# Case Study:
## Revolutionizing Viral Infectious Disease Research and Drug Discovery with Organ-on-a-Chip Technology

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Despite decades of therapeutic progress, respiratory viruses continue to pose a significant threat to global health, causing ~3 million deaths annually[1] in addition to the 7 million reported deaths from the COVID-19 pandemic[2]. All too often, the 2D cell culture and animal models traditionally used to research these diseases fall short in replicating disease pathology and therapeutic effect, leading to a continued health burden. Fortunately, Organ-on-a-Chip technology presents a path forward, allowing researchers to model the complexity of human biology and disease as well as explore the underlying pathophysiology to counteract the threat of viral infectious diseases.

This case study explores three examples of how the Wyss Institute at Harvard University is using Organ-Chips to rapidly identify promising treatments, investigate how viruses evolve through human-to-human transmission, and study the role of mechanical forces on the innate immune response to viral infection.

## Rapid Drug Repurposing with Organ-Chips

### Background

The COVID-19 pandemic triggered a race to discover effective therapeutics for preventing or treating infection. Unfortunately, traditional static cell culture often falls short in modeling infection due to poor differentiation, a lack of cellular complexity, and a static microenvironment. To overcome this challenge, researchers leveraged Organ-on-a-Chip technology, which allows for highly differentiated tissue structures, circulating immune cell response, and epithelial-endothelial crosstalk—all critical features that affect human response to viral infection.

### Objective

The Wyss Institute aimed to utilize Organ-on-a-Chip technology to rapidly evaluate existing drugs for protection against SARS-CoV-2. The goal was to streamline the drug testing process, ensuring safety and efficiency.

### Methodology

In this study, the Wyss Institute used the Airway Lung-Chip, which incorporates a co-culture of primary human lung epithelial and endothelial cells in a dynamic microenvironment and enables cells to be cultured under air-liquid interface (ALI). Inside the Airway Lung-Chip, lung epithelial cells experience higher expression of the ACE2 receptor, which SARS-CoV-2 uses to infect cells. By administering SARS-CoV-2 pseudoparticles (SARS-CoV-2pp) in the model's air channel, the team tested eight drugs that had previously shown activity against related viruses in conventional *in vitro* and *in vivo* models.

## Results

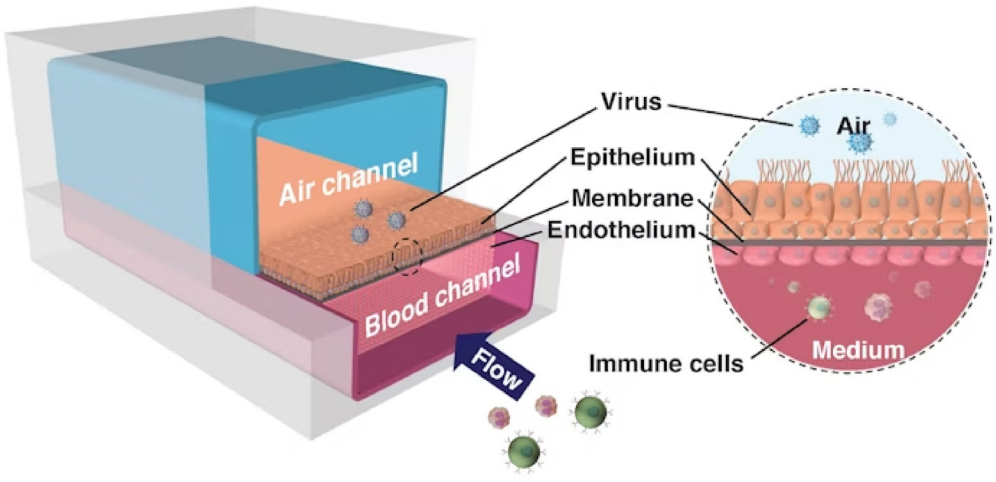
Drug testing in an established conventional cell culture model (Huh-7 cells in a 96-well-plate format) showed that all eight drugs exhibited inhibition of SARS-Cov-2pp infection in a dose-dependent manner. However, studies in the Airway Lung-Chip showed that five of the drugs, including widely discussed candidates like hydroxychloroquine, were not effective in this more human-relevant model. Three of these five drugs (hydroxychloroquine, chloroquine, and arbidol) also failed to show positive results in human clinical trials, indicating the strong clinical translatability of the Organ-Chip results.

Only three candidates showed the potential to reduce viral infection, with the most effective candidate—the antimalarial drug amodiaquine—reducing infection by around 60%. Additional studies using the actual SARS-CoV-2 virus in cell culture (Vero 6, A549) and animal (hamster) models further demonstrated the drug's efficacy to both prevent and treat infection. Amodiaquine, which is inexpensive and readily available in Africa, was subsequently included in the ANTICOV clinical trial in over 13 countries across the continent.

## Impact

This case study demonstrates how the use of Organ-on-a-Chip technology in conjunction with additional cell culture and animal model assays shows strong potential for rapid drug repurposing. The collaborative effort with partnering organizations led by the Wyss Institute identified multiple potential treatments for viral infection in just three months. From initial tests in January 2020, the researchers quickly moved to publish their findings in a preprint that April, showcasing the rapid pace of research and validation, and not long after had them fully peer reviewed to published in *Nature Biomedical Engineering.*

By simulating human organ functional units more accurately than traditional cell cultures, Organ-Chips can streamline and improve the accuracy of drug testing. Unlike conventional cell models, Organ-Chips allow for a more complex and representative recapitulation of lung biology and function in longer-term culture, including highly differentiated structures, cellular crosstalk, immune response, and ALI. This technology holds promise for the scientific community as a valuable tool for confronting future pandemics. With the continued use of technologies like Organ-Chips, researchers are now better equipped to evaluate, validate, and repurpose drugs quickly and effectively, ultimately benefiting global public health.




**Schematic of the viral and immune cell administration in the Airway Lung-Chip.**

Credit: Wyss Institute at Harvard University.

# Modeling Viral Evolution with the Airway Lung-Chip

## Background

Each year, the influenza virus causes a major health burden, with 9–41 million illnesses, 100–710 thousand hospitalizations, and 5–52 thousand deaths in the United States alone[3]. The reason influenza is so difficult for researchers to control is that it rapidly spreads from human to human and evolves constantly, causing new strains to emerge that reduce the effect of existing vaccines and treatments. Conventional cell culture approaches and often-used ferret animals fail to accurately model viral evolution, as they lack the host innate immune response which plays a critical role in host selection pressure *in vivo*, resulting in viral mutations that do not reflect what is seen in patients. To better understand this process, more human-relevant models of viral evolution are needed.

## Objective

Wyss Institute researchers aimed to utilize Organ-Chips to study how influenza evolves through human-to-human transmission in the presence of antiviral treatment. The primary goal was to obtain insights that might assist in predicting viral mutations and guide the development of more effective vaccines and treatments.

## Methodology

Like in the SARS-CoV-2 case study, researchers used the Airway Lung-Chip, which incorporates primary human lung airway epithelial cells in the top (airway) channel and human pulmonary microvascular endothelial cells in the bottom (vascular) channel. The two channels are separated by a porous membrane that allows for epithelial-endothelial crosstalk. Shear stress inside the Organ-Chip model promotes physiologically relevant mucociliary behavior, which could impact the entry and clearance of infectious viruses.

Researchers introduced influenza A virus into the airway channel of the Organ-Chip as well as antiviral drugs (amantadine, oseltamivir, nafamostat) into the vascular channel or airway channel according to their route of administration in patients via injection, digestion, or inhalation. The infected mucus droplets were then transferred to another Airway Lung-Chip, which was again co-treated with antiviral drugs. By repeating this process multiple times across Organ-Chips with five different epithelial cell donors, researchers were able to model human-to-human transmission.

## Results

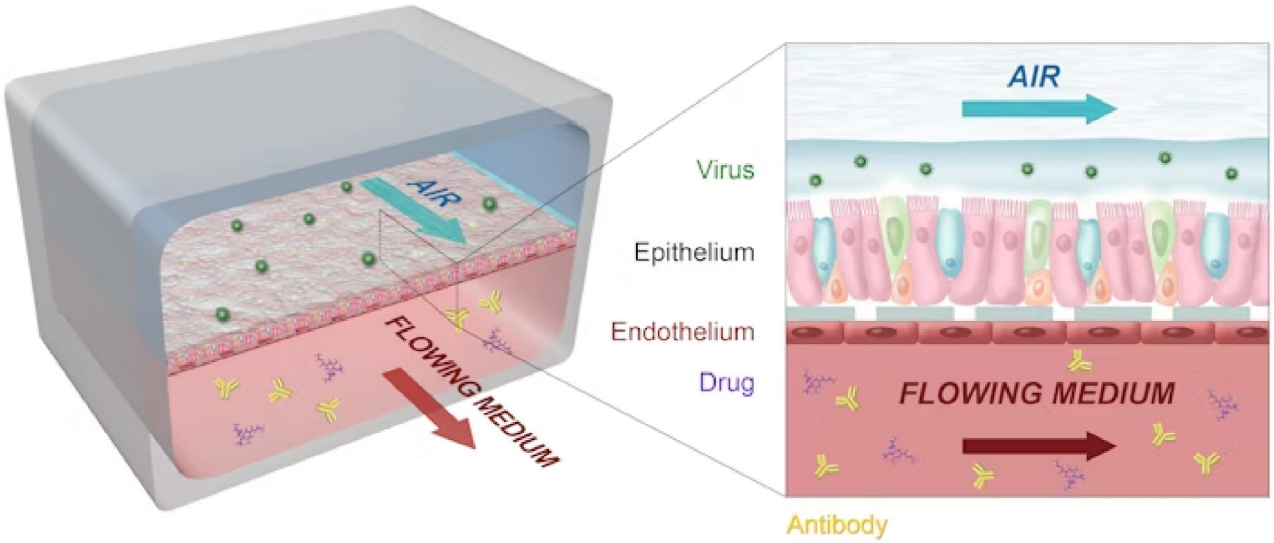
By sequentially infecting multiple Airway Lung-Chips with influenza-A-infected mucus droplets in the presence of antiviral drugs, researchers were able to successfully model spontaneous viral evolution. Importantly, these mutations included not only clinically prevalent mutations, but also some that had not previously been documented in patients.

While it took over 25 chip-to-chip transmissions for the influenza virus to become resistant to oseltamivir, it took just eight transmissions to develop significant resistance to amantadine. This difference in resistance aligns with what is seen in the human population, where influenza resistance to oseltamivir is relatively rare and resistance to amantadine is common. Meanwhile, nafamostat did not induce resistance even after 30 chip-to-chip transmissions, potentially because the drug targets the host cell response as opposed to molecular targets on the virus itself.

## Impact

This study by the Wyss Institute shows how the Airway Lung-Chip can be used to model viral evolution and the emergence of clinically relevant treatment-resistant mutations. Because Organ-Chips can recapitulate the host innate immune response seen in humans, viruses undergo host selection pressure that aligns with what is seen in patients, sharply contrasting with what is seen in conventional cell culture models.

Through broader adoption of these techniques, scientists may be able to identify treatment-resistant strains *before* they emerge in the human population and proactively develop improved vaccines and treatments.




**Schematic of viral and drug administration in the Airway Lung-Chip.**

Credit: Wyss Institute at Harvard University.

# Investigating the Role of Breathing in Viral Infection

## Background

With each breath taken, cells in the alveolus are exposed to mechanical forces that play a fundamental role in human health and disease. However, the impact of these forces on the innate immune response is poorly understood, as conventional *in vitro* models are static and incapable of recreating them. Thankfully, Organ-on-a-Chip technology, which can apply mechanical strain, allows researchers to study the role of these forces to uncover new insights into mechanisms of disease as well as the underlying biology.

## Objective

Researchers aimed to harness Organ-on-a-Chip technology to delve into the lung's immune response to viral threats, focusing on the mechanics of breathing as a potential catalyst for innate immune defenses. The project sought to uncover how mechanical stretching, akin to breathing, could influence viral replication and immune responses, with an eye towards identifying novel therapeutic targets and drugs for treating viral and inflammatory lung diseases.

## Methodology

In this study, the researchers used the Alveolus Lung-Chip, co-culturing primary human alveolar epithelial cells and endothelial cells to recreate the alveolar-capillary interface. Inside the chip microenvironment, alveolar cells form tight junctions with low permeability, alveolar type I and type II differentiation, and increased expression of ion channels, transporters, and defense responses.

The team infected the Alveolus Lung-Chip with H3N2 influenza virus in the presence or absence of cyclic stretch to investigate the effect of breathing-like motions on viral infection and the inflammatory response.

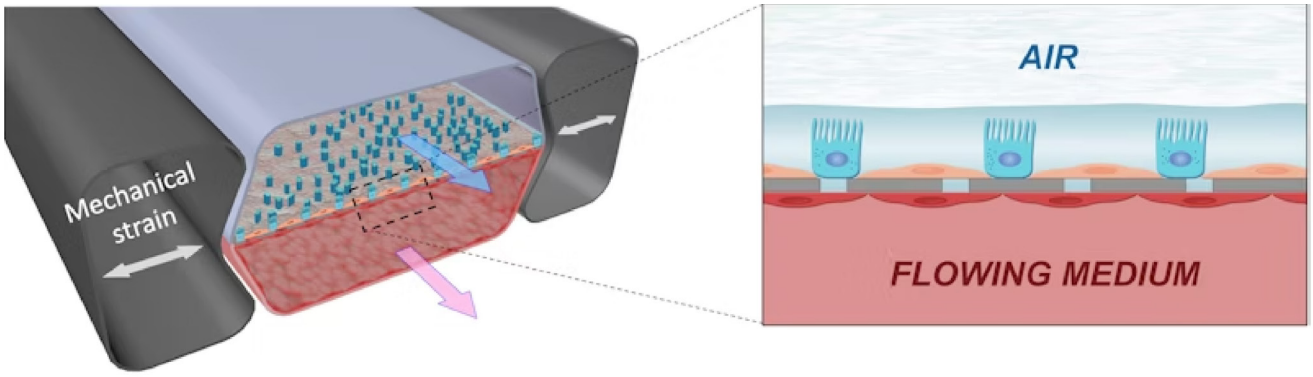
## Results

With each breath taken, alveolar cells are exposed to mechanical forces that play a fundamental role in human health and disease. However, the impact of these forces on the innate immune response is poorly understood, as conventional cell models are static and cannot recreate them. Thankfully, Organ-on-a-Chip technology can apply mechanical strain, allowing researchers to study the role of these forces to uncover new insights into mechanisms of disease as well as the underlying biology.

The study showed that the mechanical force of breathing-like stretch initiated a protective immune response that significantly suppressed viral infection, with qPCR analysis showing a reduction in viral RNA in the epithelium by 50% in the stretch versus non-stretch groups. Analysis of differentially expressed genes demonstrated that pathways related to the host defense response were activated by cyclic stretch. Additionally, endothelial channel effluent analysis showed an increase in inflammatory cytokines in the stretch group, further supporting a mechanically stimulated immune response. Based in part on data from these Organ-Chip studies, azeliragon was then licensed to Cantex Pharmaceuticals for the treatment of inflammatory lung diseases, including COVID-19.

### Impact

This research not only underscored the critical role of breathing in the innate immune response in the lung, but also led to the discovery of a promising treatment for respiratory inflammatory diseases that was quickly translated from research to commercialization—with less than one year from preprint publication to commercial licensing. These results show how Organ-Chips can enable human-relevant insights into viral infection and therapeutic efficacy due to the technology's ability to emulate the dynamic microenvironment and mechanical forces seen in the human body.




**Schematic of the Alveolus Lung-Chip, showing administration of cyclic strain to model the mechanical forces of breathing.**

Credit: Wyss Institute at Harvard University.

## Conclusion

Organ-on-a-Chip technology holds immense promise in the realm of infectious disease research. Whether it is used to rapidly identify potential drug treatments for emergent threats like SARS-CoV-2, model the evolution of viral threats, or discover the impact of mechanical forces on innate immune response, this technology provides a revolutionary tool for the scientific community. As these case studies indicate, adopting such advanced methods can significantly improve our understanding of human biology and make a tangible difference in the fight against infectious disease.

***Discover how Organ-on-a-Chip technology can help advance your research at emulatebio.com***

#### References

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