

FORMULATION, CHARACTERIZATION, AND *IN VIVO* EVALUATION OF A FIRST-IN-KIND POLYMER LUNG SURFACTANT THERAPY

By

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The recent COVID-19 pandemic has emphasized the risk of respiratory infections leading to acute respiratory distress syndrome (ARDS). A significant factor contributing to poor ARDS outcomes is the impairment of lung surfactant due to infiltrating surface-active proteins and phospholipases during lung inflammation. Lung surfactant's vital role in stabilizing alveoli by reducing air-water interfacial tension becomes evident as its dysfunction severely compromises respiratory function. Although lung surfactant (LS) replacement therapy effectively addresses neonatal LS deficiencies, its efficacy in ARDS treatment for adults remains limited. The challenge lies in the chemical similarity between current animal-extracted surfactants and human lung surfactant which are both phospholipid-based. To address this, this dissertation outlines a transformative "polymer lung surfactant (PLS)" designed to overcome the limitations of conventional exogenous surfactants in treating ARDS.

Firstly, a formulation method, referred to as equilibration-nanoprecipitation (ENP), is established which achieves reproducibility, controls sizing, and limits dispersity of the PLS formulation consisting of block copolymer (BCP) kinetically "frozen" micelles/nanoparticles suspended in water. The method uses a two-step approach of 1) equilibrating the BCP nanoparticles in a water/co-solvent mixture and 2) removing co-solvent using dialysis against a large water reservoir. Comparison of ENP with a conventional solvent-exchange technique through experimental and computational analysis yields further insights into ENP's advantages.

Next, various studies are highlighted which provide fundamental characterizations of the air-water surface behavior and physical properties of BCP nanoparticles in water. The air-water surface properties of block copolymers have been studied extensively when spread as free chains in organic solvent; however, little was previously known about air-water interfacial behavior of water-spread polymer nanoparticles. The studies address such topics as the effect of nanoparticle size, effect of nanoparticle core chemistry, and the effect of temperature on surface-mechanical behavior. Insights into nanoparticle molecular structure at the interface are provided through X-ray reflectivity and grazing incidence X-ray diffraction. The effect of temperature is further

characterized by developing novel NMR and Langmuir trough methods to determine the physical state (glassy vs rubbery) of the core domain in the nanoconfined state at temperatures above and below physiologic temperature.

Lastly, *in vivo* studies are presented which demonstrate the detailed and promising proof-of-concept results on the efficacy of the PLS technology in mouse models of lung injury. The PLS therapy not only improves biomechanical function of the lung, but it also significantly lowers the extent of lung injury as shown by histological analysis and inflammatory marker measurements. An additional *in vivo* study is presented which highlights challenges in the delivery of the liquid PLS suspension to the lungs. The *in vivo* studies ultimately provide solid motivation for continued research into the development of the PLS therapy.

Given the promising the potential of the PLS technology shown in the *in vivo* studies, the materials characterizations shared in this presentation offer valuable insights into the design of a novel PLS therapy. From these insights, key design parameters such as nanoparticle size characteristics, core chemistry, and core molecular weight can be chosen to produce the most desirable material properties. Overall, this dissertation furthers the progress of PLS therapeutic development and will hopefully ultimately contribute to improved health outcomes in patients suffering from ARDS.