The effect of nanoparticles on airway mucus functional properties, a case study

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ABSTRACT

The mucus of the conducting airways may be exposed to nanoparticles both from environmental sources and through the development of nanomedicines. Given the importance of mucus rheology to the correct functioning of the mucociliary clearance system it is of interest to study whether nanoparticles may alter mucus rheology. Here we present a case study of the effect of 200nm positively charged nanoparticles on cystic fibrosis sputum rheology and demonstrate nanoparticles are able to alter mucus rheology.

INTRODUCTION

The lungs can be divided in the respiratory zone, consisting of the alveoli where gas exchange takes place, and the conducting zone, consisting of the trachea, bronchi and bronchioles[1]. Apart from providing a low resistance pathway for airflow the conducting zone of the airways also plays a central role in maintaining the cleanliness of the lungs through the mucociliary clearance system. The epithelial surfaces of the airways are covered by cilia, which in turn are covered by a secreted mucus layer. Inhaled microbes and particulate matter are deposited on the mucus surface where they become entrapped in the sticky secretion. The coordinated beating of the cilia continually propels the mucus and the entrapped matter towards the pharynx, where it is ultimately swallowed, thus removing the entrapped matter from the lungs and maintaining the cleanliness of the gas exchange surface[1, 2].

For the mucociliary clearance system to function effectively the rheological properties of the mucus must be appropriate such that the transmission of force from the cilia tips to epithelial side of the mucus layer results in bulk transport of the entire mucus depth towards the pharynx as a cohesive mass[2, 3]. Failure to translocate the force from the epithelial to the luminal side of the mucus layer would result in failure to remove entrapped particulate matter from the lungs.

Environmental exposure to inhaled particles is not a new phenomenon, however the changing industrial and technological landscape has altered the volume and nature of the particulate matter we are exposed to with an increased proportion of particles in the nanoscale and a broader range of chemical compositions[4]. In addition to environmental exposure there is also significant interest in the potential of inhaled nanomedicines to address drug delivery challenges[5]. The fate of inhaled particles is dependent to a large extent on their size, they may be exhaled, or deposited in the mouth and pharynx, conducting airways or alveoli[4, 5]. Between 5 and 30% of inhaled nanoscale particles could be expected to be deposited in the mucus of the conducting airways[4]. On this basis it is of interest to investigate the effects of nanoparticles on mucus functional properties.

MATERIALS AND METHODS

A sample of previously frozen sputum from a cystic fibrosis (CF) patient was thawed and nanoparticles (200nm diameter, NH3+, Fluospheres© Life Technologies) were added to a concentration of 0.25 wt%. For control samples the same volume of MQ water was added.

Samples were subjected to rheological testing using a Kinexus Ultra+ rheometer. Tests included a frequency sweep 0.01-10 Hz at 1% strain, a relaxation spectrum at 5% strain and an amplitude sweep from 0.01-100% strain at 1Hz, which encompassed both small deformation and large deformation regime.

RESULTS AND DISCUSSION

The frequency sweep showed the control CF sputum behaved as a viscoelastic material with frequency dependent moduli and a phase angle that increased with decreasing frequency. This behaviour profile is likely to be linked to degradation of the macromolecular sputum components by for example DNAase or elastase[6].

Addition of 200nm positively charged nanoparticles (Fig. 1) resulted in a slight reduction in the elastic modulus, a meaningful reduction in the viscous modulus and an overall lowering of the phase angle with the introduction of a frequency independent plateau in the phase angle between about 0.5 and 5 Hz.

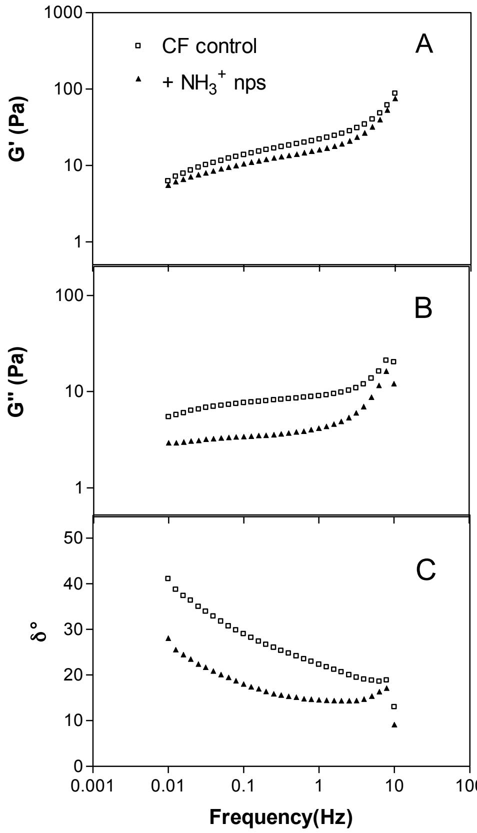

Figure 1. Frequency sweep showing the elastic modulus (A), viscous modulus (B) and phase angle (C) of control CF sputum (open squares) and 200nm NH_3^+ nanoparticle treated CF sputum (closed triangles).

As the amplitude of oscillation was increased the phase angle of the control CF sputum remained constant until a maximum strain in the oscillatory cycle of around 0.7% after which the phase angle rose steadily without evidence of dramatic gel failure. The addition of the test nanoparticles resulted in an increase in the strain at which the phase angle began to increase to around 3% (Fig. 2). As with the control the phase angle then rose steadily with no evidence of dramatic gel failure.

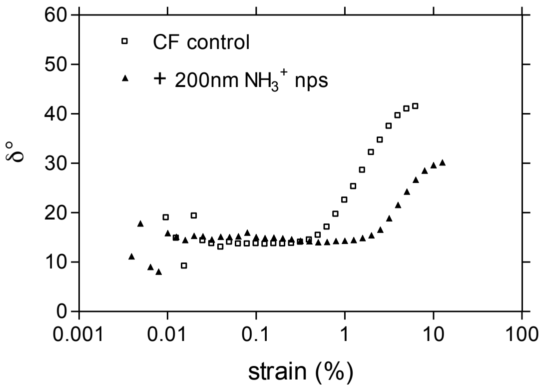

Figure 2. phase angle with increasing maximum strain in the oscillatory cycle for control CF sputum (open squares) and 200nm NH_3^+ nanoparticle treated CF sputum (closed triangles).

The relaxation spectrum (Fig.3) revealed a relatively rapid dissipation of stress within the control CF sputum with terminal decline at around 500 seconds. The addition of the test nanoparticles resulted in a substantially slower dissipation of stress (Fig. 3)

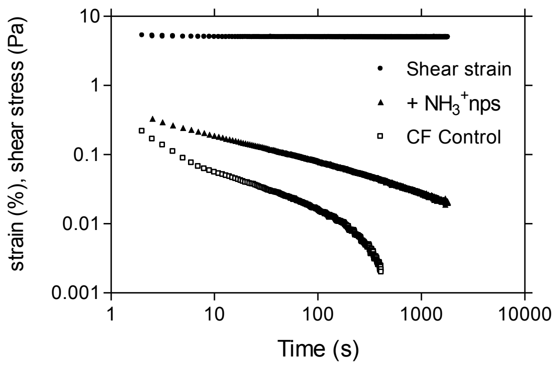

Figure 3. relaxation spectrum of control CF sputum (open squares) and 200nm NH_3^+ nanoparticle treated CF sputum (closed triangles).

Taken together these results suggest that in this particular sputum sample the addition of 200nm positively charged nanoparticles predominantly reduces the dissipation or loss of energy within the gel matrix and reduces the sputum's propensity to flow. Reduced dissipation of force is likely to be associated with transmission of the force applied to the cilia through the depth of the mucus gel and therefore better clearance of entrapped matter on the luminal surface of the mucus. However in interpreting these results it must be remembered that cystic fibrosis is a disease associated with significant mucus build up in the airways and CF sputum in general is not considered to have good rheological properties for mucociliary transport, and changes seen here may not reflect the physiological situation well. In is also worth noting that the properties of CF sputum vary considerably for example as a consequence of treatment regimes[6]. Additionally the functional consequences of nanoparticle deposition on airway mucus will also be heavily dependent on the mobility of those nanoparticles in airway mucus[7]. Poorly mobile nanoparticle will be concentrated in, and potentially alter the rheological properties of, the luminal surface of the mucus, whereas highly mobile particles, which readily diffuse in airway mucus may have the potential to alter mucus rheology through the entire depth of the mucus layer.

CONCLUSIONS

Inhaled nanoparticles can induce changes in the functional properties of airway mucus, which may have consequences for mucociliary clearance in the airways.

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