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Enrichment of immunoregulatory proteins in the biomolecular corona of nanoparticles within human respiratory tract lining fluid

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Abstract

When inhaled nanoparticles deposit in the lungs, they transit through respiratory tract lining fluid (RTLF) acquiring a biomolecular corona reflecting the interaction of the RTLF with the nanomaterial surface. Label-free snapshot proteomics was used to generate quantitative profiles of the proteins within the corona formed around silica (SiO_2) and poly(vinyl) acetate (PVAc) nanoparticles in RTLF, the latter employed as an archetype drug delivery vehicle. The evolved PVAc corona was significantly enriched compared to that observed on SiO_2 nanoparticles (698 vs. 429 proteins identified); however both coronas contained a substantial contribution from innate immunity proteins, including surfactant protein A, napsin A and complement (C1q and C3) proteins. Functional protein classification supports the hypothesis that corona formation in RTLF constitutes opsonisation, preparing particles for phagocytosis and clearance from the lungs. These data highlight how an understanding of the evolved corona is necessary for the design of inhaled nanomedicines with acceptable safety and tailored clearance profiles.

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Q4

Key words: Protein corona; Nanoparticle; Respiratory tract lining fluid; Plasma; Silica; Poly(vinyl) acetate; Proteomics

Abbreviations: NP, nanoparticles; RTLF, respiratory tract lining fluid; TEM, transmission electron microscopy; SiO_2, silica nanoparticle; PVAc, poly(vinyl) acetate nanoparticle; SP-A, surfactant protein A; SP-B, surfactant protein B; SP-D, surfactant protein D; DLS, dynamic light scattering; SEM, scanning electron microscopy; LC-MS/MS, liquid chromatography mass spectrometry; CC16, clara cell secretory protein 16; COPD, chronic obstructive pulmonary disease; BAL, bronchoalveolar lavage; NSpC, normalized spectral count.

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Background

The importance of the protein constituents of the biomolecular corona formed around nanoparticles in biofluids in determining particle–cell interactions was recently demonstrated convincingly,[1] but like much of the other published work in this area,[2,3] the corona examined was based on the incubation of nanoparticles with human plasma. To understand the interactions between nanoparticles and the lungs requires an appreciation of the precise form in which inhaled materials present to externally-facing respiratory cells. This is crucial for the design of optimised inhaled nanomedicines,[4] and also relevant for understanding effects on pharmaceutical nanocarrier biostability,[5] use for targeted drug delivery,[6] the drug release profile[7] and their clearance by macrophages.[8] Further, it is also important in determining how the lung responds to a variety of other inhaled nanomaterials, such as those derived from diesel-powered engines[9] or cigarette smoking.[10] If the many opportunities offered by inhaled nanomedicines are to be realised,[11-13] the biological and therapeutic implications of the first interactions of inhaled nanoparticles with the lungs must be appreciated.[14,15] Inhaled nanoparticle–biomolecule interactions occur during their transit through the thin aliphatic layer of respiratory tract lining fluid (RTLF) that coats the airways. As yet, however, the biomolecular corona formed in human RTLF has not been characterised at the molecular level.

To date, studies into the interactions of nanoparticles with biological fluids have focused on the protein corona formed when particles are in contact with plasma[16] or foetal bovine serum (FBS),[17] as well as on the kinetics of the protein corona formation.[18] These studies in plasma and FBS have shown that the acquisition of a plasma protein coating on particles significantly modifies surface properties, including charge, propensity to aggregate and hydrodynamic diameter, thus altering particle behaviour *in vivo*.[16,19] These non-respiratory studies indicate that the corona interface that develops forms the 'biological identity' of the particle, effectively determining downstream biological effects.[20] Examples of biological actions modified by the corona include decreasing the uptake of silica nanoparticles,[21] impairing the cell targeting capabilities of transferrin-functionalised silica nanoparticles,[22] and reducing the cytotoxicity of cationic nanoparticles.[23] The biological implications of the biomolecular corona formed around nanoparticles in plasma have been reviewed recently by Monopoli et al (2012).[3] The absence of similar work to investigate the corona formed around nanoparticles in human RTLF is a serious deficiency in the field, as the relevance of the corona formed in plasma to particle–lung interactions is highly questionable.[14]

Human RTLF differs substantially from human plasma, with the epithelial and immune cells of the airways significantly influencing its composition. With respect to inhaled nanoparticles, which deposit predominately in the lung periphery, the composition of RTLF in the alveolar region is most relevant. This surfactant-enriched compartment has significant contributions from plasma-derived proteins such as albumin and transferrin,[24] but also contains a number of lung-specific proteins with important roles in innate host defense, including surfactant proteins A and D (SP-A, SP-D), which promote the clearance of inhaled pathogens[25] from the lung. Apart from surfactant proteins, the RTLF contains other proteins, including apolipoprotein A-1, haptoglobin, uterglobin, alpha-1-antitrypsin and alpha-2-macroglobulin, which have been shown to play important roles in innate immunity.[26-30] Pioneering studies indicate that phospholipids within the RTLF adhere to the surface of inhaled particles, as demonstrated for $PM_{2.5}$ particles,[31] which aggregate following formation of a phospholipid corona.[32] This is consistent with stabilization of sedimenting metal oxide nanoparticles that has been reported in liposomal suspensions.[33] Thus, it appears that inhaled nanoparticles interact with proteins and phospholipids in human RTLF to acquire a biomolecular corona that can profoundly affect the fate of inhaled nanoparticles.[34] The aim of this study was to characterize the protein constituents of biomolecular corona that forms around nanoparticles in human RTLF.

Methods

Human respiratory tract lining fluid

Lavage was performed on healthy (n = 5, 26 ± 2 years, 2 M/3 F) subjects following an overnight fast as previously described.[35] Briefly, an initial bronchial wash was performed either in the right middle lobe or the left lingula lobe by the instillation and immediate aspiration of 2 × 20 mL of sterile saline (37 °C). These initial samples of the conducting airway RTLFs are not reported on in this paper, as the recovered volumes were insufficient for processing. Following the bronchial wash, a larger volume bronchoalveolar lavage (BAL, 3 × 60 mL) was performed at the same site. The aspirates recovered from each of the 60 mL instillations were collected and pooled into a siliconised container on ice. All lavage samples were filtered through nylon (pore diameter 100 μm) and centrifuged at 400 g for 15 min to isolate the cell free supernatant. The supernatants were then stored at −80 °C until required for further processing. Sample concentration, following sample thawing, was performed using 9 K MWCO iCON Pierce concentrators (Thermo Scientific), by centrifugation at 4,000 rpm at 4 °C, for 15 min cycles until complete. The filter retentate was resuspended in 1 mL HBSS and the protein concentration of the five individual concentrated BAL samples (reconstituted RTLF), as well as a pooled sample derived from equal volumes (200 μL) of the separate samples were determined using the bicinchoninic acid assay, as previously described.[35] The highly abundant plasma protein albumin was depleted from all the reconstituted RTLF samples, using SwellGel Blue Albumin Removal Discs (Pierce), according to the manufacturer's instructions to enhance the quantification of low abundance proteins using downstream proteomic approaches. It should be noted therefore that the concentrated BAL fluid is not a fully reconstituted RTLF because of the necessity to pre-deplete albumin. This approach is commonly applied in proteomic studies[36] and trades the underestimation of the 'true' contribution of albumin, and potentially the loss of constituents below the molecular weight cut-off, for the ability to quantify low abundance proteins. Informed written consent was obtained from all subjects prior to inclusion into this study,

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