Journal of Controlled Release 192 (2014) 202–208

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

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

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ARTICLE INFO

Article history:
Received 26 May 2014
Accepted 20 July 2014
Available online 29 July 2014

Keywords:
Drug delivery
Mucoadhesion
Mucus-penetrating particles
Surfactant
Biodegradable polymers

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 4000-fold lower in mucus than in water, regardless of the PVA 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 2500-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 that 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.

1. 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 mucoinert 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

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http://dx.doi.org/10.1016/j.jconrel.2014.07.045
0168-3659/

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.

2. Materials and methods

2.1. 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 200 ×*g* for 2 min to collect the secretions. Collected mucus samples were stored at 4 °C until used for microscopy within 4 h.

2.2. 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), and 78 kDa (88% hydrolyzed, polydispersity 1.9) were purchased from Polysciences, Inc. (Warrington, PA). To coat particles, 5 μL of PS-COOH NP (2% w/v) was incubated with 295 μL of select surfactants at either 1% or 0.01% (w/v) for 1 h, unless otherwise indicated. Particles with a dense PEG coating (PS-PEG NP) were prepared by covalently conjugating 2 kDa mPEG-NH_2 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 h to remove the organic solvent, the particles were collected by centrifugation at 11,648 ×*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 μL of the particle suspension was added to a Sephadex G-25 spin column and centrifuged at 800 ×*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 the manufacturer's instructions.

2.3. High resolution multiple particle tracking

Particle suspension (~10^{10} particles/mL) was added at 3% v/v (final ~10^8 particles/mL) to CVM to minimize dilution of the mucus gel; samples were incubated 1 h 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 100× 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), $\langle \Delta r^2(\tau) \rangle = [x(t+\tau) - x(t)]^2 + [y(t+\tau) - y(t)]^2$ (τ = time scale or time lag), from which distributions of MSDs and effective diffusivities were calculated, as previously demonstrated [19].

2.4. Statistics

Data represent mean ± 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.

3. Results

3.1. 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.

3.2. 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 tested the transport of 200 nm PS-COOH

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	9000
PS/$PVA_{25k}^{0.01\%}$	226 ± 5	−2 ± 1	4000
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 1 s. 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.