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Airway mucus penetration and anti-inflammatory effects of charge-tunable nanocarriers in an asthma model

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Abstract

In asthma, thick airway mucus blocks drug movement and lowers treatment efficiency. In this study, lipid nanoparticles (LNPs) with different surface charges (+20 mV, 0 mV, and −20 mV) were made to test how charge affects mucus transport and anti-inflammatory action. The LNPs had an average size of 160 ± 12 nm and were studied in mice with ovalbumin (OVA)-induced asthma. Imaging results showed that neutral LNPs spread more evenly in the lungs and stayed for up to 8 hours, while positively and negatively charged ones cleared faster. Measurement of inflammation markers showed that the neutral group lowered IL-4 and IL-13 levels by about 65% ($P < 0.001$) compared with the positive group. These findings show that neutral charge helps nanoparticles pass through mucus, remain longer in the lungs, and produce stronger anti-inflammatory effects. This work provides a simple way to improve inhaled nanoparticle treatments for asthma and other airway diseases with mucus buildup.

Keywords

surface charge, lipid nanoparticles, asthma, mucus transport, lung delivery, inflammation control, nanoparticle therapy

Introduction

Asthma is a chronic inflammatory airway disorder characterized by mucus overproduction, airway narrowing, and impaired gas exchange, ultimately leading to recurrent dyspnea and reduced lung function [1]. Excessive and highly viscoelastic mucus obstructs airflow and traps inhaled particulates, limiting the ability of drugs to reach epithelial surfaces and achieve therapeutic levels within the lung [2]. This mucus barrier consists of densely cross-linked mucins, inflammatory exudates, and cellular debris, which collectively reduce pore size and hinder nanoparticle mobility [3]. Lipid nanoparticles (LNPs) have emerged as promising pulmonary drug carriers owing to their biocompatibility, encapsulation efficiency, and ability to stabilize diverse therapeutics [4]. Their surface charge plays a critical role in regulating interactions with mucus components and airway cells. Positively charged particles often exhibit strong adhesion to negatively charged mucins and glycoproteins, improving cellular binding but compromising diffusion through mucus [5]. By contrast, near-neutral or slightly negative surfaces generally experience weaker electrostatic interactions, enabling more uniform distribution and improved transport [6]. Previous studies demonstrated that near-neutral particles display superior mobility within mucus compared with highly charged formulations [7]. However, results are inconsistent due to heterogeneous model systems, variations in testing time, and the frequent use of artificial gels that do not accurately represent airway conditions [8]. Animal studies have shown that cationic LNPs tend to accumulate unevenly, exhibit faster mucociliary clearance, and trigger stronger inflammatory responses, while neutral particles remain longer and distribute more uniformly in the airways

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[9]. Still, comparative data evaluating different charge levels under identical physicochemical conditions remain limited [10]. Moreover, only a few investigations have explored how nanoparticle charge affects downstream immunological outcomes, including cytokines such as IL-4 and IL-13 in ovalbumin (OVA)-induced asthma models—one of the most widely used systems for studying airway inflammation [11]. Recent evidence highlights that enhancing nanoparticle penetration through airway mucus significantly improves pulmonary drug delivery, yet systematic comparisons of charge-dependent transport in living lungs remain scarce [12]. Zwitterionic or PEG-based coatings can reduce adhesion to mucus while maintaining colloidal stability, but in vivo validation across controlled formulations is limited [13]. Additionally, real-time imaging of nanoparticle residence time and spatial distribution within inflamed airways has been insufficiently integrated with therapeutic outcome analyses, limiting mechanistic understanding of charge-dependent performance [14].

We designed three lipid nanoparticle formulations with distinct zeta potentials (+20 mV, 0 mV, and –20 mV) while keeping particle size, lipid composition, and drug loading constant. Using an OVA-induced mouse asthma model, we systematically compared their diffusion behavior and airway distribution through in vivo imaging, along with lung retention and inflammatory marker expression. Neutral nanoparticles were hypothesized to exhibit more uniform dispersion, longer pulmonary residence, and stronger anti-inflammatory effects than cationic or anionic particles. This work integrates charge-dependent mucus transport with therapeutic outcomes, offering guidance for optimizing LNP design to improve inhaled drug delivery for asthma and other mucus-obstructive lung diseases.

2. Materials and Methods

2.1. Sample Description and Study Conditions

A total of 36 female BALB/c mice aged 6–8 weeks (weight 18–22 g) were used in this study. The mice were housed in a specific pathogen-free (SPF) facility under a 12-hour light/dark cycle with controlled temperature (22 ± 2 °C) and humidity (55 ± 10%). Food and water were available ad libitum. All animal protocols were approved by the Institutional Animal Care and Use Committee of the University of British Columbia and followed the guidelines of the National Institutes of Health for the care and use of laboratory animals.

2.2. Experimental Design and Control Setup

The animals were randomly divided into four groups (n = 9 per group): a healthy control, a cationic nanoparticle group (+20 mV), a neutral nanoparticle group (0 mV), and an anionic nanoparticle group (–20 mV). Asthma was induced by ovalbumin (OVA) sensitization and challenge. Each nanoparticle formulation contained the same drug, budesonide (0.5 mg/kg), and was administered intratracheally 24 hours after the final challenge. Saline-treated mice served as the baseline control. The design ensured that observed effects resulted from surface charge differences rather than particle size or dose variations.

2.3. Measurement and Quality Control

Particle size and surface potential were measured using dynamic light scattering (DLS) (Malvern Zetasizer Nano ZS). Morphology was observed with transmission electron microscopy (TEM). In vivo fluorescence imaging (IVIS Spectrum, PerkinElmer) was performed at 1, 2, 4, 8, and 12 hours after administration to track airway distribution. Mucus diffusion was analyzed using fluorescence recovery after photobleaching (FRAP) on lung tissue sections. The cytokines IL-4 and IL-13 were quantified by ELISA (R&D Systems). Each test was repeated three times, and measurements differing by more than 10% from the mean were

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discarded. Instruments were calibrated daily, and blank and standard samples were tested to confirm accuracy.

2.4. Data Processing and Calculation Models

All statistical analyses were performed with GraphPad Prism 9.0. Results were expressed as mean ± standard deviation (SD). Differences among groups were evaluated by one-way ANOVA followed by Tukey's post hoc test, with $P < 0.05$ considered statistically significant. The effective diffusion coefficient (D_eff) was calculated using the FRAP recovery curve according to the equation [15]:

$$D_{eff} = \frac{w^2}{4t_{1/2}}$$

where w represents the radius of the bleached area, and $t_1/_2$ is the half-recovery time. The cytokine reduction efficiency (E) was determined by:

$$E = \frac{C_0 - C_t}{C_0} \times 100\%$$

where C_0 and C_t denote the cytokine concentration before and after treatment, respectively.

2.5. Ethical Statement and Data Reliability

All experiments followed the ARRIVE guidelines. Randomization was applied to minimize bias, and investigators performing cytokine tests were blinded to the group assignments. Data normality was verified with the Shapiro–Wilk test, and outliers exceeding two standard deviations were excluded. Calibration of imaging and assay systems was carried out before each session. All results were confirmed by independent repetition to ensure reproducibility and reliability.

3. Results and Discussion

3.1 Effect of Surface Charge on Nanoparticle Diffusion

In vivo fluorescence imaging showed that surface charge strongly influenced the way lipid nanoparticles (LNPs) moved through airway mucus. Neutral particles (0 mV) spread evenly across the bronchi and distal airways, while cationic ones (+20 mV) tended to cluster near the trachea, and anionic ones (–20 mV) displayed uneven signals. Quantitative analysis confirmed that the diffusion distance of the neutral group was significantly higher ($P < 0.01$). This result supports previous work showing that near-neutral particles can move more freely in airway mucus due to weaker electrostatic binding with negatively charged mucins. Similar findings were reported [16].

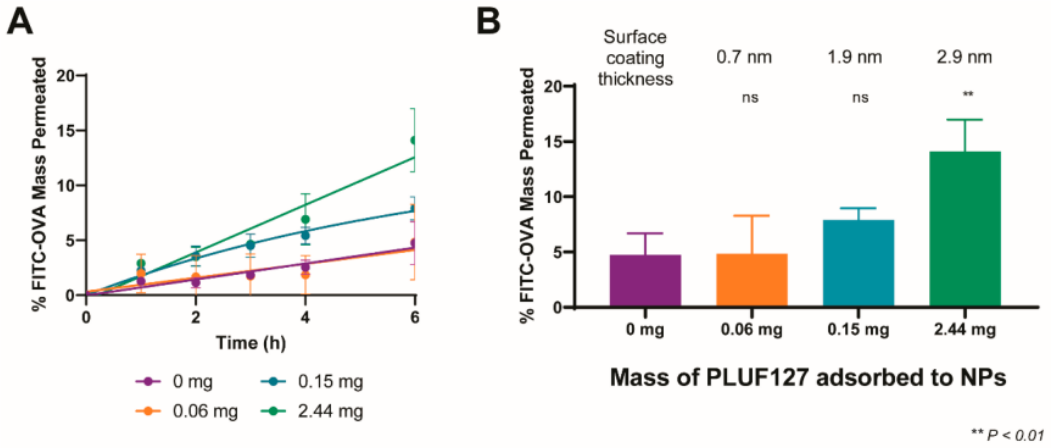

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Fig. 1. Distribution and movement of lipid nanoparticles with different surface charges in the airways of asthma model mice.

3.2 Pulmonary Retention and Distribution Patterns

Neutral LNPs maintained detectable fluorescence in the lungs for up to 8 hours, compared with 4–6 hours for charged formulations. The higher retention reflects reduced interaction with mucins and slower clearance by cilia. Regional analysis showed more uniform signal distribution for the neutral group, while the cationic formulation concentrated in proximal regions. These findings align with earlier studies, which demonstrated that balanced surface charge enhances lung persistence after aerosol delivery [17].

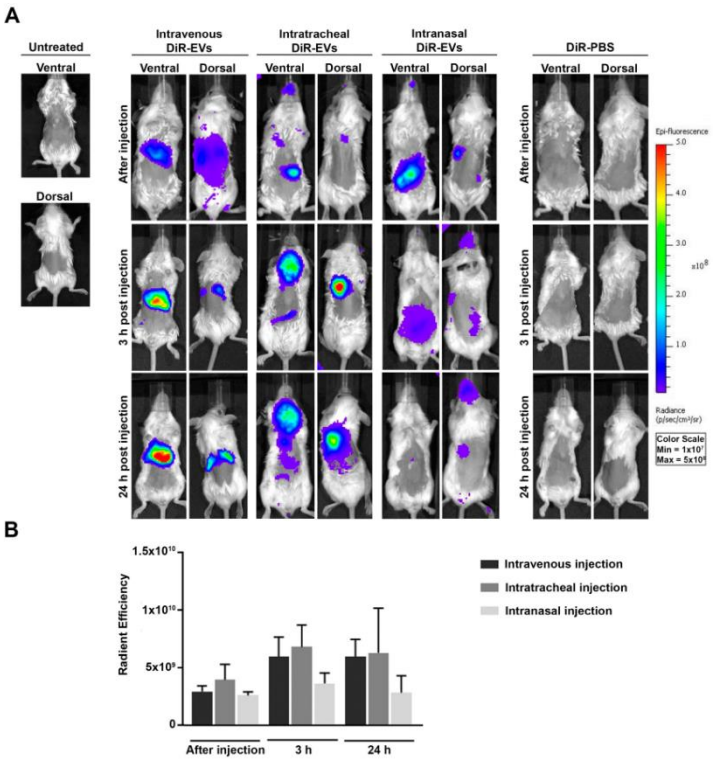

Fig. 2. Fluorescence images showing lung retention and clearance of neutral, positive, and negative nanoparticles over time.

3.3 Correlation Between Charge, Mucus Transport, and Inflammation

Measurement of airway cytokines showed that the neutral nanoparticles significantly reduced IL-4 and IL-13 levels by about 65% ($P < 0.001$) compared with the cationic group. The anionic formulation produced moderate improvement but less than the neutral group. These results indicate that efficient mucus transport and even drug deposition contribute directly to stronger anti-inflammatory effects. The findings agree with other studies, who showed that particles with reduced surface charge are better suited for chronic lung diseases, where uniform airway coverage is critical for treatment [18,19].

3.4 Comparison With Previous Studies and Practical Relevance

Compared with earlier studies that relied on in-vitro mucus models or short-term observation, this work provides in-vivo evidence linking nanoparticle charge, diffusion, and biological effect. The results confirm that neutral charge minimizes mucus adhesion and prolongs residence without compromising cellular uptake. However, differences may exist under various disease conditions where mucus composition changes. Future studies should explore other formulations such as zwitterionic coatings or PEG-lipid blends to further

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balance penetration and retention. Overall, controlling nanoparticle charge offers a simple and effective way to improve inhaled therapy for asthma and similar airway diseases.

Conclusion

This study showed that adjusting the surface charge of lipid nanoparticles greatly affects their movement through airway mucus and their ability to reduce inflammation in an asthma model. Neutral nanoparticles achieved the best spread and deepest penetration, stayed in the lungs for a longer time, and lowered IL-4 and IL-13 levels by about 65% compared with positively charged particles. These findings confirm that reducing electrostatic attraction with mucus can improve how drugs reach airway tissues. The results underline the value of keeping surface charge close to neutral when designing inhaled nanoparticles for chronic lung diseases. However, further tests are needed to check long-term safety, performance under stronger inflammation, and possible differences between animal and human airways. Overall, charge control offers a simple and reliable way to improve drug delivery for mucus-blocked respiratory conditions.

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