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# Engineering Preclinical Tools and Therapeutics to Understand and Treat COVID-19

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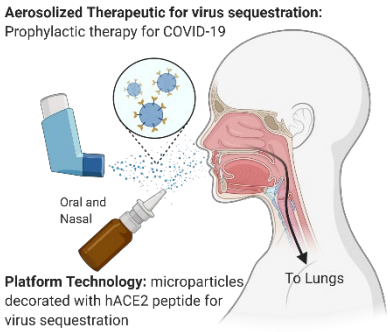
## Introduction

The novel respiratory coronavirus SARS-CoV-2, which causes the disease COVID-19, has caused a significant impact on humanity worldwide.[1] Among the many challenges to containing SARS-CoV-2 has been its high degree of spread by asymptomatic individuals and the lack of effective therapeutics capable of either preventing viral transmission or halting adverse host immune responses.[1,2] Indeed, many initial therapeutic candidates have been found to be ineffective at fighting COVID-19,[3] with only recently a report of dexamethasone demonstrating some therapeutic efficacy. To combat the lack of treatment options, our team at the University of Delaware is actively working to test a novel prophylactic, post-exposure approach as a platform inhalable technology for fighting inhaled respiratory pathogens (see Figure 1).

Figure 1. Microparticles decorated with ACE2 peptide can be delivered via oral or nasal inhalation to at-risk populations.




## Designing Inhalable Therapies as a Prophylactic Platform Technology for COVID-19 and Future Respiratory Pathogens

SARS-CoV-2 enters vulnerable cells by binding to angiotensin converting enzyme (ACE) 2 protein via spike protein on the surface of the virus.[4,5] Recent studies have implicated cells within the nasal passageway as the first site of viral entry, which gradually descends through the lung as the infection progresses.[6] To ultimately clear the virus, the adaptive immune system responds by producing neutralizing antibodies to the virus to block viral function.[7] Many of these antibodies, isolated from clinical samples, are specific against the viral spike protein, further showing that prevention of the initial binding interaction between the virus and the host ACE2 is an important step in halting the viral life cycle.[7] However, host antibodies that neutralize the virus requires anywhere from 14-30 days from the initial time of infection to be generated by the adaptive immune system.[1,8,9] This lag time from infection to neutralization allows SARS-CoV-2 with ample opportunity to continue replicating, spread to other organ systems, dysregulate the host innate immune system, and wreak havoc on the body.[9]

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A prophylactic treatment that stops viral particles from entering cells, thus mimicking the function of neutralizing antibodies produced later in the infection, would be useful in combatting COVID-19. Applied early in the infection, immediately after known exposure, or as a prophylactic treatment to high risk populations life front line healthcare workers, this strategy could act at the site of infection and be applied via nasal spray or inhaler (Figure 1). An effective post-exposure therapy would not only lessen the viral load and asymptomatic spread, but also reduce the burden of infection. SARS-CoV-2 is particularly infectious, with reports of infected people displaying no or only mild symptoms, resulting in high degree of asymptomatic spread that is further exacerbated by limited testing. So far, no post-exposure treatment regimens have proven effective in preventing the ultimate disease onset.

Delivering ACE2 to an infected individual, thus acting as a decoy for SARS-CoV-2 binding, has been suggested as a possible treatment for COVID-19. Soluble ACE2 has been shown to reduce SARS-CoV-1 and CoV-2 transduction efficiency *in vitro,* and two ongoing phase I/II clinical trials are underway to treat acute respiratory distress syndrome and COVID-19 (NCT04335136) with recombinant ACE2.[10–12] However, these clinical trials both use an intravenous route of administration as opposed to an inhalation route. Since the virus is primarily transmitted through inhalation and initial viral shedding from infected cells occurs through the epithelial apical surface into the airway lumen,[4,9] a treatment that can sequester virus from the pulmonary airspace would be able to directly limit the viral load at the primary site of infection. While this could be achieved by inhaled therapeutics, pulmonary delivery is accompanied by many delivery challenges. The complex lung mucosal microenvironment and airway anatomy, combined with low-efficiency inhalers and heterogeneity in aerosol formulations, has limited commercial inhaled therapies to a few small molecule drugs.[13–16] Significant innovation in both the formulation of inhaled therapeutic as well as the preclinical testing paradigm are needed to successfully create new inhaled medicines.

Our current research efforts are focused on designing an inhalable microparticle to deliver SARS-CoV-2 binding domains capable of sequestering virus at the site of infection (see Figure 2). The microparticles are comprised of poly(ethylene glycol) diacrylate [PEGDA], an inert material that has been previously studied in our group and shown to have high biocompatibility in the lung with tunable degradation profiles.[17–19] By modulating the base particle formulation and the attachment of SARS-CoV-2 binding fragments that mimic ACE2, we can modulate where the particle will deposit in the lung, how much virus will be sequestered, and how the medicine will be cleared from the lung. Aerosols with aerodynamic diameters around 10 μm are known to deposit in the upper respiratory tract (mouth, pharynx and trachea) and aerosols approximately 5 μm in diameter deposit efficiently in the conducting airways.[20] Our synthetic approach allows us to precisely modulate the amount of ACE binding peptide to control how well the microparticle can entrap the virus and also the degradation rate in the lung. While research is actively on-going, our overall goal is to demonstrate this approach can actively sequester free virus in the lung airspace, decrease viral load, and maintain a robust safety profile.

Figure 2. Microparticles comprised of PEGDA will block SARS-CoV-2 binding to host cells in an analogous manner to neutralizing antibodies.

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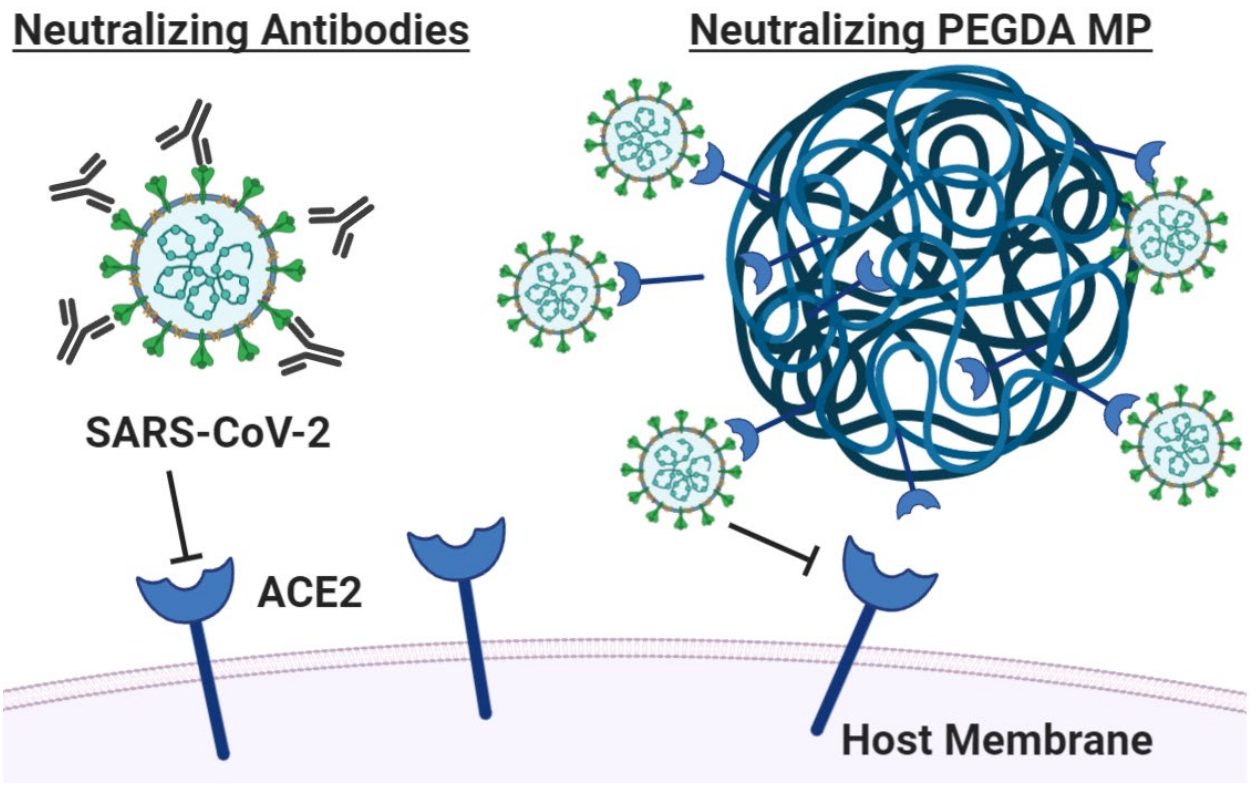


Initially designed for the current SARS-CoV-2 pandemic, we believe this can be considered a platform technology to combat a wide range of current and future respiratory pathogens. Viral infections and lower respiratory diseases continue to represent a significant global health care burden, responsible for over two million global deaths annually.[21] Development of safe and effective post-exposure and prophylactic treatments remain a high priority. Our inhalable PEGDA microparticle platform (Figure 2) is highly modular, with a plug-and-play approach for the specific binding sequence. By simply replacing the ACE2 binding peptide with a binding domain that is specific for a different pathogen, we can create a whole new line of inhalable treatments with similar method of preventing infection through viral decoys. By pursuing development of this approach for the current COVID19 pandemic, we aim to create a highly modular platform that can be rapidly deployed in future outbreaks to prevent the occurrence of any subsequent viral airborne pandemics.

## Lab-scale models of the lung for advanced testing

One of the critical aspects in the development of inhalable therapeutics is the ability to test their efficacy in relevant preclinical environments. Unfortunately, current ability to predict how well an inhaled therapy will work in humans is quite limited. Despite understanding that efficacy of inhaled medications depends on many factors ranging from device used, airway dynamics, disease state, and local microenvironment,[22,23] there are currently no preclinical tools or models capable of assessing these *a priori*, leading to generally poor *in vitro* / *in vivo* correlations (IVIVC) of inhaled therapeutics.[15] Current preclinical assays, whether experimental or computational, are stationary approaches that fail to incorporate aspects of breathing or the lung microenvironment to assess drug efficacy following deposition. Accordingly, the current paradigm of preclinical aerosol testing is *only* capable of roughly estimating deposition location

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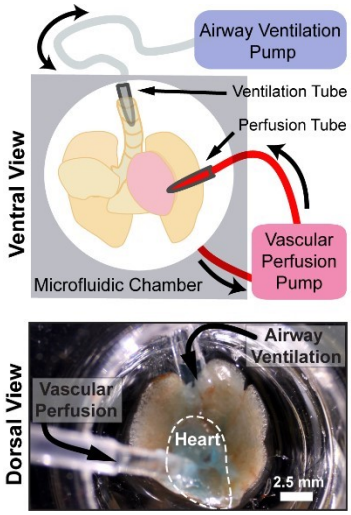
and is permanently decoupled from downstream biological response. Because of this, accurate dosing needs are poorly realized.[24]

In collaboration, our two labs have contributed significantly in this area to develop models that can represent the complete structure of the human lung. Here, we describe our on-going engineering efforts to validate new preclinical models capable of effectively evaluating our proposed inhaled PEGDA microparticle treatment.

## Gleghorn Lab: Murine *Ex vivo* Lung Explants.

The Gleghorn lab has recently demonstrated the first viable *ex vivo* culture heart-lung *en bloc* for neonatal mice (see Figure 3). By culturing *ex vivo*, this model allows for a duration and level of accessibility impractical with existing *in vivo* models and tunable breathing control. Through active perfusion of the vasculature, this system allows for tissue nutrient delivery and waste removal, while providing critical mechanical cues to the pulmonary vasculature. This is in contrast to bath culture methods that avoid necrosis by cutting the lung into blocks, preventing studies of organ-scale structure and biology. Our model allows for separate control of the mechanics acting on airway epithelium and pulmonary vascular endothelium by independently regulating ventilation parameters and vascular perfusion. This separation can be used to decouple signaling factors that are induced by different mechanical disruptions that are often clinically co-incident; *e.g.*, the effects of mechanical ventilation and pulmonary hypertension. We accomplish this control through airway intubation through the trachea and perfusion via the heart. This allows for tunable tidal volumes, breathing rates, and vascular perfusion. Using this approach, we can administer inhaled formulations directly to the airspace under highly modular and well controlled breathing conditions. Furthermore, this approach enables us to decouple the response of the lung tissue from any recruited cell components and identify new mechanisms in COVID-19 host response.

Figure 3. Ex vivo whole lung culture platform.




## Fromen Lab: Dynamic Lung Models.

The Fromen lab has developed the first breathing whole lung model to assess regional aerosol deposition in varying states of health and disease (Figure 4). Our approach leverages advances in

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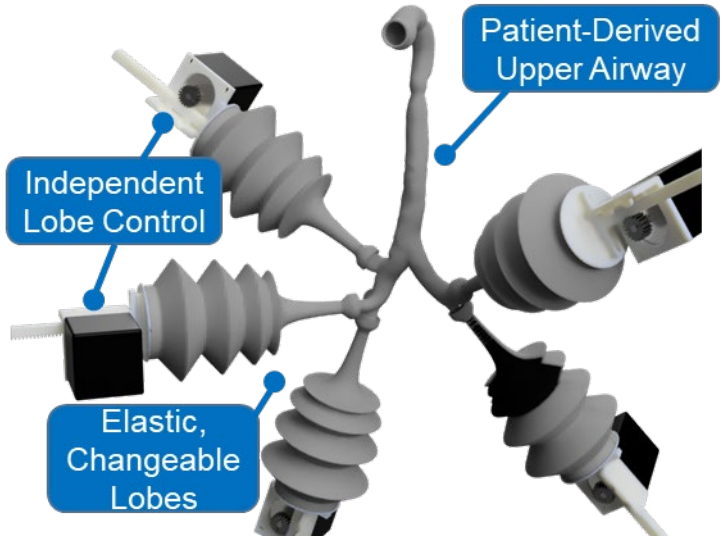
additive manufacturing and consists of a patient-derived upper airway connected to anatomically-scaled deformable lobe units. Each lobe is independently actuated by mechanical deformation for a tunable, realistic breathing profile, with an overall breathing capability matching human tidal and forced breathing metrics, as well as spatial aerosol deposition. The full range of human airways are approximated based on Weibel's Model A geometry,[25] mimicking the cross-sectional area and total volume for each airway generation in a series of frustums (Figure 4). This first-in-kind approximation resembles a cone or bell and recreates the increasing volume available with increasing lung generation. Internal structures within the lobe enable collection of spatially distributed aerosols to mimic human deposition during breathing maneuvers. Lobes are connected to a microcontroller-driven motor that generates airflow by expansion/contraction. Not only does this approach provide advantages by approximating the *full* range of human airways (volume, surface area) and dynamic breathing profiles, but it also boasts ease of fabrication, with entirely interchangeable parts for disease and patient-specific modeling. This device is the first model that incorporates realistic breathing capability to enable lobe- and generation- level deposition measurements. Using this approach, we can develop personalized formulations and/or inhaler devices that will deliver optimized doses of inhaled therapeutics, as well as assess the efficacy of new therapies, such as our prophylactic PEGDA microparticle treatment.

Figure 4. 3D-printed breathing lung model design and features.




## On-going bioengineering opportunities

Given the tremendous global challenge of SARS-CoV-2, novel treatment paradigms are desperately needed. Challenges in developing effective treatments for respiratory infection fall hand-in-hand with an on-going need for new preclinical assays that allow for rapid screening and successful clinical translation. Bioengineers have a considerable role to play in tackling these needs and others that have surfaced during the COVID-19 pandemic. As reported by our team in a recent report [CMBE review], on-going bioengineering efforts include the application of new preclinical tools toward the study of host immune response, viral transmission and replication, renin angiotensin system contributions, host factors and exacerbates, and drug transport in the lung. Similarly, there are many on-going bioengineering efforts to design new therapeutics

capable of modulating viral dynamics, host immune response, and vaccine delivery. Together, the contribution of bioengineers in this space is significant in providing tools to learn new aspects of COVID-19 disease progression and in developing novel approaches towards the mitigation and elimination of the disease. Our on-going efforts of the Gleghorn and Fromen labs at the University of Delaware are creating platform technologies for preclinical respiratory assessment and prophylactic post-exposure anti-viral treatment that will be essential components of combatting COVID-19 and future airborne respiratory pathogens.

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