Published in final edited form as:

Biomaterials. 2011 September ; 32(26): 6285–6290. doi:10.1016/j.biomaterials.2011.05.008.

Drug carrier nanoparticles that penetrate human chronic rhinosinusitis mucus

Samuel K. Lai^{1,5,7}, Jung Soo Suk², Amanda Pace¹, Ying-Ying Wang², Ming Yang², Olcay Mert¹, Jeane Chen¹, Jean Kim³, and Justin Hanes^{*,1,2,5,6}

¹Department of Chemical & Biomolecular Engineering, Johns Hopkins University, 3400 N Charles Street, Baltimore, MD 21218 (USA)

²Department of Biomedical Engineering, Johns Hopkins University School of Medicine, 720 Rutland Avenue, Baltimore, MD 21205 (USA)

³Department of Medicine, Divisions of Allergy and Clinical Immunology, and Department of Otolaryngology, Head and Neck Surgery, Johns Hopkins University School of Medicine, 3400 N. Charles St., Baltimore, MD 21218.

⁴Department of Ophthalmology, The Wilmer Eye Institute, Johns Hopkins University School of Medicine, 400 N. Broadway, Baltimore, MD 21287 (USA)

⁵Institute for NanoBioTechnology and the Center for Cancer Nanotechnology Excellence, Johns Hopkins University, 3400 N Charles Street, Baltimore, MD 21218 (USA)

⁶Center for Nanomedicine, Johns Hopkins University School of Medicine, 400 N Broadway, Baltimore, MD 21287

Abstract

No effective therapies currently exist for chronic rhinosinusitis (CRS), a persistent inflammatory condition characterized by the accumulation of highly viscoelastic mucus (CRSM) in the sinuses. Nanoparticle therapeutics offer promise for localized therapies for CRS, but must penetrate CRSM in order to avoid washout during sinus cleansing and to reach underlying epithelial cells. Prior research has not established whether nanoparticles can penetrate the tenacious CRSM barrier, or instead become trapped. Here, we first measured the diffusion rates of polystyrene nanoparticles and the same nanoparticles modified with muco-inert polyethylene glycol (PEG) coatings in fresh, minimally perturbed CRSM collected during endoscopic sinus surgery from CRS patients with and without nasal polyp. We found that uncoated polystyrene particles, previously shown to be mucoadhesive, were immobilized in all CRSM samples tested. In contrast, densely PEGylated particles as large as 200 nm were able to readily penetrate all CRSM samples from patients with CRS alone, and nearly half of CRSM samples from patients with nasal polyp. Based on the

© 2011 Elsevier Ltd. All rights reserved.

*Corresponding author: Justin Hanes, Ph.D., The Center for Nanomedicine at Johns Hopkins, 400 N Broadway; Robert H. and Clarice Smith Bldg., 4th Floor, Baltimore, MD 21287, Tel: 410-614-6512, hanes@jhu.edu.

⁷Current Address: Division of Molecular Pharmaceutics, UNC Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, CB #7362, UNC, Chapel Hill, NC 27517 (USA). lai@unc.edu

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Declaration of Conflict of Interest. The mucus penetrating particle technology described in this publication is being developed by Kala Pharmaceuticals. Dr. Hanes is co-founder of and serves on the Board of Directors of Kala. Dr. Hanes owns company stock, which is subject to certain restrictions under University policy. The terms of this arrangement are being managed by the Johns Hopkins University in accordance with its conflict of interest policies.

mobility of different sized PEGylated particles, we estimate the average pore size of fresh CRSM to be at least 150 ± 50 nm. Guided by these studies, we formulated mucus-penetrating particles (MPP) composed of PLGA and Pluronic, two materials with a long history of safety and use in humans. We showed that biodegradable MPP are capable of rapidly penetrating CRSM at average speeds up to only 20-fold slower than their theoretical speeds in water. Our findings strongly support the development of mucus-penetrating nanomedicines for the treatment of CRS.

Introduction

Chronic rhinosinusitis (CRS, also commonly referred to as chronic sinusitis) is one of the most prevalent and debilitating chronic conditions in the United States [1]. CRS encompasses a range of inflammatory diseases in the nasal and paranasal mucosa [1, 2], often exacerbated by viral infections that cause goblet cell hyperplasia and infiltration of inflammatory cells, such as eosinophils and lymphocytes [3-5]. Excess mucin secretion, and release of DNA and actin from degenerating neutrophils, together create a highly viscoelastic gel that cannot be effectively cleared from the sinuses by mucociliary action. The consequent mucus accumulation leads to further inflammation, nasal polyp formation, olfactory dysfunction, headache, chills, fever and breathing difficulties [1, 6]. Commonly used treatment modalities include glucocorticosteroids (oral and nasal sprays), antibiotics and surgery, but these treatments are largely ineffective and cause significant adverse side effects [6-8].

Local drug delivery using nanoparticles may significantly reduce systemic side effects and improve the efficacy of CRS therapies [9, 10]. To minimize removal from nasal cleansing and to reach distal nasal cavities, these systems must penetrate across extracellular CRS mucus (CRSM) [11]. Human mucus serves as a critical diffusional barrier against most foreign particles [12, 13]. In diseases characterized by hyper-viscoelastic mucus, such as CRS or cystic fibrosis (CF), the barrier properties of mucus secretions may be further enhanced by the increased solid content and elevated viscoelasticity [14, 15]. We have previously shown that the diffusion of nanoparticles in mucus is highly sensitive to nanoparticle surface chemistry. Whereas most synthetic particles are immobilized in mucus, we found that a dense coating of low molecular weight (MW) poly(ethylene glycol) (PEG) can effectively minimize particle association to mucus constituents, enabling rapid particle penetration across both human cervicovaginal mucus and cystic fibrosis sputum [13, 16, 17]. Thus, to investigate whether nanoparticles can penetrate CRSM, we first measured the transport of 100, 200 and 500 nm densely PEGylated polystyrene particles (PS-PEG) in CRSM samples collected from patients during sinus surgery. To evaluate the influence of surface chemistry on particle transport, we also tested 200 nm uncoated, carboxyl-modified polystyrene particles (PS-COOH) in the same CRSM samples. Based on the insights from our findings, we next engineered drug-carrier nanoparticles, composed of a biodegradable polymer core and an excipient with a long history of safety in humans, that are capable of rapidly penetrating highly viscoelastic CRSM.

Materials & Methods

Collection of human chronic rhinosinusitis mucus (CRSM)

All subjects studied were enrolled after obtaining informed consent under a Johns Hopkins Medicine Institutional Review Board-approved human subjects research protocol. CRS patients with nasal polyps were defined by historical, endoscopic, and radiographic criteria, and by meeting the definition of the American Academy of Otolaryngology-Head and Neck Surgery (AAO-HNS) Chronic Rhinosinusitis Task Force [2]. Specifically, patients with CRS alone had continuous symptoms of rhinosinusitis as defined by the Task Force report

for greater than 12 consecutive weeks, associated with computed tomography (CT) of the sinuses revealing isolated or diffuse sinus mucosal thickening and or air fluid level. CRS subjects with nasal polyps were defined by endoscopic exam findings of polyps and post-treatment CT scan confirmation of persistent bilateral and diffuse paranasal sinus mucosal thickening. Surgery for patients was only performed if a patient's symptoms and radiographic findings failed to resolve despite at least 6 weeks of treatment with oral antibiotics, topical corticosteroids, decongestants, and/or mucolytic agents in accordance with the accepted standards of medical care. However, all CRS subjects chosen for these studies had no immediate preoperative steroids within 14 days prior to obtaining any specimen. Mucus samples were removed from the maxillary sinus cavities of each subject using a curved suction under stereotactic CT image-guidance. CRSM was transported on ice and stored at 4°C prior to use.

Multiple particle tracking

Particle transport rates were measured by analyzing 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 (N.A., 1.3). Experiments were carried out in custom synthesized glass chambers. Particle solutions were added to ~35 μ L of CRSM to a final concentration of 3 % v/v and incubated for 2 h before microscopy. Trajectories of n~150 particles were analyzed for each experiment, and 3-4 experiments were performed for each condition. Movies were captured with Metamorph software (Universal Imaging, Glendale, WI) at a temporal resolution of 66.7 ms for 20 s and tracking resolution of 10 nm. The coordinates of nanoparticle centroids were transformed into time-averaged 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 demonstrated [13, 18, 19].

Preparation of muco-inert probe particles

Carboxyl-modified red or green fluorescent polystyrene (PS) nanoparticles (100-500 nm) were purchased from Molecular Probes (Eugene, OR) and covalently coated with low M.W. PEG (Nektar Therapeutics, San Carlos, CA) as previously described [13]. Size and ζ -potential were determined at pH 7 by dynamic light scattering and laser Doppler anemometry, respectively, using a Zetasizer Nano ZS90 (Malvern Instruments, Southborough, MA). The dense PEG coating on PS-PEG particles was confirmed by their near neutral surface charge (Table 1); in contrast, uncoated PS-COOH were highly negatively charged.

Formulation of biodegradable, mucus-penetrating particles

Doxorubicin (NetQem, Durham, NC), used here as a fluorescent marker, was chemically conjugated to poly(lactide-co-glycolide) (PLGA; M.W. 11,000 Da, 50:50) (Alkermes Inc., Cambridge, MA) as previously described [20]. Nanoparticles composed of the labeled PLGA polymers were prepared by using a solvent diffusion method. Briefly, 10 mg of the polymer was dissolved in 1 mL of tetrahydrofuran, and added dropwise into 40 mL of ultra pure water. Particles were collected, washed twice in ultrapure water, and resuspended in 0.4 mL of ultrapure water. For Pluronic-coated particles, we replaced the ultra pure water by 0.1% F127 aqueous solution during the washing steps, and the PLGA/F127 particles were resuspended in 0.4 mL of 0.1% F127. The particle suspensions were subsequently centrifuged at 92 xg (MicroA Marathon centrifuge, Fisher Scientific, Pittsburgh, PA) for 2 min to remove any potential aggregates, and the supernatants (containing PLGA/F127 particles) were purified by gel permeation chromatography. The hydrodynamic size and surface charge of particles were characterized as described above.

Results

Transport of model nanoparticles in CRSM

PS-PEG sized 100 nm (Figure 1A) and 200 nm (Figure 1B) in diameter underwent rapid diffusion in the first several CRSM samples tested, as evident by the Brownian nature of their trajectories compared to the highly constrained trajectories of 500 nm PS-PEG (Figure 1C) and uncoated 200 nm PS-COOH (Figure 1D). We quantified the extent of the impediment to particle transport by the time scale (τ) dependent ensemble mean squared displacements $\langle \text{MSD} \rangle$ of nanoparticles in CRSM [13, 18]. The rapid mobility of 100 and 200 nm PS-PEG is reflected by their markedly higher $\langle \text{MSD} \rangle$ across all time scales compared to 500 nm PS-PEG and 200 nm PS-COOH (Figure 1E). At $\tau = 1$ s, the $\langle \text{MSD} \rangle$ of 200 nm PS-PEG was ~2-fold and ~110-fold higher than that of 100 nm and 500 nm PS-PEG, respectively; however, the difference between the $\langle \text{MSD} \rangle$ of 100 and 200 nm PS-PEG was not statistically significant. Overall, the average diffusivities of 100, 200 and 500 nm PS-PEG in CRSM were 80-, 20- and 140-fold lower than their theoretical speeds in water, respectively (Table 1). In comparison, 200 nm PS-COOH were slowed by more than 2300-fold in CRSM compared to in water. The difference in transport rates between 200 nm PS-COOH and 200 nm PS-PEG particles in the same CRSM samples were all statistically significant ($p < 0.001$). The slow transport of uncoated particles was also reflected by the slope α from the logarithmic $\langle \text{MSD} \rangle$ versus logarithmic time scale plots ($\alpha = 1$ for pure unobstructed Brownian diffusion, e.g., particles in water, and α becomes smaller as obstruction to particle diffusion increases): the average α value was 0.77 for 200 nm PS-PEG, but only 0.39 for 200 nm PS-COOH (Figure 1E).

Fast moving “outlier” nanoparticles represent a subpopulation of interest, as they are more likely to penetrate deep into CRSM layers. We thus examined the distribution of individual particle effective diffusivities at a time scale of 1 s (Figure 2A-D). There were substantial fractions of fast-moving 100 nm and 200 nm PS-PEG. The average speeds of the fastest 20% of 100 and 200 nm PS-PEG were only 10- and 4-fold lower, respectively, than their average speeds in water (Figure 3B). In contrast, we did not observe fast outlier particles for 200 nm PS-COOH, and a large fraction of 500 nm PS-PEG were immobilized and hindered to the same extent as the uncoated 200 nm PS-COOH. The distribution of speeds of different sized PS-PEG also allows us to quantitatively estimate the effective pore sizes in CRSM [21]. By fitting an empirically-derived obstruction scaling model, applicable to entangled and cross-linked gels such as mucus [22-25], to the measured diffusion rates of 200 nm and 500 nm probe particles, we estimated the average pore size of fresh CRSM to be at least 150 ± 50 nm (Figure 3). Nearly 20% of the pore sizes probed were in excess of 200 nm.

A biodegradable, CRSM-penetrating particle platform

Based on the rapid transport observed with ~200 nm non-degradable, coated latex beads, we next sought to formulate drug carrier nanoparticles ~200 nm in size. Conventional biodegradable polymeric nanoparticles for drug delivery, including those composed of the widely used poly(lactide-co-glycolide) (PLGA) [26], are typically extensively immobilized in human mucus [12, 13]. We recently discovered that coating PLGA particles with specific Pluronics, another polymer/excipient with a long history of use and safety in humans [27-29], can effectively block adhesive interactions between the PLGA core and mucus constituents to an extent similar to covalent conjugation of PEG to latex beads [30]. We thus sought to test whether Pluronics-coated PLGA nanoparticles can penetrate CRSM. The physicochemical properties of sub-200 nm Pluronic F127-coated PLGA (PLGA/F127) particles and uncoated PLGA particles are shown in Table 1. The Pluronic coating on PLGA/F127 particles was confirmed by the near neutral surface charge of the coated particles compared to the negative surface charge for PLGA particles.

Whereas uncoated PLGA particles were strongly immobilized (Figure 4A), PLGA/F127 particles in the same CRSM samples exhibited movements characteristic of freely diffusive Brownian particles (Figure 4B). The $\langle \text{MSD} \rangle$ for PLGA/F127 particles were 280-fold higher than uncoated PLGA particles at a time scale of 1 s ($p < 0.05$), with a substantially greater α value (0.85 vs. 0.35) and a near uniform increase in individual particle speeds (Figure 4C-D). Using a standard method that classifies the mechanism of particle transport via time scale-dependent effective diffusion coefficients [13, 19], we found a ~ 13 -fold increase in the fraction of coated particles that underwent diffusive transport compared to uncoated particles ($p < 0.05$) (Figure 4E). Importantly, the average speed for PLGA/F127 particles was only ~ 20 -fold reduced compared to their theoretical speed in water (uncoated PLGA particles were slowed by > 1000 -fold; Table 1), and the fastest 20% of the PLGA/F127 particles was only slowed 6-fold in CRSM compared to their average speeds in water.

Heterogeneity in particle penetration

The transport measurements of PS-PEG and PLGA/F127 described above were performed in CRSM samples collected from six individual patients (Figure 4A). Since we anticipated that there may be substantial patient-to-patient variations in the properties of CRSM analogous to what we previously observed for CF sputum, we further expanded our observations by measuring the transport of 200 nm PS-PEG in seven additional CRSM samples. We found that in 5 of the 13 total samples tested, the diffusion of 200 nm PS-PEG was substantially hindered (Figure 5A). The hindered motion of PS-PEG in these samples is unlikely attributed to particle quality, since the same batch of 200 nm PS-PEG was used throughout the study and there was no clear chronological dependence (in the last seven samples, the same particles rapidly penetrated samples #9 and #12). To determine whether the disease state of CRS could perhaps account for our observations, we classified CRSM samples based on whether the patient was suffering from CRS alone or CRS together with nasal polyp. Coated particles were able to uniformly and rapidly penetrate all CRSM samples from patients with CRS alone ($n = 4$). However, we observed substantial heterogeneity in particle penetration of mucus collected from CRS patients with nasal polyps, with rapid particle transport found in only 4 of 9 samples (Figure 5B). This suggests that in a subset of CRS patients with nasal polyps, densely coating particles with low MW PEG coatings alone may not facilitate rapid particle penetration. We were unable to correlate particle transport to the bulk (macroscopic) viscoelasticity of the CRSM samples due to the small volume of CRSM samples collected from most patients.

Discussion

Hyper-viscoelastic CRSM secretions not only play a key role in the pathogenesis of CRS disease, but also serve as a critical diffusional barrier that blocks particles from reaching the underlying epithelium and/or distal sinus cavities. Here, we show that synthetic nanoparticles at least as large as ~ 200 nm in diameter, including biodegradable nanoparticles composed of commonly used biomaterials, can be engineered to rapidly penetrate a majority of CRSM samples. CRSM-penetrating particles must have surfaces that minimize adhesion to CRSM constituents, which can be achieved via a dense covalent coating of low MW polyethylene glycol (PEG), or via a non-covalent coating with Pluronic F127. Without coatings that can minimize adhesive interactions between particles and CRSM constituents, nanoparticles are extensively trapped in CRSM. Importantly, the average speed of the biodegradable PLGA/F127 particles was only ~ 20 -fold reduced compared to their theoretical speed in water, with the fastest 20% of the particles only slowed 6-fold compared to their average speeds in water. The ability to engineer nanoparticle-based carriers, using likely safe materials, that rapidly penetrate the formidable CRSM barrier and slowly release encapsulated therapeutics over time may lead to new generations of nanomedicines with

tailored pharmacokinetics for local treatment of CRS. This includes CRSM-penetrating particles that, upon nasal instillation, can slowly release drugs like corticosteroids or antibiotics locally in the sinuses, thereby potentially mitigating much of the adverse side effects associated with systemic exposure of both drugs.

The PLGA/F127 particle platform reported here likely represents a safe delivery vehicle to the sinuses. PLGA, one of the most widely used polymers in drug delivery, has a well established biocompatibility and biodegradability profile [26]. PLGA is FDA-approved for use in various biomedical devices, such as resorbable sutures and drug delivery devices, including the Lupron Depot® (intramuscular injection) and Atridox® (periodontal gel) [31, 32]. Likewise, Pluronic has a long history of safety for use in oral, intravenous and ophthalmic applications [29], including toothpastes and mouthwashes, laxatives, and pharmaceutical products such as Oraqix® (periodontal gel), Differin® (a topical acne cream or gel) and Lariam® (oral tablet) [33-36]. Reflecting their biocompatibility when applied to mucosal tissues and systemically, both PLGA and Pluronics are classified as Generally Regarded As Safe (GRAS) materials by the FDA. It is important to note that the concept of GRAS does not automatically imply biocompatibility, which must be considered in the context of a specific application since biocompatibility is not a property of the material. Nevertheless, the ability to engineer mucus-penetrating particles composed entirely of GRAS materials, without generating any new chemical entity (NCE), may facilitate quicker and more cost-effective clinical development of this drug delivery platform.

In addition to their safety profile, we anticipate that the PLGA/F127 particles will also provide sustained delivery of a wide array of therapeutics, since many therapeutic molecules can be encapsulated and released from PLGA particles [37]. Importantly, our current method involves only incubation of pre-formulated PLGA particles with Pluronic, and does not require altering the formulation process of drug-loaded PLGA particles; the simplicity of the process should help ensure ease of manufacturing and production scalability.

The rapid diffusion of coated particles in CRSM suggests that CRSM, like CVM and CFS, possesses a mesh structure with a low viscosity, water-like, interstitial fluid between the structural elements through which properly engineered particles can penetrate [11, 13, 15-17]. Nevertheless, there appear to be distinct differences in the barrier properties of CRSM against nanoparticles compared to other human mucus secretions. CRSM slowed the transport of all PS-PEG particles to a greater extent than CVM, especially to 100 nm and 500 nm PS-PEG than 200 nm PS-PEG [13, 21]. Since 200 nm PS-PEG exhibited faster transport than 100 nm PS-PEG in the same CRSM samples, the slower transport of 100 nm PS-PEG particles cannot be attributed to steric obstruction. Instead, 100 nm PS-PEG must be slowed by adhesive interactions with CRSM, perhaps a consequence of less effective PEG coating on smaller particles due to the higher degree of curvature. Nevertheless, the same 100 nm PS-PEG particles rapidly penetrated CVM [38], suggesting that CRSM most likely possess greater adhesivity than CVM. The elevated adhesivity may also be reflected by the slower speeds of 200 nm PS-PEG in CRSM compared to CVM, which was slowed on average only 4-fold in CVM compared to their theoretical speeds in water, compared to 20-fold in CRSM [13]. The hindered transport of 500 nm PS-PEG in CRSM vs. CVM likely reflects a combination of increased adhesivity and elevated steric obstruction from a denser mesh, similar to what we have previously observed with CFS [16]. Interesting, the same trend for the speeds of 100 – 500 nm PS-PEG particles in CRSM (200 nm > 100 nm > 500 nm) was also observed with CFS, although the absolute speeds of PS-PEG particles in CRSM are faster than those in CFS, suggesting that CRSM may pose an overall less tenacious diffusional barrier than CFS [16]. The similar barrier properties of CRSM and CFS may be attributed to the similar pathogenesis of the two diseases, in which the formation of an increasingly viscoelastic mucus gel is exacerbated by pathogenic infections

and chronic inflammation [4, 39]. Indeed, CRS occurs extremely frequently in CF patients, and carriers of a single CF mutation have a higher prevalence of CRS than the general population, suggesting the potential genetic linkage between the two diseases may have led to similarities in their nanoscale barrier properties [40, 41].

Although we were able to engineer densely PEGylated particles to rapidly penetrate all CRSM samples from patients without nasal polyps, the particles were not able to penetrate a subset of CRSM samples from patients with nasal polyps. While the actual biochemical differences between the two groups of CRSM samples remain unclear, the disparity is likely related to polyp-induced alteration of the mucus barrier. Since polyp formation likely reflects greater prior exposure to inflammatory stress, the greater adhesivity and viscoelasticity in these CRSM samples may be attributed to increased DNA and actin content from degenerating inflammatory neutrophils and elevated mucin content from goblet cell hyperplasia. This motivates the use of adjuvant therapies, such as mucolytics, to reduce the barrier properties of CRSM and improve particle penetration across these samples [42].

Conclusion

Nanoparticles up to 200 nm in diameter that are coated with a dense layer of non-mucoadhesive PEG polymers readily penetrate CRSM, including drug carriers composed entirely of GRAS components. The development of polymeric particles with improved sinus mucus penetration capability should encourage the commercial development of new generations of nanoparticle-based drug delivery systems for CRS and other diseases affecting the sinuses.

Acknowledgments

This work was supported in part by NIH grant (R21HL089816), a Croucher Foundation Fellowship (S.K.L) and an NSF Graduate Research Fellowship (Y.-Y.W). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Heart, Lung, And Blood Institute or the National Institutes of Health.

References

- [1]. Dalton P. Olfaction and Anosmia in Rhinosinusitis. *Current Allergy and Asthma Reports*. 2004; 4:230–6. [PubMed: 15056406]
- [2]. Benninger MS, Ferguson BJ, Hadley JA, Hamilos DL, Jacobs M, Kennedy DW, et al. Adult chronic rhinosinusitis: definitions, diagnosis, epidemiology, and pathophysiology. *Otolaryngol Head Neck Surg*. 2003; 129:S1–32. [PubMed: 12958561]
- [3]. Ponikau J. Striking deposition of toxic eosinophil major basic protein in mucus: Implications for chronic rhinosinusitis. *The Journal of allergy and clinical immunology*. 2005; 116:362–9. [PubMed: 16083791]
- [4]. Heinecke L, Proud D, Sanders S, Schleimer RP, Kim J. Induction of B7-H1 and B7-DC expression on airway epithelial cells by the Toll-like receptor 3 agonist double-stranded RNA and human rhinovirus infection: In vivo and in vitro studies. *The Journal of allergy and clinical immunology*. 2008; 121:1155–60. [PubMed: 18378285]
- [5]. Ponikau JU, Sherris DA, Kephart GM, Kern EB, Gaffey TA, Tarara JE, et al. Features of airway remodeling and eosinophilic inflammation in chronic rhinosinusitis: is the histopathology similar to asthma? *The Journal of allergy and clinical immunology*. 2003; 112:877–82. [PubMed: 14610473]
- [6]. Raviv JR, Kern RC. Chronic rhinosinusitis and olfactory dysfunction. *Advances in oto-rhinolaryngology*. 2006; 63:108–24.
- [7]. Mott AE, Cain WS, Lafreniere D, Leonard G, Gent JF, Frank ME. Topical corticosteroid treatment of anosmia associated with nasal and sinus disease. *Archives of otolaryngology--head & neck surgery*. 1997; 123:367–72. [PubMed: 9109781]

- [8]. Dykewicz MS, Fineman S, Skoner DP, Nicklas R, Lee R, Blessing-Moore J, et al. American Academy of Allergy, Asthma, and Immunology. Diagnosis and management of rhinitis: complete guidelines of the Joint Task Force on Practice Parameters in Allergy, Asthma and Immunology. *Ann Allergy Asthma Immunol.* 1998; 81:478–518. [PubMed: 9860027]
- [9]. LaVan DA, McGuire T, Langer R. Small Scale Systems for in vivo drug delivery. *Nature Biotechnology.* 2003; 21:1184–91.
- [10]. Rosen H, Abribat T. The rise and rise of drug delivery. *Nat Rev Drug Discovery.* 2005; 4:381–5.
- [11]. Lai SK, Wang YY, Hanes J. Mucus-penetrating nanoparticles for drug and gene delivery to mucosal tissues. *Advanced drug delivery reviews.* 2009; 61:158–71. [PubMed: 19133304]
- [12]. Cone, R. Mucus. In: Lamm, Michael E.; McGhee, Jerry R.; Mayer, Lloyd; Mestecky, Jiri; Bienenstock, John, editors. *Mucosal Immunology.* 3ed.. Academic Press; San Diego: 1999. p. 43-64.WS
- [13]. Lai SK, O'Hanlon DE, Harrold S, Man ST, Wang YY, Cone R, et al. Rapid transport of large polymeric nanoparticles in fresh undiluted human mucus. *Proc Natl Acad Sci U S A.* 2007; 104:1482–7. [PubMed: 17244708]
- [14]. Dawson M, Wirtz D, Hanes J. Enhanced viscoelasticity of human cystic fibrotic sputum correlates with increasing microheterogeneity in particle transport. *J Biol Chem.* 2003; 278:50393–401. [PubMed: 13679362]
- [15]. Lai SK, Wang YY, Wirtz D, Hanes J. Micro- and macrorheology of mucus. *Advanced drug delivery reviews.* 2009; 61:86–100. [PubMed: 19166889]
- [16]. Suk JS, Lai SK, Wang YY, Ensign LM, Zeitlin PL, Boyle MP, et al. The penetration of fresh undiluted sputum expectorated by cystic fibrosis patients by non-adhesive polymer nanoparticles. *Biomaterials.* 2009; 30:2591–7. [PubMed: 19176245]
- [17]. Wang, YY.; Lai, SK.; Suk, JS.; Pace, A.; Cone, R.; Hanes, J. *Angewandte Chemie (International ed. Vol. 47.* 2008. Addressing the PEG mucoadhesivity paradox to engineer nanoparticles that “slip” through the human mucus barrier; p. 9726-9.
- [18]. Suh J, Dawson M, Hanes J. Real-time multiple-particle tracking: applications to drug and gene delivery. *Advanced drug delivery reviews.* 2005; 57:63–78. [PubMed: 15518921]
- [19]. Suk JS, Suh J, Lai SK, Hanes J. Quantifying the intracellular transport of viral and nonviral gene vectors in primary neurons. *Exp Biol Med.* 2007; 232:461–9.
- [20]. Yoo HS, Oh JE, Lee KH, Park TG. Biodegradable nanoparticles containing doxorubicin-PLGA conjugate for sustained release. *Pharmaceutical research.* 1999; 16:1114–8. [PubMed: 10450940]
- [21]. Lai SK, Wang YY, Hida K, Cone R, Hanes J. Nanoparticles reveal that human cervicovaginal mucus is riddled with pores larger than viruses. *Proc Natl Acad Sci U S A.* 2010; 107:598–603. [PubMed: 20018745]
- [22]. Amsden B. Solute diffusion within hydrogels. Mechanisms and models. *Macromolecules.* 1998; 31:8382–95.
- [23]. Amsden B. An obstruction-scaling model for diffusion in homogeneous hydrogels. *Macromolecules.* 1999; 32:874–9.
- [24]. Olmsted SS, Padgett JL, Yudin AI, Whaley KJ, Moench TR, Cone RA. Diffusion of macromolecules and virus-like particles in human cervical mucus. *Biophys J.* 2001; 81:1930–7. [PubMed: 11566767]
- [25]. Shen H, Hu Y, Saltzman WM. DNA diffusion in mucus: effect of size, topology of DNAs, and transfection reagents. *Biophys J.* 2006; 91:639–44. [PubMed: 16632500]
- [26]. Shive MS, Anderson JM. Biodegradation and biocompatibility of PLA and PLGA microspheres. *Advanced drug delivery reviews.* 1997; 28:5–24. [PubMed: 10837562]
- [27]. Ignatius AA, Claes LE. In vitro biocompatibility of bioresorbable polymers: poly(L, DL-lactide) and poly(L-lactide-co-glycolide). *Biomaterials.* 1996; 17:831–9. [PubMed: 8730968]
- [28]. Visscher GE, Robison RL, Maulding HV, Fong JW, Pearson JE, Argentieri GJ. Biodegradation of and tissue reaction to 50:50 poly(DL-lactide-co-glycolide) microcapsules. *Journal of biomedical materials research.* 1985; 19:349–65. [PubMed: 4077887]
- [29]. Poloxamer. U.S. Food and Drug Administration; Inactive Ingredient Search for Approved Drug Products.

- [30]. Yang, M.; Lai, SK.; Wang, YY.; Zhong, W.; Happe, C.; Zhang, M., et al. *Angewandte Chemie* (International ed. Vol. 50. 2011. Biodegradable Nanoparticles Composed Entirely of Safe Materials that Rapidly Penetrate Human Mucus; p. 2597-600.
- [31]. Crawford ED, Sartor O, Chu F, Perez R, Karlin G, Garrett JS. A 12-Month Clinical Study of LA-2585 (45.0 MG): A New 6-Month Subcutaneous Delivery System for Leuprolide Acetate for the Treatment of Prostate Cancer. *The Journal of Urology*. 2006; 175:533–6. [PubMed: 16406989]
- [32]. Kim TS, Klimpel H, Fiehn W, Eickholz P. Comparison of the pharmacokinetic profiles of two locally administered doxycycline gels in crevicular fluid and saliva. *Journal of clinical periodontology*. 2004; 31:286–92. [PubMed: 15016257]
- [33]. Donaldson D, Gelskey SC, Landry RG, Matthews DC, Sandhu HS. A placebo-controlled multi-centred evaluation of an anaesthetic gel (Oraqix) for periodontal therapy. *Journal of clinical periodontology*. 2003; 30:171–5. [PubMed: 12631173]
- [34]. Emanuele RM. FLOCOR: a new anti-adhesive, rheologic agent. *Expert opinion on investigational drugs*. 1998; 7:1193–200. [PubMed: 15992024]
- [35]. Na-Bangchang K, Karbwang J, Palacios PA, Ubalee R, Saengtertilapachai S, Wernsdorfer WH. Pharmacokinetics and bioequivalence evaluation of three commercial tablet formulations of mefloquine when given in combination with dihydroartemisinin in patients with acute uncomplicated falciparum malaria. *European journal of clinical pharmacology*. 2000; 55:743–8. [PubMed: 10663453]
- [36]. Thiboutot DM, Shalita AR, Yamauchi PS, Dawson C, Kerrouche N, Arsonnaud S, et al. Adapalene gel, 0.1%, as maintenance therapy for acne vulgaris: a randomized, controlled, investigator-blind follow-up of a recent combination study. *Archives of dermatology*. 2006; 142:597–602. [PubMed: 16702497]
- [37]. McGinity JW, O'Donnell PB. Preparation of microspheres by the solvent evaporation technique. *Advanced drug delivery reviews*. 1997; 28:25–42. [PubMed: 10837563]
- [38]. Lai SK, Wang YY, Cone R, Wirtz D, Hanes J. Altering mucus rheology to “solidify” human mucus at the nanoscale. *PLoS ONE*. 2009; 4:e4294. [PubMed: 19173002]
- [39]. Boucher RC. Evidence for airway surface dehydration as the initiating event in CF airway disease. *Journal of internal medicine*. 2007; 261:5–16. [PubMed: 17222164]
- [40]. Wang X, Kim J, McWilliams R, Cutting GR. Increased prevalence of chronic rhinosinusitis in carriers of a cystic fibrosis mutation. *Archives of otolaryngology--head & neck surgery*. 2005; 131:237–40. [PubMed: 15781764]
- [41]. Wang X, Moylan B, Leopold DA, Kim J, Rubenstein RC, Togias A, et al. Mutation in the gene responsible for cystic fibrosis and predisposition to chronic rhinosinusitis in the general population. *Jama*. 2000; 284:1814–9. [PubMed: 11025834]
- [42]. Suk JS, Lai SK, Boylan NJ, Dawson MR, Boyle MP, Hanes J. Rapid transport of muco-inert nanoparticles in cystic fibrosis sputum treated with N-acetyl cysteine. *Nanomedicine (Lond)*. 2011; 6:365–75. [PubMed: 21385138]

Figure 1.

Transport of different sized uncoated (PS-COOH) and minimally mucoadhesive PEG-coated (PS-PEG) latex particles in fresh, undiluted human chronic sinusitis mucus (CRSM).

Representative trajectories of (A) 200 nm PS-COOH, and (B) 100 nm, (C) 200 nm and (D) 500 nm PS-PEG in CRSM from 20 s movies. The particle traces reflect 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. Data represent four independent experiments, with $n > 100$ particles per experiment.

Figure 2.

Distributions of the logarithms of individual particle effective diffusivities ($Deff$) at a time scale of 1 s for (A) 100 nm PS-PEG, (B) 200 nm PS-PEG, (C) 500 nm PS-PEG and (D) 200 nm PS-COOH particles.

Figure 3. Distribution of effective pore sizes in human chronic sinusitis mucus estimated by obstruction scaling model, with average pore size $\sim 150 \pm 50$ nm.

Figure 4.

Transport of biodegradable polymeric nanoparticles composed of poly(lactide-co-glycolide) (PLGA) in CRSM. Representative trajectories of (A) uncoated (PLGA) and (B) Pluronic F127-coated (PLGA/F127) PLGA particles in CRSM traced over 20 s. The particle traces reflect effective diffusivities within one s.e.m. of the ensemble mean. (C) Ensemble-averaged geometric mean square displacements ($\langle \text{MSD} \rangle$) as a function of time scale. Data represent three independent experiments with $n > 150$ particles per experiment. (D) Distributions of the logarithms of individual particle effective diffusivities (Deff) at a time scale of 1 s. (E) Fractions of PLGA and PLGA/F127 undergoing diffusive motion in CRSM. Bars represent standard errors. * denotes statistical significance ($p < 0.05$).

Figure 5. Ensemble-averaged geometric mean square displacements (<MSD>) at a time scale of 1 s for 200 nm PS-PEG (cross mark x) and ~200 nm PLGA/F127 (closed circle •) particles for (A) each individual CRSM sample tested, and (B) CRSM samples grouped by whether the patient has nasal polyps (+NP) or not (-NP).

Table 1

Physicochemical properties of various particles and their effective diffusivity in CRSM (D_m) compared to in water (D_w). Samples include uncoated polystyrene particles (PS), PEG-coated polystyrene particles (PS-PEG), uncoated poly(lactic-co-glycolic acid) particles (PLGA) and Pluronic F127-coated PLGA particles (PLGA/F127). Effective diffusivity in CRSM is calculated at time scale of 1s and water diffusivity is calculated from Stokes-Einstein equation using average particle diameter.

Formulation	Diameter (nm)	ζ -potential (mV)	D_w/D_m
Uncoated PS	217 ± 5	-59 ± 4	2300
PS-PEG	114 ± 4	-3 ± 1	80
PS-PEG	231 ± 6	-2 ± 2	20
PS-PEG	515 ± 17	-4 ± 3	140
PLGA	129 ± 22	-52 ± 4	1000
PLGA/F127	188 ± 7	-7 ± 1	20