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COMMUNICATION

Biodegradable mucus-penetrating nanoparticles composed of diblock copolymers of polyethylene glycol and poly(lactic-*co*-glycolic acid)

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Abstract Mucus secretions coating entry points to the human body that are not covered by skin efficiently trap and clear conventional drug carriers, limiting controlled drug delivery at mucosal surfaces. To overcome this challenge, we recently engineered nanoparticles that readily penetrate a variety of human mucus secretions, which we termed mucus-penetrating particles (MPP). Here, we report a new biodegradable MPP formulation based on diblock copolymers of poly(lactic-*co*-glycolic acid) and poly(ethylene glycol) (PLGA-PEG). PLGA-PEG nanoparticles prepared by a solvent diffusion method rapidly diffused through fresh, undiluted human cervicovaginal mucus (CVM) with an average speed only eightfold lower than their theoretical speed in water. In contrast, PLGA nanoparticles were slowed more than 12,000-fold in the same CVM secretions. Based on the measured diffusivities, as much as 75% of the PLGA-PEG nanoparticles are expected to penetrate a 10-μm-thick mucus layer within 30 min, whereas virtually no PLGA nanoparticles are expected to do so over the same duration. These results encourage further development of PLGA-PEG nanoparticles as mucus-penetrating drug carriers for improved drug and gene delivery to mucosal surfaces.

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Mucus is a viscoelastic and adhesive substance that coats and protects nearly all exposed surfaces of the human body not covered by skin, including the respiratory, gastrointestinal and female reproductive tracts, as well as the surface of the eye. In particular, mucus traps a variety of pathogens and other foreign particles via hydrophobic, electrostatic, and/or hydrogen-bonding adhesive interactions with its dense mesh network of mucin fibers and readily eliminates trapped particulates via natural mucus clearance mechanisms [1–3]. Unfortunately, mucus also acts as a significant barrier to controlled delivery of therapeutics at mucosal surfaces, since conventional drug and gene carriers are easily immobilized in mucus and eliminated typically on the order of seconds to minutes, long before they can reach the underlying epithelium. To address this challenge, our group recently pioneered the development of "mucus-penetrating particles" (MPP), achieved by coating nanoparticles with a dense coverage of low molecular weight (MW) poly(ethylene glycol) (PEG), a hydrophilic and uncharged polymer routinely used in pharmaceutical applications. A dense PEG coating effectively shields particles from adhesive interactions with mucus constituents, allowing them to rapidly penetrate mucus by diffusion through low-viscosity fluid between mucin fibers [4, 5]. MPP move only a few-fold slower in fresh, undiluted human mucus than in water, enabling a large fraction of the particles to quickly penetrate the mucus gel. MPP may thus achieve improved distribution and retention at mucosal surfaces, as well as closer proximity to and greater uptake by epithelial cells.

Despite recent progress, only a few biodegradable MPP formulations have been reported to date that can penetrate highly viscoelastic human mucus samples [6, 7], limiting the range of therapeutics that can be efficiently encapsulated and the tunability of drug release kinetics. Here, we report a biodegradable MPP formulation composed of a diblock copolymer of PEG (MW, 2 kDa) and poly(lactic-*co*-glycolic acid) (PLGA; MW, 18 kDa; L/G=50:50). The chemical structure of the PLGA-PEG diblock copolymer (Jinan Daigang Biomaterials Co., Shandong, China) was confirmed by ^{1}H-NMR (Online resource 1). We covalently labeled the polymer with Alexa Fluor 647 (Molecular Probes, Eugene, OR), using a method similar to that described previously [8], and formulated fluorescent nanoparticles using a conventional solvent displacement method whereby PLGA-PEG dissolved in acetonitrile was added dropwise to ultrapure water with stirring [9]. As the acetonitrile diffuses away, the hydrophilic PEG segments partition to the surfaces of the particles, forming a dense brush layer that shields the hydrophobic PLGA core. The size and ζ-potential of the resulting nanoparticles were characterized by dynamic light scattering and laser Doppler anemometry, respectively, using a Zetasizer Nano ZS90 (Malvern Instruments, Southborough, MA) following manufacturer instructions. As shown in Table 1, PLGA and PLGA-PEG nanoparticles possessed an average hydrodynamic diameter of ~130 and 90 nm, respectively, with a narrow size range (Fig. 1a). The size and sphere-like morphology of PLGA-PEG nanoparticles were further confirmed by transmission electron microscopy (Fig. 1b). The near-neutral surface charge of PLGA-PEG nanoparticles (−0.4 mV), compared to the negative charge of PLGA particles (−20 mV), confirms the presence of a dense PEG coating.

To test whether the PEG coating on PLGA-PEG nanoparticles was sufficiently dense, we next measured the mobility of PLGA-PEG nanoparticles in fresh human cervicovaginal mucus (CVM). Undiluted, non-ovulatory CVM was obtained from women with acidic, lactobacilli-dominated vaginal flora using a self-sampling menstrual collection device [10], following a protocol approved by the Johns Hopkins Institutional Review Board. Particles were added to CVM samples, and their motions were recorded using high-resolution epifluorescence microscopy, as previously described [4, 5, 11]. PLGA-PEG nanoparticles exhibited Brownian-like, freely diffusive time-lapse trajectories over the course of 20-s movies (Fig. 2a). In contrast, PLGA nanoparticles were strongly hindered, as evident by their highly constrained, non-Brownian traces (Fig. 2b). We further quantified the transport rates of the nanoparticles in the form of ensemble-averaged mean-squared displacements (<*MSD*>) using multiple particle tracking, a technique that enables quantitative measurements of the diffusion of hundreds of individual particles [12]. As shown in Fig. 2c, the <*MSD*> of PLGA-PEG nanoparticles was at least 200-fold higher than that of PLGA nanoparticles over all time scales tested. At a time scale of 1 s, the average effective diffusivity (<D_{eff}>) of PLGA-PEG nanoparticles was only eightfold lower than their theoretical speed in water, whereas that of PLGA nanoparticles was more than 12,000-fold lower (Table 1). This difference in transport rates was also reflected by the slope α of the *MSD* vs. τ plot (α=1 represents unconstrained Brownian transport, while decreasing α corresponds to increased obstruction to particle movement); the average α

Table 1 Characterization of PLGA-PEG and PLGA nanoparticles, and ratios of the ensemble average diffusion coefficients in water (D_w) compared to in cervicovaginal mucus (D_m)

Formulation	Diameter (nm)	ζ-potential (mV)	D_w/D_m[a]
PLGA-PEG	90±6	−0.4±0.3	8
PLGA	130±2	−20±4	12,000

Data represent mean ± standard error of the mean (s.e.m.)

[a] D_m values are obtained at a time scale of 1 s. D_w is calculated from the Stokes–Einstein equation.

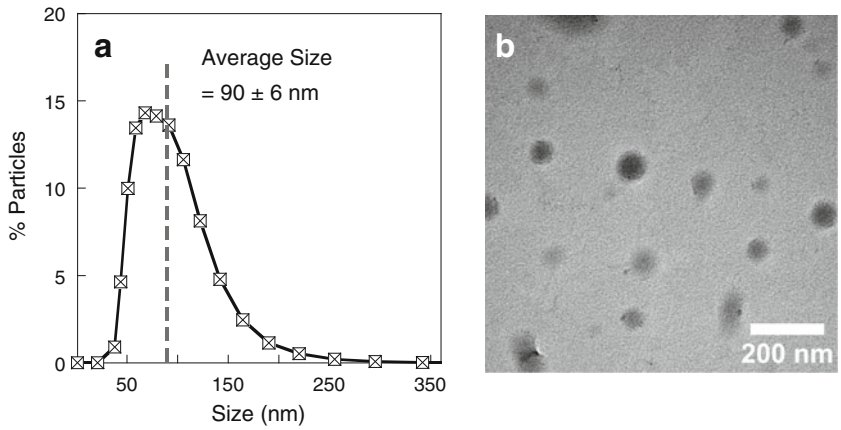

Fig. 1 Size and morphology of PLGA-PEG nanoparticles. **a** Particle number size distribution as measured by dynamic light scattering; data represent the average of four independent experiments. **b** Transmission electron microscopy images. PLGA nanoparticles exhibited similar size distribution (average=130 nm) and morphology as PLGA-PEG nanoparticles (data not shown)

was 0.78 for PLGA-PEG nanoparticles, but only 0.06 for PLGA nanoparticles. Since both PLGA and PLGA-PEG nanoparticles were comparable in size and significantly smaller than the average pore size of CVM (~340±50 nm [11]), the rapid transport of PLGA-PEG nanoparticles compared to PLGA nanoparticles must be attributed to the PEG coating effectively minimizing any adhesive interactions with the mucus mesh constituents, and not to significant differences in the extent of steric obstruction.

To ensure the observed average transport rate of biodegradable PLGA-PEG nanoparticles was not biased by a small fraction of fast-moving outlier nanoparticles, we evaluated the heterogeneity in particle transport rates by examining the distribution of particle effective diffusivities at a time scale of 1 s (Fig. 3a). PLGA-PEG nanoparticles exhibited uniformly rapid transport, the fastest 50% with $\langle D_{eff} \rangle$ only ~sixfold reduced in mucus compared to in water. While $\langle D_{eff} \rangle$ for the fastest 10% of PLGA-PEG nanoparticles was only ~threefold lower in mucus than in water, the fastest 10% of PLGA nanoparticles were slowed more than 1,000-fold. Based on the measured diffusion rates for hundreds of nanoparticles, we used Fick's second law to estimate the fraction of particles that may penetrate a physiologically thick mucus layer over time, as previously reported [6, 7]. Within 30 min, almost 75% of PLGA-PEG nanoparticles are expected to penetrate a mucus layer ~10-μm-thick, a relevant thickness for the mucus layer in the lung airways [6, 13], whereas <1% of PLGA nanoparticles are expected to penetrate over the same duration (Fig. 3b). We further estimated the fraction of particles capable of penetrating a 100-μm-thick mucus layer, which approximates the mucus layer thickness in the cervicovaginal (CV) and gastrointestinal (GI) tracts [13]. Over 4 h, ~40% of PLGA-PEG nanoparticles are predicted to be capable of diffusing across the mucus layer, while again <1% of PLGA nanoparticles would be expected to do so over the same period of time (Fig. 3c). Mucus clearance typically occurs over minutes to a few hours in the respiratory, CV, and GI tracts [13]. Thus, these results suggest that a significant fraction of PLGA-PEG nanoparticles will likely be able to penetrate lung, CV, and GI mucus layers before luminal mucus layers are cleared.

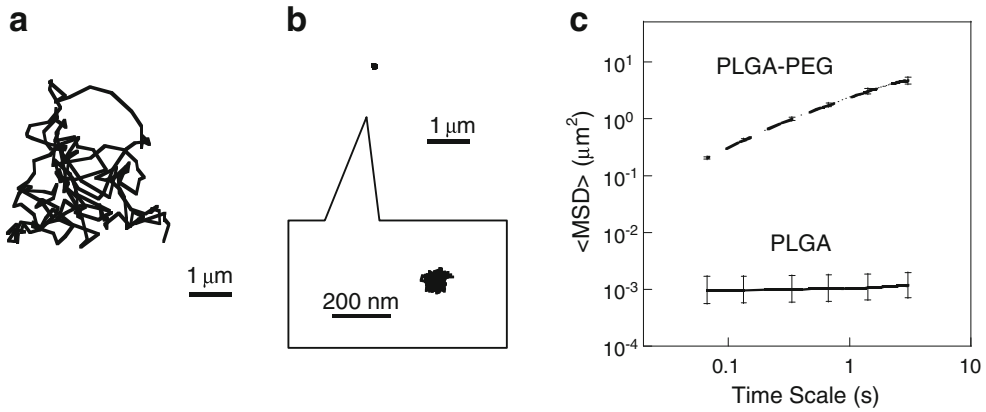

Fig. 2 Transport rates of PLGA-PEG nanoparticles and PLGA nanoparticles in human cervicovaginal mucus. **a**–**b** Representative trajectories for **a** PLGA-PEG nanoparticles and **b** PLGA nanoparticles, with effective diffusivities within 1 s.e.m. of the ensemble average. **c** Ensemble-averaged geometric mean square displacements (<*MSD*>) as a function of time scale. Data represent the ensemble average (mean ± s.e.m.) of six independent experiments, with $n \geq 100$ particles for each experiment

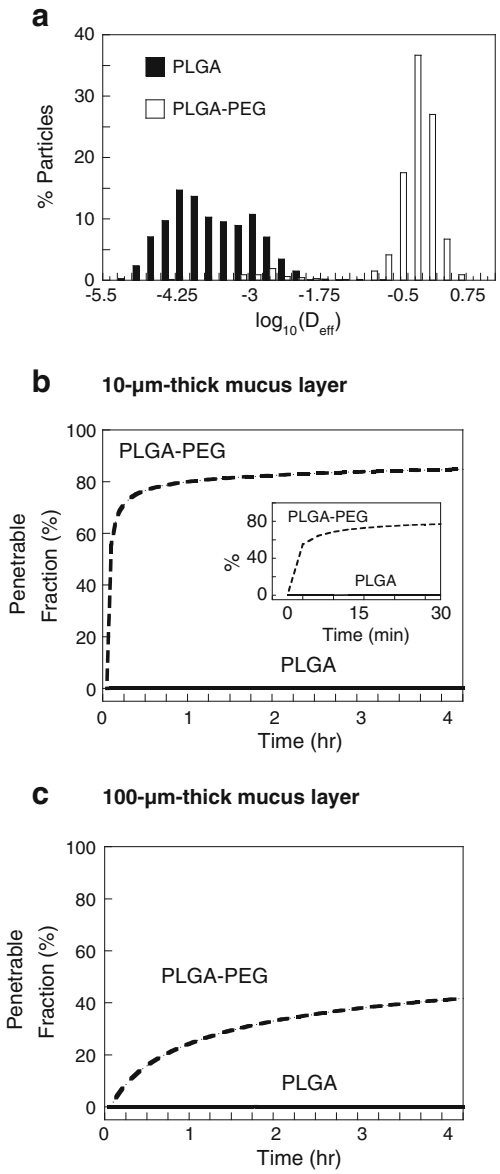

Fig. 3 **a** Distributions of the logarithms of individual particle effective diffusivities (D_{eff}) at a time scale of 1 s. **b**–**c** Fraction of particles predicted to be capable of penetrating a **b** 10- and **c** 100-μm-thick mucus layer over time. *Inset* in **b** shows the estimated fraction over the first 30 min

We previously developed two other MPP drug carriers composed of biodegradable polymers. One formulation is composed of a diblock copolymer of poly(sebacic acid) (PSA) and PEG (PSA-PEG) [6], which offers efficient encapsulation and sustained release for a number of drugs [6, 14, 15]. We used PLGA-PEG here to develop MPP drug carriers with a polyester core, which provides excellent stability and release kinetics for a wide range of therapeutic molecules, including peptides and proteins [16]. Our work with PSA-PEG and PLGA-PEG suggests that the use of amphiphilic diblock copolymers, composed of PEG conjugated to a hydrophobic polymer, to produce nanoparticles by the solvent diffusion method may be a general approach for developing MPP drug carriers. This method contrasts with another MPP formulation we previously developed, based on non-covalent coating of PLGA or poly(ε-caprolactone) (PCL) with select amphiphilic, PEG-containing poloxamers (PLGA/poloxamer or PCL/poloxamer, respectively) [7]. An advantage of the PLGA/poloxamer and PCL/poloxamer formulations is the use of entirely GRAS materials, since no new chemical entities are generated through covalent linkages. Pharmaceutical formulations composed entirely of GRAS materials may require less time and lower costs for clinical development. Nevertheless, it is possible that non-covalent surface PEG coatings may be less stable than PEG coatings covalently associated to the particle core, since poloxamer molecules may desorb over time. In addition, PEG surface density may be more easily controlled with covalently conjugated coatings, which may also allow precise tuning of ligands attached to PEG molecules for targeted drug delivery applications.

PLGA and PEG both have a long history of safety and use in humans, and PLGA-PEG derivatives have emerged as an important family of biocompatible polymers for pharmaceutical and biomedical applications, including nanoparticulate drug delivery systems [9, 17–19]. Nanoparticles formulated from these polymers feature improved stability and combine the safety profiles of their component parts: (1) PLGA, which is used in humans in the form of biodegradable sutures, oral implants, and other drug delivery platforms/devices [20–22] and (2) PEG, which is widely used in foods, cosmetics, and pharmaceuticals, including oral, topical, and systemically administered formulations [23, 24]. Here, we show additionally that PLGA-PEG nanoparticles can rapidly penetrate human mucus secretions, suggesting they could diffuse through surface mucus layers that are quickly eliminated. With a large repertoire of different core polymers and surface coatings, MPP formulations may be tailored to achieve different release profiles and pharmacokinetic properties for the prophylaxis or treatment of mucosal diseases, including sexually transmitted infections, cancer, COPD, and inflammation.

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Conflicts of interest The mucus-penetrating particle technology described in this publication is being developed by Kala Pharmaceuticals. Dr. Hanes is co-founder, consultant, and member of the Board of Directors of Kala; Dr. Lai and Dr. Fu are consultants for Kala. Drs.

Hanes, Lai, and Fu own company stock, which is subject to certain restrictions under university policy. The terms of this arrangement are being managed by the Johns Hopkins University (Drs. Hanes and Fu) and the University of North Carolina (Dr. Lai) in accordance with their respective conflict of interest policies.

Declaration of ethical standards The experiments in this work comply with the current laws of the USA.

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