viscoelastic, adhesive gel that coats all exposed epithelial surfaces not covered by skin, such as those of the respiratory, gastrointestinal (GI), and female reproductive tracts, and the surface of the eye. Mucus protects the underlying epithelium by both lubricating the surface and trapping and removing foreign particulates. Mucus has been shown to be highly adhesive to pathogens[1, 2], environmental fine particles[3], and conventional particle-based drug delivery systems[4–6], facilitating their removal in a matter of minutes to hours by mucus turnover. Thus, mucus is not only vital for human health, but it also significantly limits the potential for localized and sustained drug and gene delivery to mucosal surfaces[7]. Penetrating deep into the mucus barrier without compromising its protective properties could lead to improved prophylactic and therapeutic particle-based treatments for diseases and conditions such as cystic fibrosis (CF), sexually transmitted infections (STI), degenerative eye diseases, lung cancer, and irritable bowel disease.

Mucus forms adhesive interactions with particulates via hydrophobic, electrostatic, and hydrogen bonding interactions[8]. Additionally, mucus gel is composed of highly cross-linked mucin fibers, creating a dense porous structure[9]. Therefore, to penetrate mucus, nanoparticles must be small enough to avoid steric obstruction and have a hydrophilic, net-neutral surface to avoid adhesion[7]. Recently, we demonstrated that nanoparticles as large as 500 nm can diffuse rapidly through undiluted human cervicovaginal mucus (CVM), if densely coated with low molecular weight polyethylene glycol (PEG)[4, 9]. These mucus penetrating particles (MPP) diffuse through human mucus only a few-fold slower than their theoretical diffusion in water[4]. In this Research News article, we discuss MPP as vehicles for drug and gene delivery to mucosal surfaces and as a tool for probing and characterizing mucus structure and rheology.

2. The importance of surface characteristics for mucus penetration

Nanoparticle-based systems have many desirable properties for mucosal drug delivery, including protection and sustained release of the drug cargo[10]. However, even the most potent extended release nanoparticle will be rapidly cleared from the body if it adheres to the superficial layers of mucus, reducing delivery of the drug to the target tissues[11]. Thus, our group has investigated surface characteristics of nanoparticles that enable mucus penetration to reach the more slowly cleared mucus that adheres to the epithelial surface. Previous literature had indicated that PEG coatings actually cause mucoadhesion, presumably by interpenetrating polymer network (IPN) effects between the PEG and the mucus mesh[12–14] and/or hydrogen bonding[15]. However, we discovered that nanoparticles with a dense surface coating of 2 kDa PEG penetrated CVM only a few fold slower than their theoretical diffusion rates in water[4]. To reconcile these seemingly paradoxical results for PEG interaction with mucus, we investigated the effects of PEG molecular weight and degree of surface coverage on the rate of nanoparticle diffusion in mucus[16].

Starting with conventional mucoadhesive nanoparticles (CP), we covalently conjugated either 2 kDa or 10 kDa PEG to the particle surface via free carboxylic acid groups. To characterize the surface coating, we measured the surface charge (zeta potential). As previously demonstrated[4], particles coated with a high density of 2 kDa PEG had a near-neutral surface charge (−2 ± 4 mV) and rapid mucus penetration. On the other hand,

particles well-coated with a high density of 10 kDa PEG were mucoadhesive. Also in contrast to particles coated with a high density of 2 kDa PEG, particles coated with a low density of 2 kDa PEG had a surface charge of −10 ± 3 mV and were mucoadhesive. It was apparent that a low molecular weight (2 kDa) PEG coating at a high surface density was able to shield the hydrophobic polystyrene core from adhesive interactions with mucus. In contrast, neither a high density coating of 10 kDa PEG or a low density coating of 2 kDa PEG could prevent mucoadhesion due to interactions between nanoparticles and mucus via exposed hydrophobic surfaces or interpenetration of PEG into the mucus mesh[16]. Although only a few studies report surface charge measurements, most studies involving hydrophobic, anionic core particles with a surface charge more negative than −10 mV report significant mucoadhesion (Figure 1). The only paper we found that did not observe mucoadhesion, despite apparently incomplete PEG surface coverage, investigated the association of the particles with mucus *in vitro*[17]. Cells in culture are unlikely to produce mucus gels with the structure and characteristic adhesivity of human mucus.

Although 10 kDa PEG chains are short compared to the average mesh spacing of mucus (see Section 5), 10 kDa PEG chains may be long enough to entangle sufficiently with mucins, as suggested by Peppas and coworkers[14, 18]. Additionally, we have determined that sufficient coatings with ≤ 5 kDa PEG, both covalent[11, 16] and non-covalent[19], also confer mucus penetrating ability.

3. Mucus penetrating particles for drug delivery

For drug delivery purposes, nanoparticles must be formulated from biodegradable components that will break down in the body. To this end, we sought to create biodegradable MPP from block copolymers synthesized by covalently attaching low molecular weight PEG to polymers such as poly(lactic-co-glycolic acid) (PLGA-PEG) and polysebacic acid (PSA-PEG). PLGA-PEG provides excellent stability and release kinetics for a wide range of therapeutic molecules, including peptides and proteins[20]. Poly(ether-anhydrides), such as PSA-PEG, degrade by surface erosion, thereby minimizing burst release of encapsulated drugs[21]. Both block copolymers have been formulated into MPP that diffuse rapidly in CVM[6, 22]. Additionally, biodegradable MPP that rapidly penetrate human mucus have been made entirely out of Generally Regarded as Safe (GRAS) materials, PLGA and Pluronic® triblock copolymer[6]. No new chemical entities are generated by covalent linkages in this approach, since the Pluronic® coating physically adsorbs to the PLGA core. MPP composed entirely of GRAS materials will likely facilitate more rapid clinical development. Lastly, MPP have also been developed using a novel PEG-based surfactant that significantly improves the loading of a front-line chemotherapy drug, paclitaxel[23]. These nanoparticles contained ~8% Paclitaxel by weight, and released drug in a sustained manner over a 5 day period. Biodegradable MPP can be formulated from a wide range of materials, allowing the possibility for tailoring loading and release characteristics.

By penetrating the outer, rapidly cleared mucus layers to reach the more slowly cleared layers, MPP could improve epithelial distribution and retention times[8]. We recently tested this hypothesis for vaginal delivery, with an emphasis on developing microbicides for prevention of STIs[11]. The vaginal epithelium contains numerous folds (rugae) that are typically collapsed due to intra-abdominal pressure and, as a result, are less accessible to drugs and drug carriers[24, 25]. Poor drug distribution into the rugae, even after simulated intercourse, has been implicated in failure to protect susceptible vaginal surfaces from infection[26]. After confirming that MPP rapidly penetrate mouse vaginal mucus, we demonstrated that MPP coat the vaginal and cervical epithelium, whereas mucoadhesive CP aggregated in the lumen unless the mucus was removed prior to particle administration (Figure 2A/B). By penetrating into the more slowly cleared mucus layer, including those in

the rugae, 60% of MPP were retained in the vaginal tract after 6 h, as compared to only 10% of the CP. To demonstrate that MPP could deliver drugs more effectively than current microbicide gel formulations, a model drug was vaginally administered either in gel or encapsulated in biodegradable MPP composed of GRAS ingredients. After 24 h, the vaginal surface was completely coated with model drug when delivered in MPP, while only patches of drug sparsely covered the vaginal epithelium when administered in vaginal gel (Figure 2C). We then tested whether the improved distribution and retention of vaginally administered drug would improve efficacy in a mouse model for vaginal HSV-2 infection. Administered 30 min prior to viral challenge, anti-HSV MPP protected mice better than even 10-fold more free drug, whereas the free drug at the same concentration as given in anti-HSV MPP provided no significant protection. Importantly, cytokine levels indicating epithelial injury were indistinguishable between HSV MPP and hydroxyethylcellulose (HEC) universal placebo gel after daily dosing for 7 days. HEC gel is routinely used in clinical trials, indicating that HSV MPP may be safe for repeated vaginal dosing[11].

In addition to demonstrating that MPP are able to rapidly penetrate healthy human mucus, we have also demonstrated that MPP can rapidly penetrate human mucus in diseased states. Both polystyrene-based and biodegradable MPP composed of entirely GRAS ingredients were able to rapidly penetrate chronic rhinosinusitis mucus (CRSM)[4], and sputum expectorated from Cystic Fibrosis (CF) patients[6, 27].

4. Mucus penetrating particles for gene delivery

Topical delivery of genetic materials via nanoparticle systems may be an attractive strategy to combat a variety of diseases affecting epithelial surfaces, including genetic disorders, infectious diseases and cancers. Cystic fibrosis (CF) gene therapy is of a great interest since it is a monogenetic disorder that requires correction of a single gene to cure the disease. Numerous gene delivery systems based on viral and non-viral gene platforms have been developed and tested in the lungs of CF patients without promising therapeutic outcomes[28]. In the CF lungs, mucus dehydration and subsequent chronic infection and inflammation lead to hyperviscoelastic sputum secretions[29, 30]. The reduced water content, as well as the increased amounts of cells debris and macromolecules released from bacterial and immune cells, is believed to reinforce the barrier property of this sputum layer[8, 31, 32]. We previously found that diffusion rates of 200 and 500 nm MPP were ~15-fold and ~400-fold slower in CF sputum than their rates in CVM, likely due to increased adhesivity and steric obstruction from the sticky and dense CF sputum mesh[4, 6, 33]. We estimated the average mesh spacing of CF sputum to be ~145 ± 50 nm[27], which is substantially smaller than that of human cervicovaginal mucus (~340 ± 70 nm). These findings led to our hypothesis that unsatisfactory results in CF gene therapy clinical trials to date may be attributed to the inability of gene delivery systems to penetrate CF sputum and reach the underlying epithelium. In support of this hypothesis, we recently showed that the diffusion of leading viral[34] and non-viral[33, 35] gene carriers is strongly hindered in sputum freshly expectorated by CF patients. The average diffusion rates of adenovirus, adeno-associated virus serotype 5 and a clinically-tested polymeric DNA nanoparticle were at least ~3000-fold slower in CF sputum than their theoretical speeds in water[33–35]. Likewise, Sanders and coworkers showed that clinically tested lipid-based gene carriers are unable to efficiently penetrate a sputum layer in a diffusion chamber study[36]. These findings suggest that developing strategies to overcome the CF sputum barrier is a prerequisite to effective CF gene therapy.

Highly compacted DNA nanoparticles, composed of single molecules of plasmid DNA compacted with block copolymers of poly-L-lysine and 10 kDa polyethylene glycol ($CK_{30}PEG_{10k}$), have been shown to mediate effective gene transfer to brain[37], eyes[38], and

lungs[33, 39] in animals. However, these DNA nanoparticles do not efficiently penetrate human CF sputum[33, 34]. We recently showed that diffusion of these DNA nanoparticles in sputum pretreated with N-acetylcysteine (NAC) was significantly improved compared to their diffusion in untreated sputum, as evident by the greater travel distance (Figure 3A) and higher individual (Figure 3B) and ensemble-averaged (Figure 3C) diffusion rates of DNA nanoparticles in NAC-treated sputum. It is most likely that the enhanced diffusion is attributed to enlarged pores in sputum treated with NAC; we previously showed that NAC treatment markedly increases the average mesh spacing of CF sputum, to 230 ± 50 nm, by cleaving disulfide cross-links among mucin fibers[27]. In a mucus-hypersecreting mouse model where the airway mucus production (Figure 3D) and secretion (Figure 3E) was substantially elevated, we showed that NAC treatment enhanced diffusion of DNA nanoparticles on *ex vivo* mouse tracheal tissue and mediated improved airway gene transfer (Figure 3F)[33]. Nevertheless, the average diffusion rate of DNA nanoparticles remained ~40-fold lower than that of substantially larger, spherical PEG-coated polystyrene nanoparticles (diameter ~500 nm) in NAC-treated sputum[27, 33], suggesting diffusion of these highly compacted DNA nanoparticles in CF sputum is strongly hindered by their adhesive interaction with sputum constituents. Based on our previous finding that nanoparticles densely coated with low MW PEG (2–5 kDa) are muco-inert, whereas particles coated with 10 kDa PEG are mucoadhesive[16], we formulated highly compacted DNA nanoparticles with low MW PEG coatings ($CK_{30}PEG_{2k}$ and $CK_{30}PEG_{5k}$). We found that reducing PEG MW from 10 kDa to 5 kDa did not affect the particle stability, ability to protect cargo DNA, traffic through a beneficial intracellular route, nor mediate gene transfer to mouse lungs[35, 40]. However, $CK_{30}PEG_{5k}$ DNA nanoparticles, similar to $CK_{30}PEG_{10k}$ DNA nanoparticles, were essentially immobilized in human CF sputum[35]. The immobilization of $CK_{30}PEG_{5k}$ DNA nanoparticles is most likely due to insufficient PEG surface coverage that leads to adhesion of nanoparticles to sputum constituents. We estimated the PEG surface density on $CK_{30}PEG_{5k}$ DNA nanoparticles to be ~12–30-fold less than that on 200 nm MPP shown to rapidly penetrate CF sputum[35]. We previously showed that only ~40% of reduction in PEG surface coverage led to a 700-fold decrease in particle transport in human mucus[16]. These findings suggest that sputum-penetrating gene carriers must be small enough to diffuse through sputum mesh and possess highly dense PEG surface coatings to avoid adhesion to sputum constituents.

5. Mucus-penetrating particles as a biophysical tool

MPP can be used as probes to investigate micro- and nanoscale properties of complex fluids that cannot always be adequately resolved with conventional macroscopic techniques. In particular, our lab has focused on studying the mesh structure and microrheology of mucus secretions. Mucus is a highly viscoelastic polymer network composed of mucin proteins, creating a mesh-like structure that can have an average mesh spacing (pores) less than 1 μm in diameter[41]. Common rheological techniques, like cone and plate rheometry, can only provide bulk or macroscopic rheological properties. Macroscopic rheology is beneficial for understanding lubrication and clearance mechanisms, but does not provide information about the rheology experienced by nanoscale objects, such as particles or viruses.

The mechanical or “rheological” properties of gels, e.g. viscosity and elasticity, depend strongly on the length scale of interest. For example, entities that adhere to the gel and/or are larger than the gel pores become trapped. In contrast, particles that are smaller than the average pore size of the gel and do not stick to gel fibers can pass through as if diffusing through a purely viscous fluid, with a viscous drag roughly comparable to that imposed by water. Using MPP, both the viscosity and elasticity experienced by particles in gels can be probed by using a technique called particle tracking microrheology (PTM), which extracts local mechanical properties from the time scale-dependent mean square displacements of

individual probe particles[42]. Microrheological data is extracted from the amplitude and time scale-dependence of the geometrically averaged ensemble mean-squared displacements[9]. These values can be used to calculate the viscoelastic spectrum via Laplace transform and the generalized Stokes-Einstein equation, followed by Fourier transformation to extract the elastic and shear moduli as a function of frequency[43]. For a more detailed explanation of the calculation of the viscous and elastic moduli, see[8]. For a more in depth discussion on mucus microrheology, see[9].

Recently, we examined the effects of the addition of a nonionic detergent (nonoxynol-9 or N9) commonly used in vaginal gels, lubricants, and condoms on the microrheology of mucus experienced by different sized MPP probe particles[8]. Fresh, undiluted CVM mucus was found to be an impermeable elastic barrier to non-adhesive probes 1 μm in size or larger, whereas it behaved as a viscoelastic liquid to non-adhesive objects smaller than 500 nm in diameter. The phase angle (δ) of a gel, represented by the equation

$$\delta = tan\left(\frac{G''}{G'}\right) \quad (1)$$

where G' is the elastic modulus and G“ is the viscous modulus, determines whether the gel is a viscoelastic solid (δ<45°, G'> G“) or a viscoelastic liquid (δ>45°, G'< G“). MPP probes 500 nm or smaller experienced a viscoelastic liquid with δ>60° and, therefore, a greater viscous than elastic modulus at a frequency of 2π rad/s. Particles with a size of 1 μm experienced a viscoelastic solid (δ≈30°), with mucus exhibiting a much greater elastic modulus compared to viscous modulus at the same frequency when probed with these larger particles. However, mucus treated with N9 behaved as an impermeable elastic barrier to both 200 and 500 nm particles. The change in microrheology was likely due to the disruption of hydrophobic interactions between mucin fiber bundles by N9, creating a finer elastic mucin mesh[8]. Thus, smaller particles experienced elastic recoil rather than viscous drag. Interestingly, the bulk rheology (macroscopic rheology) was unchanged by addition of N9, potentially indicating that the reduction in adhesive interactions within mucin bundles was likely balanced by an increase in mucin entanglements[8].

The use of MPP probes smaller than the average mesh spacing of a gel allows the determination of the microviscosity experienced by these particles in the mucus gel pores. Previous studies by our group using unmodified, mucoadhesive polystyrene (PS) beads found that the microviscosity of CF sputum were only 15- and 7-fold lower than the bulk viscosity measured by a cone and plate rheometer, as measured using 100 and 200 nm PS particles, respectively[44]. However, because these studies were performed with particles that are mucoadhesive, the observed transport rates in mucus are strongly affected by adhesive interactions with the mucus mesh, and therefore may more accurately reflect the bulk rheology. More recently, we used densely PEG-coated polystyrene MPP probes to evaluate the microviscosity of CF sputum and found the microviscosity experienced by 200 nm MPP was 300–1500 times lower than the bulk viscosity, in stark contrast to the results observed with unmodified PS beads[45].

In addition to microrheology measurements, MPP probes can be used to estimate the mesh structure of mucus gels. Using techniques such as fluorescence recovery after photobleaching (FRAP) and multiple-particle tracking (MPT), the diffusion coefficients of probe particles with varying size can be evaluated in mucus samples. The diffusion coefficients can then be used to estimate the average mesh spacing of a mucus sample using an obstruction scaling model. Importantly, this model is only valid when there is no adhesive interaction between probe particles and the gel mesh. Thus, mucus-penetrating particles are excellent probe particles because their dense PEG coatings minimize adhesive

interactions with mucus constituents. The obstruction scaling model[46, 47] represents the ratio of diffusion in a gel (e.g. mucus) and diffusion in pure water as

$$\frac{D_g}{D_o} = e^{\left(-\frac{\pi}{4}\right)\left(\frac{r_s + r_f}{r_g + r_f}\right)^2} \quad (2)$$

where D_g is the diffusion coefficient of the particle in the polymer gel, D_o is the diffusion coefficient in water, r_s is the particle radius, r_f is the gel fiber radius, and r_g is the effective radius of the pore. Using a set of experimentally determined D_g and r_s (and biochemical estimates of r_f), a fit can be performed to estimate r_g, the average mesh spacing of the mucus sample. In the case of multiple-particle tracking, the diffusivities of individual particles can be assessed[48] and application of the same model generates a distribution of pore sizes, in addition to an average pore size.

We have used the MPT technique to estimate the average mesh spacing and pore size distribution of a variety of native and chemically treated mucus samples. Previously, the average mesh spacing of human cervical mucus was estimated to be 100 nm based on SEM[49] and DMSO-mediated glutaraldehyde fixation and transmission electron microscopy (TEM)[5], 500–800 nm by using freeze substitution and TEM[50], and even up to 1,000–10,000 nm or larger by using various EM procedures[51–53]. We recently explored the mesh spacing of freshly collected human CVM with MPP probe particles[54]. The average mesh spacing was found to be 340 ± 70 nm with pores ranging in size from 50 nm to nearly 2 μm. However, when treated with the nonionic surfactant N9, the average mesh spacing of the mucus was significantly reduced to 130 ± 50 nm with a relatively narrow distribution of pore sizes. The pore size reduction suggests that the mucus mesh is formed by mucin "cables" at least three times larger in diameter than individual fibers, and that a surfactant such as N9 is able to debundle mucin fibers by disrupting hydrophobic interactions between fibers, creating a finer mesh. These results were contrary to the idea that human CVM was a very fine mesh that might trap viruses and virus-sized particles via steric obstruction. Rather, mucus may pose a significant adhesive barrier to therapeutic nanoparticles and some viruses at mucosal surfaces[1, 54]. Finally, the results with N9 demonstrate that MPP probes can be helpful in understanding the underlying biophysical interactions that give rise to the microstructure of mucus gels. We have similarly used MPP probe particles to investigate the microstructure of other mucus secretions, such as cystic fibrosis sputum[45], chronic sinusitis mucus[55], mouse vaginal mucus[11], and CF sputum treated with a commonly used mucolytic[27].

The inhalation of non-degradable engineered ultrafine particles is a major concern in the field of nanotoxicology, as occupational exposure is likely to increase as engineered nanoparticle production (such as ZnO, which is commonly used in sunscreens and other products) continues to increase. To investigate the potential effects of environmental ultrafine particles on human mucus, we used MPP probes to estimate the mesh structure of native human mucus and human mucus exposed to mucoadhesive polystyrene particles (MAP) as a mimic for environmental ultrafine particles[56]. Interestingly, we found that a low concentration of MAP had little to no effect on the microstructure of the mucus, but a higher concentration led to an increase in the average pore size of mucus from 380 nm to 470 nm. This was evidenced by a 10-fold increase in the average effective diffusivity of 1μm MPP probes in MAP-treated samples. Despite changes in the microstructure of mucus, the bulk rheology was unchanged by addition of MAP at any concentration. These findings suggest that mucoadhesive ultrafine particles can substantially alter the microstructure of mucus, potentially making exposed individuals more susceptible to infection by pathogens and to further exposure to foreign materials.

In summary, MPP probes can be used to probe the microrheology and microstructure of complex gel systems, providing important structural information and allowing the testing of chemical treatments on gel structure. The use of MPP rather than other particle probes is crucial, as mucoadhesion can be falsely interpreted as steric obstruction, leading to inaccurate estimations of microrheology and pore structure. Multiple-particle tracking provides an additional level of detail compared to techniques such as FRAP because individual particle trajectories can be used to obtain distribution profiles in addition to average values. Finally, although this technique has been used by our lab solely for investigation of mucus structure to date, it is applicable to other gels.

5. Conclusions and Outlook

Mucus layers coating exposed epithelial surfaces of the body has vital protective and lubrication properties. On the macroscale, mucus is a shear-thinning gel with excellent characteristics for coating and protecting epithelial surfaces, while also being rapidly and regularly cleared. On the microscale, mucin proteins form a porous mesh that traps foreign particulates via both steric and adhesive interactions. It is these properties that can reduce the efficiency of drug and gene delivery to mucosal surfaces. Understanding and overcoming the mucus barrier, while maintaining native barrier function, can significantly improve prophylactic and therapeutic treatments for a wide array of epithelial diseases and conditions afflicting the eye, female reproductive tract, the respiratory tract, and the gastrointestinal tract.

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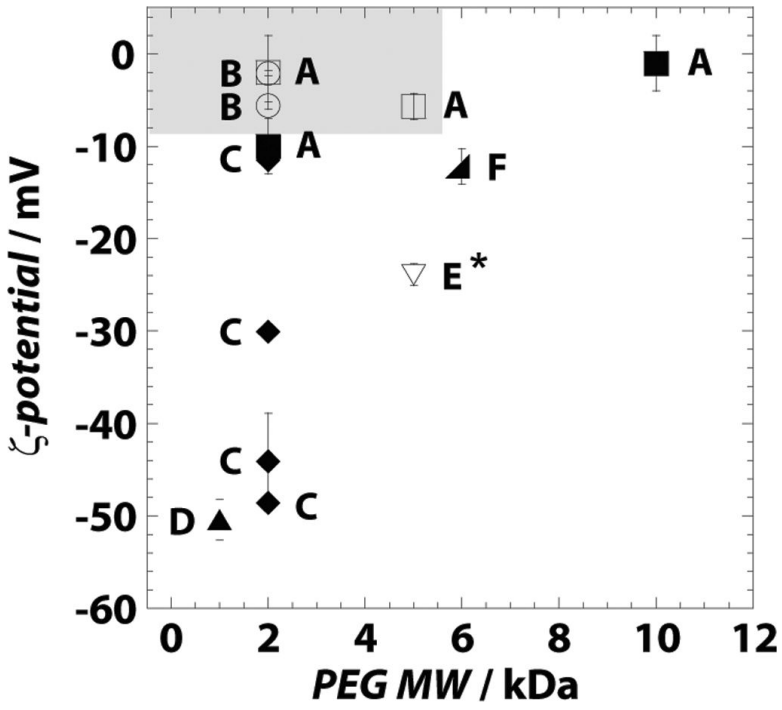

Figure 1.

Phase diagram correlating mucoinert versus mucoadhesive particle behavior to surface charge and PEG MW for various PEG-coated nanoparticles (200–500 nm in size). 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 mucoinert coating. Reproduced with permission from[16].

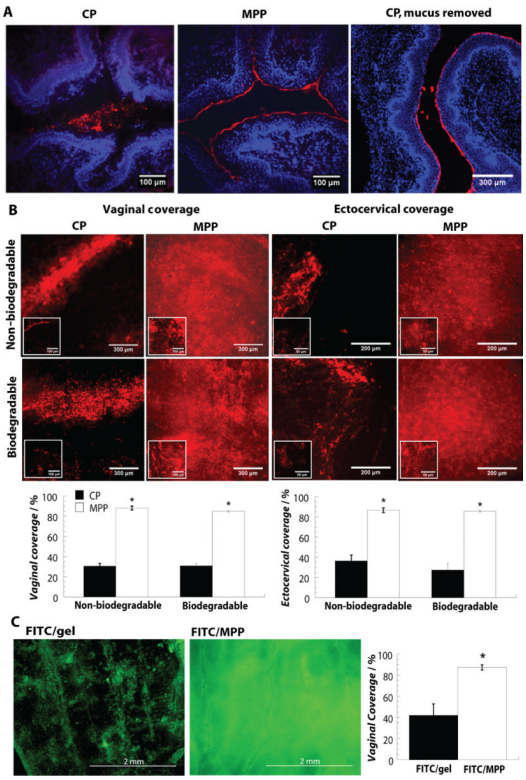

Figure 2.

(A) Distribution of red fluorescent non-biodegradable CPs and MPPs in transverse cryosections of mouse vaginal tissue with an intact mucus layer or mucus removed by lavage and swabbing (mucus removed). (B) Distribution of non-biodegradable and biodegradable CPs and MPPs on flattened mouse vaginal and ectocervical tissue. Insets are images of dark areas at higher magnification. (C) Distribution and retention of a model drug, FITC, in the mouse vagina delivered in gel form or encapsulated in biodegradable MPPs. Fluorescent images of flattened mouse vaginal tissue after 24 h. $*P < 0.05$ compared to CP or FITC/gel, Student's t test. Adapted with permission from[11].

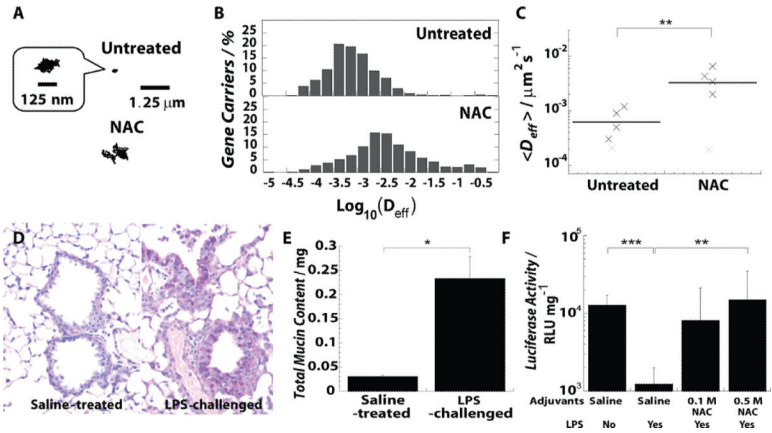

Figure 3.

Effect of NAC on the sputum penetration and airway gene transfer by $CK_{30}PEG_{10k}$/DNA nanoparticles (NPs). (A – C) Diffusion of NPs in untreated and NAC-treated CF sputum. (A) Representative trajectories of NPs in untreated and treated CF sputum during 20 s movies. The logarithmic effective diffusivities (D_{eff}) of individual traces are within one standard deviation of the logarithmic ensemble-averaged D_{eff}. (B) Ensemble-averaged geometric mean effective diffusivity ($<D_{eff}>$) of gene carriers in different CF sputum samples at a time scale (τ) of 1 s. Difference in $<D_{eff}>$ of gene carriers in untreated sputum versus sputum treated with NAC is statistically significant when one outlier sample (light gray cross mark) is excluded from the analysis (**$p < 0.01$). (C) Distribution of the logarithmic D_{eff} of individual NPs at $\tau = 1$ s. Data represents 5 independent experiments, with an average of $n > 100$ NPs per experiment. (D – F) *In vivo* gene transfer to airways of C57 mice intranasally challenged with *P. aeruginosa* lipopolysaccharide (LPS). Periodic acid Schiff (PAS) staining of lung tissues from mice treated/challenged twice either with (D, left panel) isotonic saline or (D, right panel) 2 mg/ml LPS in the interval of 2 days. On day 4, PAS-positive mucus cells (magenta color) were detected in the airways of LPS-challenged mice whereas no PAS-positive cells were detected in the airways of saline-treated mice. (E) Total mucin contents in BALF collected from mice treated/challenged with isotonic saline or LPS. (F) *In vivo* gene transfer to airways of mice treated/challenged twice either with saline or LPS. On day 4, mice were treated with adjuvants, either saline ($n = 7$) or NAC solution ($n = 10$) with varying concentrations (0.1 and 0.5 M) 30 min prior to the administration of $CK_{30}PEG_{10k}$/DNA NPs carrying pd1GL3-RL (luciferase). Subsequently, $CK_{30}PEG_{10k}$/DNA NPs (DNA dose of 50 μg / mouse) were intranasally instilled to each mouse and the luciferase activity was measured 24 h after the administration. The differences are statistically significant between the conditions indicated with asterisks (*) (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). Mean ± SEM. Adapted with permission from[33].