DIFFUSION OF LIPID NANOPARTICLES THROUGH AIRWAY MUCUS

Mohammad-Reza Rokhforouz [1,*], Belal Tafech[2,*], Jerry Leung[3], Pieter Cullis[3], Don D Sin[4,5], and Sarah Hedtrich[2,6,7], James J Feng[1,8]

[1] Department of Chemical and Biological Engineering, University of British Columbia, Vancouver, British Columbia, Canada

[2] Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, British Columbia, Canada

[3] Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, British Columbia, Canada

[4] Centre for Heart Lung Innovation, University of British Columbia, Vancouver, British Columbia, Canada

[5] Faculty of Medicine, University of British Columbia, Vancouver, British Columbia, Canada

[6] Department of Infectious Diseases and Respiratory Medicine, Charité – Universitätsmedizin Berlin, Corporate member of Freie Universität Berlin and Humboldt Universität zu Berlin, Germany

[7] Center of Biological Design, Berlin Institute of Health at Charité – Universitätsmedizin Berlin, Germany

[8] Department of Mathematics, University of British Columbia, Vancouver, British Columbia, Canada

ABSTRACT

Successful transmucosal delivery promises great therapeutic opportunities for the treatment of various diseases such as cystic fibrosis and chronic obstructive pulmonary disease (COPD). Despite promising results in preclinical studies, nanoparticle (NP) for drug delivery still remains insufficiently explored, thereby limiting effective clinical translation. Using Brownian dynamics (BD) simulations, we investigated lipid nanoparticle (LNP)-mucus interactions under the influences of steric, electrostatic, and hydrogen bond interactions. The mucus is modeled as a cubic periodic box comprised of straight, rigid, infinitely long rods. To obtain statistically reliable diffusion coefficients, we considered one thousand non-interacting spherical particles over at least 10^6 steps. The results revealed that removal of mucin's sialic acid results in higher diffusivity. However, LNP exhibit a non-monotonic behaviour as a function of mucus pH. Besides, both the electrostatic repulsion and the hydrogen-bonding, if acting alone, will hinder diffusivity. But one factor can mitigate the effect of the other to raise the diffusivity. The simulation results are compared with experimental measurements where possible, and the two generally agree. Our results may provide new insights into rational design for mucus-penetrating nanoparticles.

Brownian dynamics simulations

To simulate NP diffusion through mucus, we model the cross-linked mucin chains as rigid edges of a periodic cubic lattice, with the lattice size corresponding to the average mesh size of the mucus [1]. The Brownian diffusion of NPs can thus be tracked within a unit cell with periodic boundary conditions imposed on its faces. For each spherical particle, a Langevin equation is written out that includes a Brownian force, a drag force, and a pairwise interaction potential between the particle and the mucins:

$$m\frac{d\mathbf{v}}{dt} = F^B - \varsigma\mathbf{v} - \nabla U \quad (1)$$

where $m = \frac{\pi}{6}\rho_p d_p{}^3$ is the mass of particle of density ρ_p and diameter d_p, $\mathbf{v}$ is its velocity, t is the time, F^B is the Brownian force, ς is the drag coefficient, μ_f is the viscosity of the solvent, ∇ the spatial derivative, and U is the total pairwise interaction potential.

Charges on mucin: sialic acid cleavage

Sialic acid (SA) is responsible for most of the negative charge of the mucins [2]. To modify the charges on mucin chains, we have used an enzyme neuraminidase (NA) to cleave some of the negatively charged sialic acid side chains from the mucin backbone. We treated the mucus with NA for 10 min or 4 hours to remove different amounts of SA; the resulting mucus samples are called "semi-cleaved" and "cleaved" for short, with 23% and 56% of SA cleaved, respectively. As a control, an untreated mucus sample was also included. LNP was added separately to each of the three mucus samples, and nanoparticle tracking analysis was performed. As shown in **Fig. 1A**, the median diffusion coefficient (D) of LNP tends to increase as SA is removed by NA, but the data show a large amount of scatter. To model this in BD simulations, we use the untreated mucus as a baseline to determine the Debye length k and the electrostatic potential U_e. From this baseline, we estimate U_e for the semi-cleaved and cleaved mucus in proportion to the amount of charges left on the mucin polymer chains. Thus, we obtain the numerical diffusivity shown by the blue dots in Fig. 1A, in reasonable agreement with the experimental data.

Effect of pH

To understand the impact of pH on NP diffusion through the mucus, we decreased pH from 7 to 5, and 3. A change in pH results in a change in the surface charge density of both the mucin polymer chains and the LNP [3] (See **Table 1**). The measured zeta potential values indicate that by pH reduction from 7 to 3, a transition from electrostatic repulsion to attraction would occur. **Fig. 1B** depicts the diffusion coefficient D at different pH levels. Notably, the variation is non-monotonic, with the highest D value for the intermediate pH=5. This trend is also captured by our BD simulations, which offer an explanation for this behavior. As pH decreases from 7 to 5, D increases because the electrostatic repulsion is weakened. Further reduction to pH = 3 incurs electrostatic attraction, which in our case tends to trap the LNPs near the corners of the lattice and suppress their diffusion greatly.

Effect of hydrogen-bond attraction

Using BD simulations, we studied the interaction between electrostatic repulsion and H-bond attraction. Given the shorter-ranged nature of H-bond interaction, we deliberately chose weak electrostatic repulsions where particles can be influenced by H-bond attractions. As expected,

in the absence of H-bonds, increasing the strength of electrostatic interaction leads to lower diffusivities, as the particles tend to remain in the center of the simulation box. When we have two competing factors, one factor would reduce the effects of the other one, so that a nonmonotonic U_e dependence trend is observed (See **Fig. 1C**). For stronger electrostatic repulsions, the diffusivity will eventually fall again because of too much repulsive forces.

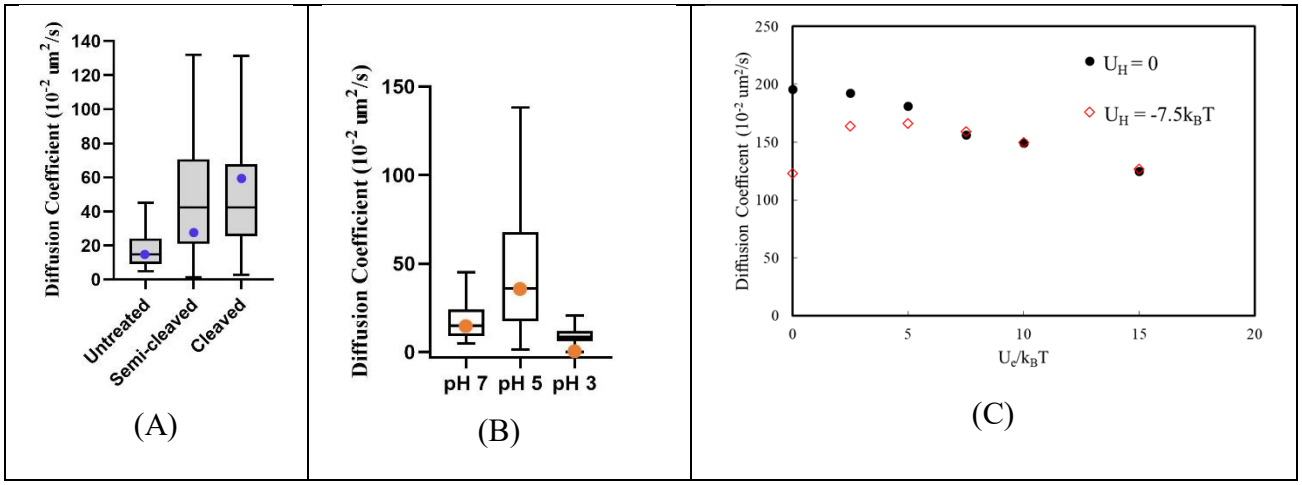

Figure 1: Variation of the diffusion coefficient due to (A) cleavage of sialic side chains from mucin; (B) change in medium pH; (C) hydrogen-bonding mitigating electrostatic repulsion.

Table 1: Zeta potential of mucin and LNP-mRNA at different pH values

	Zeta potential (pH=7)	Zeta potential (pH=5)	Zeta potential (pH= 3)
Mucin[4]	-7.7 mV	-4.8 mV	-2.2 mV
LNP-mRNA	- 6.21 mV	- 7.24 mV	+ 13.0 mV
U_e / k_BT	-76.3	-55.4	+45.6
Type of interaction	Strong repulsion	Weak repulsion	Weak attraction

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