

ENGINEERED NANOTHERAPEUTICS FOR PULMONARY AEROSOL DELIVERY

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Despite centuries of use and widespread application, aerosol delivery of therapeutics remains limited to a small subset of diseases and active pharmaceutical ingredients, mainly restricted to small molecule delivery for asthma management. Respiratory diseases which would benefit from direct and localized treatment span a much larger landscape; chronic obstructive pulmonary disease (COPD), lower respiratory infections, and lung cancers alone globally contribute 7.8 million annual deaths, with a reported 117 million pulmonary cases (~37% of population, 2012) and over \$88 billion in health care costs in the US[1, 2]. To expand the application of aerosol delivery, novel approaches are needed. To address this need, we have explored various applications of nanoparticle immune engineering for respiratory therapeutics[3]. Incorrect immune responses lie at the heart of most respiratory diseases and advances in these therapeutic areas requires consideration of the unique environment. Notably, the lung has an abundance of antigen presenting cells (APCs), such as macrophages and dendritic cells (DC), which phagocytose foreign materials at the air-lung interface. There are a number of lung-specific APC populations[4, 5]. Some subsets are well understood, however, other specialized subsets have only recently been identified due to historic challenges in differentiating these populations[6, 7]. Thus, there are many remaining questions as to the division of labor between these cells, their significance in different disease conditions, and their interactions with other adjacent cell populations at the mucosal interface[8]. Advancing this understanding is critical to develop new therapeutics; APCs are poised as the gatekeepers to lung regulation and lung DC-subset specifically are likely cellular targets for therapeutic intervention[9]. In order to better understand how these lung innate immune cells respond to inhaled particle therapeutics, we have developed sets of engineered particles with defined physical properties that originate at the molecular level. We have developed a series of metal organic framework (MOF) nanoparticle carriers with independently tunable particle size and internal porosity, enabling systematic investigation of the effect of particle pore structure on cellular interactions. These UIO-66 MOF derivatives have not only been optimized as pulmonary aerosol carriers but provide critical insight on the role of internal particle porosity following cellular internalization. To further modulate the lung immune environment and evaluate the role of ligand surface density on immune-modulation, we simultaneously developed a series of degradable polymeric nanoparticle carriers with controlled surface densities of two Toll-like receptor (TLR) ligands, lipopolysaccharide (LPS), corresponding to TLR-4, and CpG oligodeoxynucleotide, corresponding to TLR-9[10]. Our *in vitro* results with murine bone marrow derived macrophages and *in vivo* studies following a direct instillation to murine airways both support a trade-off between particle dosage and optimal surface density; proinflammatory cytokine production was driven by the distribution of the adjuvant dose to a maximal number of innate cells, whereas the upregulation of costimulatory molecules on individual cells required an optimal density of TLR ligand on the particle surface. Taken together, results from these two sets of particle types demonstrate that both particle porosity and ligand surface density are critical parameters for tight control of immune stimulation and association with lung APCs and provide a foundation to build pathogen mimicking particle (PMP) vaccines and immunostimulatory therapeutics.

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