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Nanoparticle penetration of human cervicovaginal mucus: The effect of polyvinyl alcohol

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Abstract

Therapeutic nanoparticles must rapidly penetrate the mucus secretions lining the surfaces of the respiratory, gastrointestinal and cervicovaginal tracts to efficiently reach the underlying tissues. Whereas most polymeric nanoparticles are highly mucoadhesive, we previously discovered that a dense layer of low MW polyethylene glycol (PEG) conferred a sufficiently hydrophilic and uncharged surface to effectively minimize mucin-nanoparticle adhesive interactions, allowing well-coated particles to rapidly diffuse through human mucus. Here, we sought to investigate the influence of surface coating by polyvinyl alcohol (PVA), a relatively hydrophilic and uncharged polymer routinely used as a surfactant to formulate drug carriers, on the transport of nanoparticles in fresh human cervicovaginal mucus. We found that PVA-coated polystyrene (PS) particles were immobilized, with speeds at least 4,000-fold lower in mucus than in water, regardless of the PVA

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molecular weight or incubation concentration tested. Nanoparticles composed of poly(lactide-co-glycolide) (PLGA) or diblock copolymers of PEG-PLGA were similarly immobilized when coated with PVA (slowed 29,000- and 2,500-fold, respectively). PVA coatings could not be adequately removed upon washing, and the residual PVA prevented sufficient coating with Pluronic F127 capable of reducing particle mucoadhesion. In contrast to PVA-coated particles, the similar sized PEG-coated formulations were slowed only ~6- to 10-fold in mucus compared to in water. Our results suggest incorporating PVA in the particle formulation process may lead to the formation of mucoadhesive particles for many nanoparticulate systems. Thus, alternative methods for particle formulation, based on novel surfactants or changes in the formulation process, should be identified and developed in order to produce mucus-penetrating particles for mucosal applications.

Keywords

Drug delivery; mucoadhesion; mucus-penetrating particles; surfactant; biodegradable polymers

Introduction

Mucus secretions serve as the body's first line of defense against pathogens [1, 2], toxins [3], and environmental ultrafine particles [4] at exposed surfaces not covered by skin, such as the eye and the respiratory, gastrointestinal and cervicovaginal tracts. The barrier properties of mucus are rooted in its dense network of mucin fibers, which contain highly glycosylated (negatively charged) segments interspersed by hydrophobic, lipid-coated domains [5]. These features enable mucus to trap foreign particles through steric obstruction and/or adhesion to mucus constituents via hydrophobic, electrostatic and hydrogen bonding interactions. Trapped particles are typically rapidly eliminated via natural mucus clearance mechanisms, thus limiting their effective exposure to mucosal tissues [6, 7]. Numerous studies have shown that mucus represents a critical hurdle for drug and gene delivery to mucosal tissues [7]. Thus, particles that can overcome mucus barriers are receiving increasing attention for controlled delivery of therapeutics to mucosal tissues [7, 8].

We previously discovered that coating nanoparticles with muco-inert materials, such as polyethylene glycol (PEG) or Pluronic F127, can transform otherwise mucoadhesive nanoparticles into mucus-penetrating particles (MPP). Polyvinyl alcohol (PVA) is an uncharged and relatively hydrophilic polymer [9, 10] that has been widely used as a carrier polymer in drug delivery devices, such as sustained-release particles and hydrogels [11, 12], or as a surfactant for formulating polymeric drug carriers, such as the Lupron Depot® [13, 14]. We observed that using PVA as a surfactant during particle formulation by the nanoprecipitation or emulsion methods leads to the formation of mucoadhesive nanoparticles [15–17]. Here, we sought to study the effect of PVA coating on the transport of nanoparticles in fresh human cervicovaginal mucus (CVM). We expect that our results will provide insight into the interactions of PVA-coated particles with mucus, and their suitability for applications in mucosal delivery.

Materials and Methods

Collection of human cervicovaginal mucus

Human CVM collection was performed as previously described [18, 19]. Briefly, undiluted cervicovaginal secretions were collected from women with normal vaginal flora (with healthy vagina microbiota, vaginal pH 4, no sexual intercourse within the past 24 h, and no history of being treated for sexually transmitted infections or bacterial vaginosis) using a self-sampling menstrual collection device, following a protocol approved by the Johns Hopkins University Institutional Review Board. Participants inserted the device into the vagina for approximately 30 s and then placed it into a 50 mL centrifuge tube upon removal. The tubes were centrifuged at 1,000 rpm for 2 min to collect the secretions. Collected mucus samples were stored at 4°C until used for microscopy within 4 hr.

Particle preparation and characterization

Carboxyl-modified 200 nm green and red fluorescent polystyrene (PS-COOH) NP were purchased from Molecular Probes (Eugene, OR). PVA of MW 2 kDa (75% hydrolyzed) was purchased from Acros Organics (Fisher Scientific, Hampton, NH); PVA of MW 6 kDa (80% hydrolyzed), 25 kDa (88% hydrolyzed, polydispersity 1.9), 78 kDa (88% hydrolyzed, polydispersity 1.9) were purchased from Polysciences, Inc. (Warrington, PA). To coat particles, 5 μ L of PS-COOH NP (2% w/v) was incubated with 295 μ L of select surfactants at either 1% or 0.01% (w/v) for 1 hr, unless otherwise indicated. Particles with a dense PEG coating (PS-PEG NP) were prepared by covalently conjugating 2 kDa mPEG-NH₂ to the surface of PS-COOH NP, as previously reported [20].

Doxorubicin (NetQem, Durham, NC) was chemically conjugated to the hydroxyl terminal group of poly(lactic-co-glycolic acid) (PLGA; MW 15 kDa, LA:GA 50:50; SurModics Pharmaceuticals, Inc., Birmingham, AL) and poly(ethylene glycol)-co- poly(lactic-co-glycolic acid) (PEG-PLGA; PEG MW 2 kDa, 10% w/w content; Daigang Biomaterials Inc., Jinan, China) as previously described [21]. Unless otherwise stated, fluorescent NP were prepared using a nanoprecipitation method [22]. Briefly, 20 mg of the labeled polymer was dissolved in 1 mL of acetonitrile, and added dropwise into 40 mL of 0.1% PVA (MW 25 kDa, 88% hydrolyzed) or water. After stirring for 3 hr to remove the organic solvent, the particles were collected by centrifugation at 11,648 $\times$ g (Sorvall Legend X1R centrifuge; Thermo Fisher Scientific Inc., Asheville, NC) for 20 min and washed twice in water to remove PVA.

PS, PLGA and PEG-PLGA particle suspensions were purified by size exclusion chromatography. Briefly, ~250 μ L of the particle suspension was added to a Sephadex G-25 spin column and centrifuged at 800 $\times$ g for 2 min (Microcentrifuge 5424; Eppendorf AG, Hamburg, Germany). Size and ζ -potential of diluted particle solution in 10 mM NaCl (pH 7) were determined by dynamic light scattering and laser Doppler anemometry, respectively, using a Zetasizer Nano ZS90 (Malvern Instruments, Southborough, MA) following manufacturer instructions.

High resolution multiple particle tracking

Particle suspension ($\sim 10^{10}$ particles/mL) was added at 3% v/v (final $\sim 10^8$ particles/mL) to CVM to minimize dilution of the mucus gel; samples were incubated 1 hr at 37 °C before microscopy. Particle transport rates were measured by analyzing the trajectories of fluorescent particles, recorded using a silicon-intensified target camera (VE-1000; Dage-MTI, Michigan, IN) mounted on an inverted epifluorescence microscope equipped with 100X oil-immersion objective (numerical aperture 1.3). Movies were captured with MetaMorph software (Molecular Devices, Inc., Sunnyvale, CA) at a temporal resolution of 66.7 ms for 20 s. The tracking resolution was ~ 10 nm, as determined by tracking the displacements of particles immobilized with a strong adhesive [19]. Trajectories of $n > 100$ particles were analyzed for each experiment and at least three experiments in CVM from different donors were performed for each condition. The coordinates of nanoparticle centroids were transformed into time-averaged mean squared displacements (MSD), $\langle r^2(t) \rangle = [x(t + \tau) - x(t)]^2 + [y(t + \tau) - y(t)]^2$ (τ = time scale or time lag), from which distributions of MSDs and effective diffusivities were calculated, as previously demonstrated [19].

Statistics

Data represent mean $\pm$ standard error of the mean (S.E.M.). A one-tailed, unequal variance Student's *t*-test was used to evaluate the statistical significance of differences between the MSDs of particle formulations with and without PVA; *P* values < 0.05 were considered statistically significant.

Results

PVA readily associates to the surface of PS particles

We first investigated whether PVA of various MW (2–25 kDa), and at different incubation concentrations (0.01–1%), could coat the surface of PS-COOH NP (~ 200 nm), which are normally immobilized by human mucus [19]. In addition to their stability in aqueous solutions and well-controlled sizes, PS-COOH NP exhibit highly negative surface charge at pH 7 (due to carboxylic acid groups), providing a convenient measure of coating efficiency by uncharged PVA polymers. All PVA-coated PS-COOH NP (PS/PVA NP) had roughly neutral surface charge (measured by ζ -potential, Table 1), similar to muco-inert PEG-coated PS nanoparticles (PS-PEG NP; PEG MW 2 kDa) [19, 23]. The extent of PVA coating was also reflected by an increase in the hydrodynamic diameters of the various PVA-coated nanoparticle formulations, as measured by dynamic light scattering (Table 1). Longer incubation (over 2 weeks) in PVA (25 or 78 kDa, 88% hydrolyzed; 1% solution) did not further affect the particle size or surface charge (Table S1). These results suggest that PVA readily coats the surfaces of PS-COOH NP.

PVA-coated PS particles are immobilized in human CVM

PVA with MW 25 kDa is commonly used for drug delivery particle formulations; thus, we first 0.01% tested the transport of 200 nm PS-COOH NP pre-incubated with a low 0.01% (PS/PVA_{25k}^{0.01%} NP) and high 1% (PS/PVA_{25k}^{1%} NP) concentration of 25 kDa PVA. We

added PS/PVA_{25k}^{0.01%} NP or PS/PVA_{25k}^{1%} NP to fresh undiluted human CVM collected from donors with healthy vaginal flora, and observed nanoparticle mobility after 1 hr incubation. Similar to those of PS-COOH NP (Figure 1A), the time-lapse traces of fluorescently-labeled PS/PVA_{25k}^{0.01%} NP or PS/PVA_{25k}^{1%} NP (Figure 1B and 1C, respectively) were highly constrained and non-Brownian. In contrast, the traces of similarly sized PS-PEG NP (Figure 1D) resembled freely-diffusive Brownian motion, spanning large distances over the course of 20 s movies, in good agreement with our previous findings [19].

We measured the mobility of PS/PVA NP using multiple particle tracking (MPT), which allows quantitative measurements of the motions of hundreds of individual particles [19]. MPT measurements are presented in the form of time-scale dependent ensemble averaged mean squared displacements ($\langle \text{MSD} \rangle$). The $\langle \text{MSD} \rangle$ values for both PS/PVA_{25k}^{0.01%} NP and PS/PVA_{25k}^{1%} NP, averaged over 3 independent experiments at a time scale of 1 s, were 600- and 1,300-fold lower than that for PS-PEG NP, respectively (Figure 1E). There was no statistically significant difference between $\langle \text{MSD} \rangle$ values for the two PS/PVA_{25k} NP formulations and that for PS-COOH NP. Overall, PS-COOH NP, PS/PVA_{25k}^{0.01%} NP and PS/PVA_{25k}^{1%} NP were slowed in CVM ~9,000-, 4,000- and 10,000-fold on average, respectively, compared to their theoretical speeds in water (Table 1), while PS-PEG was slowed only ~6-fold in CVM. PS/PVA NP prepared with longer incubation time (over 2 weeks) showed similarly slowed mobility in CVM (Table S1 and Figure S1A). To determine whether the strongly hindered average transport rates of PS/PVA_{25k}^{0.01%} NP and PS/PVA_{25k}^{1%} NP were due to a population of slow outliers, we plotted the distributions of the logarithms of individual particle effective diffusivities, and found that PS/PVA_{25k}^{0.01%} NP and PS/PVA_{25k}^{1%} NP, similar to PS-COOH NP, were uniformly slower compared to PS-PEG NP (Figure 1F–I).

We previously found that coating PS-COOH NP with high MW PEG (10 kDa) resulted in significant NP mucoadhesion compared to coatings with low MW PEG (2–5 kDa), an observation that could be potentially explained by interpenetration network effects with higher MW PEG [19]. To investigate whether reducing the PVA MW may reduce the mucoadhesion of PVA-coated particles, we tested PS-COOH NP incubated in 1% solutions of PVA with MW 2 kDa (PS/PVA_{2k}^{1%} NP) or 6 kDa (PS/PVA_{6k}^{1%} NP). Despite the 12-fold span in the PVA MW tested, the motions of all PVA-coated formulations were strongly hindered in human CVM to a similar extent as uncoated PS-COOH NP and PS/PVA_{25k}^{1%} NP (Figure 2A–D). The $\langle \text{MSD} \rangle$ of PS/PVA_{2k}^{1%} and PS/PVA_{6k}^{1%} NP formulations, at a time scale of 1 s, were 3,000- and 1,900-fold lower than that for PS-PEG NP (Figure 2E), respectively. All PS/PVA NP formulations were uniformly slowed in CVM, in a manner similar to PS-COOH NP (Figure 2F–I). Overall, PS-COOH NP, PS/PVA_{2k}^{1%} NP, PS/PVA_{6k}^{1%} NP and PS/PVA_{25k}^{1%} NP were slowed in CVM ~9,000-, 23,000-, 14,000- and 10,000-fold on average, respectively, compared to their theoretical speeds in water (Table 1). Similarly, increasing the MW of PVA (i.e., 78 kDa) did not improve the mobility of PS-COOH NP in CVM (Table S1 and Figure S1A). These results indicate that all PVA-coated PS-COOH NP, regardless of incubation concentration or MW of PVA, are immobilized in human CVM.

PVA-coated PLGA nanoparticles are also immobilized in human CVM

We next tested PVA coating on biodegradable NP composed of PLGA, an FDA-approved polymer widely used in a variety of drug delivery devices. We chose to focus this and subsequent investigations on 25 kDa PVA at a concentration of 0.1%, since it is a commonly used PVA MW. Similar to PS-COOH NP, the presence of carboxylic acid groups on the surface of uncoated PLGA particles leads to a substantially negative surface charge [24]. PVA-coated PLGA nanoparticles (PLGA/PVA NP) prepared by nanoprecipitation exhibited nearly neutral ζ -potential (Table 1), suggesting that PVA coated the PLGA nanoparticles. The PVA coating was resistant to washing, as evidenced by the consistently near neutral ζ -potential of the particles following washing twice in ultrapure water (PLGA/PVA(w) NP).

Similar to various PS/PVA NP formulations, the time-lapse traces of PLGA/PVA NP and PLGA/PVA(w) NP in CVM were highly constrained and non-Brownian (Figure 3A and B). The $\langle \text{MSD} \rangle$ of PLGA/PVA NP and PLGA/PVA(w) NP (both ~ 270 nm) were $\sim 8,500$ - and $13,000$ -fold lower than that of similarly-sized PS-PEG NP (Figure 3F and 1E), respectively. The distributions of the logarithms of effective diffusivities of individual particles also confirmed that both PLGA/PVA NP and PLGA/PVA(w) NP formulations were uniformly slower compared to PS-PEG NP (Figure 3G, 3H and 1E). Overall, PLGA/PVA NP and PLGA/PVA(w) NP were slowed in CVM $29,000$ - and $43,000$ -fold on average, respectively, compared to their theoretical speeds in water (Table 2). Additionally, PLGA/PVA NP prepared via a single emulsion approach showed similarly restricted mobility in CVM (Table S1 & Figure S1B) [15], suggesting the observation was independent of the fabrication method.

PVA coating causes otherwise mucus-penetrating PEG-PLGA particles to become immobilized in human CVM

We previously showed that nanoparticles composed of biodegradable PEG-containing diblock copolymers (poly(ethylene glycol)-co-poly(sebacic acid), PEG-PSA; or PEG-PLGA) rapidly penetrate human mucus [25, 26]. Nevertheless, to stabilize the formation of nanoparticles, surfactants such as PVA are typically required during particle formulation. We thus sought to test whether the use of PVA with similar PEG-PLGA diblock copolymers could yield a biodegradable MPP platform. PEG-PLGA NP formulated without surfactants exhibited unobstructed Brownian transport in human CVM (Figure 3C), consistent with our recent work [25, 26]. In contrast, the time-lapse traces of PEG-PLGA nanoparticles formulated in PVA (PEG-PLGA/PVA NP), as well as PEG-PLGA/PVA NP washed twice in water (PEG-PLGA/PVA(w) NP), were highly constrained and non-Brownian (Figure 3D and E). The $\langle \text{MSD} \rangle$ of PEG-PLGA/PVA NP and PEG-PLGA/PVA(w) NP were 500 - and $1,000$ -fold lower than that of PEG-PLGA NP (Figure 3F), respectively. Both PEG-PLGA/PVA NP and PEG-PLGA/PVA(w) NP formulations were uniformly slowed compared to PEG-PLGA NP (Figure 3J, K and I). Overall, PEG-PLGA/PVA NP and PEG-PLGA/PVA(w) NP were slowed on average $2,500$ - and $4,000$ -fold in CVM, respectively, compared to their theoretical speeds in water, while surfactant-free PEG-PLGA NP were slowed only ~ 6 -fold compared to in water (Table 2). Our results suggest inclusion of PVA in the particle formulation process can cause firm mucoadhesion even for PEG-PLGA NP that otherwise rapidly penetrate mucus gels.

PVA prohibits muco-inert coatings by Pluronic

Since we were unable to adequately remove PVA from nanoparticles to minimize mucoadhesion, we next sought to determine whether we could coat PVA-coated particles with a muco-inert material. We previously discovered that Pluronic[®] - tri-block copolymers containing two terminal PEG moieties flanking a hydrophobic poly(propylene oxide) (PPO) segment - can readily associate to hydrophobic particle surfaces and form a sufficiently dense PEG coating to allow mucus penetration if the PPO segment MW is ≥ 3000 Da. These Pluronic[®] densely coat otherwise mucoadhesive polymeric particles prepared in the absence of PVA, including those composed of PS-COOH, PLGA and poly(ϵ -caprolactone) (PCL), and enable rapid penetration of mucus barriers [24]. We thus incubated PLGA/PVA(w) and PEG-PLGA/PVA(w) NP with Pluronic[®] F127 (PLGA/PVA(w)/F127 NP and PEG-PLGA/PVA(w)/F127 NP), and measured their mobility in CVM. Despite incubation with Pluronic[®] F127, PLGA/PVA(w)/F127 NP and PEG-PLGA/PVA(w)/F127 NP were effectively immobilized in mucus, whereas PLGA/F127 NP exhibited traces indicative of Brownian motion in the same mucus samples (Figure 4A–C). The $\langle \text{MSD} \rangle$ of PLGA/PVA(w)/F127 NP and PEG-PLGA/PVA(w)/F127 NP were comparable to their counterparts without Pluronic F127 coating (Figure 3F and 4D), and overall the average speeds of the particles were reduced $\sim 50,000$ - and $4,800$ -fold compared to their theoretical speeds in water, respectively (Table 3). In contrast to PLGA/F127 NP, both PLGA/PVA(w)/F127 and PEG-PLGA/PVA(w)/F127 NP formulations were uniformly immobilized (Figure 4E–G). These results suggest that muco-inert Pluronic[®] molecules are unable to sufficiently coat PLGA and PEG-PLGA particles already coated in PVA, precluding transformation of these particles into MPP.

Discussion

PVA is one of the most commonly used emulsifiers for the preparation of biodegradable polymeric particle systems [27]. It has an excellent safety profile, and provides reproducibility and control over the stability, size and size distribution, drug loading and drug encapsulation efficiency of polymeric particles [28, 29]. The widespread use of PVA as a surfactant in particle formulations, including those targeting mucosal tissues [30, 31], prompted us to investigate the effect of PVA coating on the transport of nanoparticles in human mucus. In addition to our previous observations that PVA-coated PLGA and PEG-PLGA nanoparticles were immobilized in human CVM [15–17], we found that PVA-coated particles were strongly mucoadhesive in human CVM regardless of PVA MW, PVA concentration, or particle core materials. Our results underscore the importance of efforts to identify or develop surfactants that can simultaneously afford efficient drug encapsulation and rapid mucus-penetration in engineering biodegradable, drug-loaded MPP.

Nanoparticles may be immobilized by the mucus mesh via steric obstruction, or adhesion via mechanisms such as hydrophobic forces, hydrogen bonding, polymer chain interpenetration, and/or electrostatic/ionic interactions [7]. All PVA-coated particles tested in this study were smaller than the average pore size in CVM (~ 340 nm, range 50–1800 nm [32]), and their sizes were comparable to that of mucus-penetrating PS-PEG NP, suggesting steric obstruction should be minimal. The immobilization of particles coated with PVA as

low as 2 kDa (Table 1) suggests that polymer chain interpenetration (typical for polymers of high MW [33]) was unlikely to be the cause. In addition, the neutrality of PVA should also minimize particle electrostatic/ionic interactions with mucus. Therefore, we hypothesize that the PVA-coated particles tested here were likely trapped by hydrogen bonding and/or hydrophobic interactions with mucus constituents.

PVA is manufactured by hydrolysis of hydrophobic polyvinyl acetate, removing the acetyl side groups to form hydrophilic hydroxyl groups [34]. Compared to fully hydrolyzed PVA (i.e., 98% or above), partially hydrolyzed PVA (i.e., 88% or below) is a more efficient emulsifier that is commonly used to stabilize emulsions to synthesize polymeric particles [35], thus our studies primarily focused on partially hydrolyzed PVA. Both the hydroxyl groups and the residual acetyl groups in partially hydrolyzed PVA may form hydrogen bonds with mucus constituents (e.g., highly glycosylated mucin fibers [6]). In addition, the residual acetyl groups are also capable of establishing hydrophobic interactions with the mucus mesh due to the hydrophobic terminal methyl group. To test whether blocking these interactions between particles and mucus may reduce particle immobilization, we pretreated CVM with PVA solution before addition of PVA-coated PS-COOH particles (Figure S2). Particles coated with PVA (25 kDa) of two different hydrolysis levels (88% or 98%) were tested. Interestingly, the diffusivity of the PS/PVA (25k Da, 98% hydrolyzed) particles was increased significantly (~2,000-fold), whereas that of PS/PVA (25k Da, 88% hydrolyzed) particles was only marginally enhanced. In comparison to 88% hydrolyzed PVA, 98% hydrolyzed PVA has much fewer residual acetyl groups, and thus its ability to interact with mucus, particularly through hydrophobic interactions, should be weaker. It is likely that pretreating mucus with PVA solution partially shielded binding sites in mucus, allowing the relatively weakly adherent PS/PVA (25k Da, 98% hydrolyzed) particles to diffuse but leaving the PS/PVA (25k Da, 88% hydrolyzed) particles stuck. These results support the role of hydrophobic and/or hydrogen bonding interactions in causing the immobilization of PVA-coated nanoparticles in CVM.

Densely PEGylated surfaces are generally considered to be effective in resisting non-specific adsorption of proteins and other biomacromolecules [36]. PEG-PLGA copolymers self-assemble into nanoparticles by phase separation and present a dense PEG coating on the particle surface, allowing PEG-PLGA NP to rapidly penetrate human mucus [26]. However, muco-inert PEG-PLGA NP, prepared by either the nanoprecipitation method or the single emulsion method, became immobilized in mucus after coating with PVA despite multiple washes to remove residual PVA (Figure 3). These results suggest that PVA is capable of associating with particles even in the presence of PEG, possibly through hydrogen bonding, hydrophobic interactions, or polymer chain entanglement, or in the case of the single emulsion formulation, competing with PEG to occupy the interface of the oil-in-water emulsion [15]. Sheng et al. similarly observed that PVA could coat on top of the PEG coating of PEGylated particles, and further showed that PVA-coated PEG-PLA particles had relatively short circulation half-life (~1.1 h), suggesting that the 'stealth' properties of PEG were disrupted [37]. In an attempt to convert PVA-coated particles into MPP, we incubated PVA-coated particles in a solution of Pluronic® F127, which can associate with hydrophobic particle surfaces via their poly(propylene oxide) (PPO) segments [24, 38] and enable them to diffuse in CVM [24]. However, PLGA/PVA(w)/F127 and PEG-PLGA/PVA(w)/F127

particles remained immobilized in human CVM. This may be partially due to the PVA coating shielding the hydrophobic particle surface sufficiently and preventing F127 from associating with the particle core. In addition, PVA itself, with residual acetyl groups present in only ~12% of the monomers and randomly distributed along the polymer chain, may not present a sufficiently hydrophobic surface for F127 PPO segments to associate with and produce a sufficiently dense F127 coating to prevent mucoadhesion.

Until recently, PEG coatings were considered highly mucoadhesive, but this was shown to be true primarily for particles coated with too low a density of PEG [19, 23]. Although incorporation of PVA in the particle formulations described here all produced mucoadhesive particles, it is possible that the PVA coatings achieved in this work do not shield the particle core as well as covalently conjugated, high surface density PEG coatings. This may allow sufficient adhesive interactions to form between the particle core and mucus to immobilize the particles, thus certain formulations, e.g., those with dense coatings of PVA free of residual acetyl groups, may be less mucoadhesive or even non-mucoadhesive. In addition, variations in mucus constituents caused by disease or individual differences may also affect the transport of PVA-coated particles in mucus [39]. Nevertheless, our results show that the use of PVA in the standard formulation processes investigated here prevents particles diffusion in human CVM.

Conclusion

Surfactants are widely used during formulation of nanoparticle drug delivery systems, since they stabilize emulsions formed during particle synthesis and prevent particle aggregation [40], as well as influence particle size, morphology, drug encapsulation efficiency and drug release kinetics [41]. However, surfactant coatings may also change the surface properties of particles, altering their biodistribution, cellular uptake, and ultimately the pharmacokinetics of the encapsulated drug in the body [28, 42]. In order for biodegradable drug carriers to efficiently penetrate mucus secretions, their surfaces must be non-adhesive to mucus [7, 19], as can be achieved with a dense coating of a muco-inert material. Our finding that conventionally formulated PVA-coated particles are strongly immobilized in human CVM provides implications for identifying or developing new muco-inert surfactants or new processes for formulating MPP.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Transport of uncoated and 25 kDa PVA-coated PS-COOH nanoparticles in fresh human CVM. (A–D) Representative trajectories of (A) PS-COOH, (B) PS/PVA_{25k}^{0.01%}, (C) PS/PVA_{25k}^{1%}, and (D) PS-PEG nanoparticles in CVM, with effective diffusivities within one S.E.M. of the ensemble mean at a time scale of 1 s. (E) Ensemble-averaged geometric mean square displacements ($\langle \text{MSD} \rangle$) as a function of time scale. (F–I) Distributions of the logarithms of individual particle effective diffusivities (D_{eff}) at a time scale of 1 s. PS-PEG data is obtained from reference [19] and included as comparison for speeds of similar-sized particles without significant mucoadhesion. Data represents three experiments for PVA-

coated particles and six experiments for uncoated particles, with $n = 100$ and average $n = 184, 183, 186,$ and 102 for PS-COOH, PS/PVA_{25k}^{0.01%}, PS/PVA_{25k}^{1%}, and PS-PEG nanoparticles, respectively. * denotes statistically significant difference compared to particle formulations containing PVA (P value < 0.05).

Figure 2.

Transport of uncoated and PVA-coated (2–25 kDa) PS-COOH nanoparticles in fresh human CVM. (A–D) Representative trajectories of (A) PS-COOH, (B) PS/PVA_{2k}^{1%}, (C) PS/PVA_{6k}^{1%}, and (D) PS/PVA_{25k}^{1%} nanoparticles in CVM, with effective diffusivities within one S.E. of the ensemble mean at a time scale of 1 s. (E) Ensemble-averaged geometric mean square displacements (<MSD>) as a function of time scale. (F–J) Distributions of the logarithms of individual particle effective diffusivities (D_{eff}) at a time scale of 1 s in CVM. PS-PEG data is obtained from reference [19] and included as comparison for speeds of similar-sized particles without significant mucoadhesion. Data represents three experiments for PVA-coated particles and six experiments for uncoated particles, with $n = 150$ and average $n = 184, 193, 188, 186,$ and 102 for PS-COOH, PS/PVA_{2k}^{1%}, PS/PVA_{6k}^{1%}, PS/PVA_{25k}^{1%}, and PS-PEG nanoparticles, respectively. * denotes statistically significant difference compared to particle formulations containing PVA (P value < 0.05).

Figure 3.

Transport of 25 kDa PVA-coated PLGA and PEG-PLGA nanoparticles in fresh human CVM. (A–E) Representative trajectories of (A) PLGA/PVA, (B) PLGA/PVA/(w), (C) PEG-PLGA, (D) PEG-PLGA/PVA, and (E) PEG-PLGA/PVA/(w) in CVM, with effective diffusivities within one S.E. of the ensemble mean at a time scale of 1 s. (F) Ensemble-averaged geometric mean square displacements ($\langle \text{MSD} \rangle$) as a function of time scale. (G–K) Distributions of the logarithms of individual particle effective diffusivities (D_{eff}) at a time scale of 1 s in CVM. Data represents three experiments for each particle formulation, with n 100 and average $n = 123, 122, 111, 105$ and 133 for PLGA/PVA, PLGA/PVA/(w), PEG-

PLGA, PEG-PLGA/PVA, and PEG-PLGA/PVA(w) nanoparticles, respectively. * denotes statistically significant difference compared to particle formulations containing PVA (P value < 0.05).

Figure 4.

Transport of 25 kDa PVA-coated PLGA and PEG-PLGA nanoparticles with and without incubation in F127 in fresh human CVM. (A-C) Representative trajectories of (A) PLGA/F127, (B) PLGA/PVA(w)/F127, and (C) PEG-PLGA/PVA(w)/F127 in CVM, with effective diffusivities within one S.E. of the ensemble mean at a time scale of 1 s. (D) Ensemble-averaged geometric mean square displacements ($\langle\text{MSD}\rangle$) as a function of time scale. PLGA/F127 data is obtained from reference [24] and included as comparison for F127-coated particles without significant mucoadhesion. (E-G) Distributions of the logarithms of individual particle effective diffusivities (D_{eff}). Data represents three experiments for each particle formulation, with $n = 100$ and average $n = 146, 145$ and 124 for PLGA/F127, PLGA/PVA(w)/F127, and PEG-PLGA/PVA(w)/F127 nanoparticles, respectively. * denotes statistically significant difference compared to particle formulations containing PVA (P value < 0.05).

Table 1

Characterization of PS-COOH nanoparticles uncoated and coated with PVA or PEG, and ratios of the ensemble average diffusion coefficients in water (D_w) compared to in human cervicovaginal mucus (D_m).

Formulation	Diameter (nm)	ζ -potential [mV]	D_w / D_m [a]
PS-COOH	196 ± 5	-46 ± 1	9,000
PS/PVA _{25k} ^{0.01%}	226 ± 5	-2 ± 1	4,000
PS/PVA _{25k} ^{1%}	249 ± 7	-5 ± 1	10,000
PS/PVA _{2k} ^{1%}	229 ± 4	-3 ± 1	23,000
PS/PVA _{6k} ^{1%}	228 ± 5	-3 ± 1	14,000
PS-PEG [b]	232 ± 7	-2 ± 1	6.3

[a] Effective diffusivity values are calculated at a time scale of 1s. D_w is calculated from the Stokes-Einstein equation.

[b] PS-PEG (2 kDa) data from [19] is included as a control for comparison of PS/PVA formulations to similar-sized particles that exhibit minimal mucoadhesion.

Table 2

Characterization of PLGA and PEG-PLGA nanoparticles uncoated and coated with PVA, and ratios of the ensemble average diffusion coefficients in water (D_w) compared to in human cervicovaginal mucus (D_m).

Formulation	Diameter (nm)	ζ -potential [mV]	D_w / D_m ^[a]
PLGA/PVA	270 $\pm$ 2	-2 $\pm$ 1	29,000
PLGA/PVA(w)	267 $\pm$ 3	-1 $\pm$ 1	43,000
PEG-PLGA	115 $\pm$ 10	-6 $\pm$ 1	6
PEG-PLGA/PVA	130 $\pm$ 8	-6 $\pm$ 1	2,500
PEG-PLGA/PVA(w)	151 $\pm$ 8	-7 $\pm$ 3	2,000

^[a] Effective diffusivity values are calculated at a time scale of 1s. D_w is calculated from the Stokes-Einstein equation.

Table 3

Characterization of PVA-coated PLGA and PEG-PLGA nanoparticles subsequently coated with Pluronic[®] F127, and ratios of the ensemble average diffusion coefficients in water (D_w) compared to in human cervicovaginal mucus (D_m).

Formulation	Diameter (nm)	ζ -potential [mV]	D_w / D_m [a]
PLGA/F127 [b]	138 ± 2	-5 ± 2	10
PLGA/PVA(w)/F127	269 ± 7	-1 ± 1	50,000
PEG-PLGA/PVA(w)/F127	140 ± 8	-4 ± 1	4,800

[a] Effective diffusivity values are calculated at a time scale of 1s. D_w is calculated from the Stokes-Einstein equation.

[b] PLGA/F127 data from [24] is included as a control for comparison of PVA(w)/F127 formulations to F127-coated particles that exhibit minimal mucoadhesion.