Examples of Human Relevant Technology in the Battle Against COVID-19

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Human Tissues, organs-on-a-chip, organoids, immune models are being used in multiple scenarios for COVID-19 research. According to the World Health Organization, "The ideal animal model for studying routes of virus transmission, pathogenesis, antiviral therapy, vaccine and immune responses has yet to be found. "[1]

In fact, a recent review of newly created animal models for COVID-19 suggests a wide gap between COVID-19 in humans and animal models. No severe illness associated with mortality was observed in review of peer-reviewed and pre-print research publications. Nonhuman primates, mice, ferrets, hamsters and cats were included in the research.[2]

Animal tests for possible human therapies and vaccines may be hindering, not helping the effort. A growing number of scientists believe that accelerated COVID-19 research is "exposing animal modeling for what many have long claimed it to be: a scientific anachronism."[3] Looking to alternatives to animal testing to accelerate therapeutics can save millions from sickness and death, while saving millions of animals who are the unwilling victims of archaic science.

- A Harvard Wyss Institute-led collaboration used the Institute's organ-on-a-chip (Organ Chip) technology to identify the antimalarial drug amodiaquine as a potent inhibitor of infection with SARS-CoV-2, the virus that causes COVID-19. The Organ Chip-based drug testing streamlines the process of evaluating the safety and efficacy of existing drugs for new medical applications, and provides a proof-of-concept for the use of Organ Chips to rapidly repurpose existing drugs for new medical applications, including future pandemics.[4]
- The NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and collaborators have organized a working group, the MPS for COVID Research (MPSCoRe) working group, that will coordinate the use of MPS to reduce animal use in studies of COVID-19 and future emerging infectious diseases.[5]
- The US FDA and Emulate, Inc. are collaborating to apply lung-chip technology to evaluate the safety of COVID-19 vaccines and protective immunity against SARS-CoV-2.[6]
- The Chemical Biological Center at the U.S. Army Combat Capabilities Development Command (CCDC) is working to better understand how COVID-19 attacks lung cells using the Emulate Alveolus Lung-Chip that recreates human biology systems. Emulate has been working on microphysiological systems for nearly a decade and now the US Defense Threat Reduction Agency (DTRA) has provided funding through the FY20 Coronovirus Aid, Relief, and Economic Security (CARES) Act. "In the past, the closest researchers could get to something like this was by introducing a virus into animals and then dissect them. With this, there is no need for animals in performing toxicological research, " according to Dan Angelini, PhD, a Center research biologist.[7]

- The University of Toronto is using organ-on-a-chip technology to discover the details for how COVID-19 invades the human body.[8]
- Lung Models - respiratory 3-D models derived from human cells and differentiated into lung epithelial cells present a promising platform for studying COVID-19 pathogenesis and host-virus interactions.
- Harvard Wyss is working to identify FDA approved drugs and novel compounds to be tested using Organ-on-a-Chip technology for the COVID-19 therapeutic repurposing pipeline.[9] Wyss has signed a one-year agreement worth up to $16 million with the US Defense Advanced Research Projects Agency (DARPA) to identify and test FDA-approved drugs that could be repurposed to prevent or treat COVID-19. [10]
- Epithelix is evaluating antiviral drugs or therapeutic strategies against COVID-19 based on fully differentiated *in vitro* 3D human airway epithelia.[11]
- Draper and University of Massachusetts Medical School uses organ-on-a-chip lung models that can be configured into existing industry standard multiwell cell culture plated systems. This airway model is used to evaluate influenza, coronavirus, or other respiratory viruses in vitro. This new capability has the potential to address a gap in the rapid assessment of therapeutic efficacy of various small molecules and antiviral agents against influenza and coronaviruses.[12]
- Using organoids - researchers have shown that human recombinant ACE2 is able to block the infection of cells by SARS-CoV-2. These same researchers also show that vascular and kidney organoids can be infected by SARS-CoV-2 and that human ACE2 blocks this infection. The next stages of testing for these proteins would be lung organoid studies.[13]
- The New York Stem Cell Foundation is leveraging the power of human stem cells as disease models to help better understand COVID-19 and find urgently needed treatments, in collaboration with top scientists worldwide.[14]
- The Human Cells Atlas project, including hundreds of scientists world wide used information from more than 4 million human cells from people of many different ages, to identify places COVID-19, may invade the body. Their findings suggest that the virus may be able to enter more kinds of cells than previously thought. The work also offers hints about two central questions: How the virus can harm so many different organs and why some people are more vulnerable to infection. The team posted a preliminary analysis on bioRxiv on April 20, 2020.[15]
- Viscient Biosciences uses 3D bioprinting technology to create lung tissue to support viral infectivity research and search for an effective therapy against SARS-CoV-2. Using the paradigm developed for liver as well as previous work in lung tissue, 3D bioprinted and other 3D tissue models made with lung cells, including a patient's own cells, are expected to be used as a "clinical trial in a dish," helping test potential COVID-19 therapies quickly and with highly accurate biology. [16]
- Weill Cornell Medicine, is testing drugs on the human cell models susceptible to SARS-CoV-2 infection to see which drugs may help combat the disease.[17]
- An in vitro model, termed "MIMIC" (Modular Immune In vitro Construct), was designed and developed to reflect the human immune system. The MIMIC System is a laboratory-based methodology that replicates the human immune system response. It is highly

automated, and can be used to simulate a clinical trial for a diverse population, without putting human subjects at risk. The MIMIC System uses the circulating immune cells of individual donors to recapitulate each individual human immune response by maintaining the autonomy of the donor. Thus, an in vitro test system has been created that is functionally equivalent to the donor's own immune system and is designed to respond in a similar manner to the in vivo response.[18]

- YUMAB, a German antibody development company, generated and characterized the first fully human antibodies with receptor blocking activity against the new coronavirus strain SARS-Co-V2 in collaboration with Boehringer Ingelheim by applying phage display technology. A human antibody discovery platform was used to identify a set of novel fully human antibody candidates in less than four weeks. The antibodies bind to a surface protein of SARS-CoV-2 including candidates that inhibit the interaction with the host cell receptor, thereby potentially blocking the virus from infection.*[19]*
- EURL ECVAM, the European government organization recommends against using animals to produce antibodies. Animal antibodies are a leading cause of the reproducibility problems in research.[20] Animal-free recombinant antibodies are being used to develop life-saving vaccines and treatments.
- Institute for In Vitro Sciences has test systems and assays to detect adverse effects of pharmaceuticals targeting COVID-19. The Respiratory Toxicology Program uses 3D pulmonary models and human 2D primary cells and lines allow the detection of acute toxic responses such as oxidative stress, cytotoxicity, and inflammation (among others), as well as long term chronic exposure responses not possible using other models.[21]
- MatTek's EpiAirway, human bronchial tissue model was used to study remdesivir. The study showed that remdesivir inhibits SARS-CoV-2 in human lung cells.[22]
- Reconstituted human airway epithelial models of nasal or bronchial origin were used to characterize viral infection kinetics, tissue-level remodeling of the cellular ultrastructure and transcriptional immune signatures induced by SARS-CoV-2. The results underline the relevance of this model for the preclinical evaluation of antiviral candidates. This model was used provide evidence on the antiviral efficacy of remdesivir and the therapeutic potential of the remdesivir-diltiazem combination as a rapidly available option to respond to the current unmet medical need imposed by COVID-19. [23]
- MatTek's EpiAirway, EpiOral and EpiIntestinal models were used to screen cannabis sativa extracts and identified indentified CBD statife extracts modulate ACE2 gene expression and ACE2 protein levels, a critical protein required for SARS-CoV-2 entry into host cells.[24]
- Hesperos Human-on-a-Chip in vitro systems, using the immune-system-on-a-chip is being used to uncover how severe acute respiratory syndrome SARS-CoV-2 directly affects multi-organ systems by activating the cytokine storm from inflammatory macrophages and to support the rapid development of therapeutics. As the global pandemic of COVID-19 continues to grow, this system has the potential to quickly evaluate antiviral and repurposed drugs to help combat this devastating disease.[25]
- Researchers from Hubrecht Institute and Maastrict University in the Netherlands used human intestinal organoids to demonstrate that SARS-CoV2 easily replicates in the gut

lining resulting in the production of large amounts of infective particles in the intestine. Many COVID-19 patients present with gastrointestinal symptoms.[26]

- COVID-19 patients have shown neurological symptoms, suggesting the virus is neurotropic under unknown circumstances. Researchers at Johns Hopkins University School of Medicine and School of Public Health used a human induced pluripotent stem cell (iPSC)-derived BrainSphere Model to demonstrate, for the first time, potentially critically important neurotropism of SARS-CoV-2.[27]
- COVID-19 patients have experienced heart damage due to the virus. Data regarding cardiac safety are urgently needed to provide better and safer treatments. The TechMed Centre, the MESA+ Institute of the University of Twente, and Leiden University Medical Center in the Netherlands are: using human pluripotent stem cell derived cardiac models for rapid evaluation of therapies; modifying heart models to become SARS-CoV-2 disease models to understand how the virus affects the heart.[28]
- Drug induced liver injury (DILI) is one of the leading causes of drug failures in clinical trials and is the most frequently citied reason for withdrawals of medications from the marketplace.[29],[30] Insphero's 3D liver organoids can test the safety of potential treatments. The 3D in vitro liver toxicology platform using primary human hepatocytes and non-parenchymal cells (which are important for capturing the immune response), mimics the human liver, making it more predictive of human response that animal tests. This human-relevant platform is especially useful for researching RNA-based vaccines for COVID-19. This technology is fast to ensure drug development programs move forward quickly.[31]
- The Human Emulation System comprised of organ-chips, hardware and apps, offers researchers a new standard for predicting human response to therapies and vaccines with greater precision and control than cell culture or animal-based test methods.[32]
- Novoheart has created a human ventricular cardiac organoid chamber that is being used by drug companies to test COVID-19 therapies on the heart. The miniature hearts allow researchers to observe mechanisms by which drugs cause arrhythmias without testing in humans. These models also provide an avenue to study COVID-19's direct effect on the heart.[33]
- Organ-on-a-chip systems serve as a valuable bridge between traditional cell culture, animal models, and human studies of disease. Researchers used a multi-system lung chip model to study the immune response to viral infection in lung cells. The system was used to examine how circulating immune cells may be causing lung injury through aberrant inflammatory response, mirroring what is known based on human data.[34]

Additionally, four testing programs for drug and vaccine discovery that can accelerate the process are outlined in a recent paper[35]:

1. **Efficacy**: There is no animal disease model for COVID-19. The automatic reflex of researchers is to use non-human primates, however this has not helped HIV or hepatitis C.[36] The next reflex of researchers is to breed or genetically modify animals to attempt to render them susceptible to the disease, as was done for SARS. Doing this, however, skips

over the opportunity to use microphysiological systems (MPS) that have become available over the past decade. Viral infections can be studied in human BrainSpheres (mini-brains).[37]

2. **Safety**: During a pandemic, the important issue is time to market. A traditional full safety package based on animals includes long-term chronic dosing (up to two years). Shortcuts are possible using human-relevant test methods. What needs to be shown?
    - The drug should not be acutely toxic – accepted alternative methods are available for most acute and topical toxicities.[38] Acute systemic toxicity may be predicted, possibly in combination with limited animal tests.[39]
    - The drug should not cause cancer – it is necessary to distinguish genotoxic and non-genotoxic carcinogenicity. Genotoxicity can be established using non-animal methods which have been available for decades. These methods are fast (days) and highly sensitive. Non-genotoxic carcinogenicity is harder to predict in animals and alternatives. However, COVID-19 treatment and vaccines will not require long exposure, these testing requirements can be waived since the treatment is less than 6 months. A repeat dose toxicity test of one-month duration. This could be done in a Microphysiological System (MPS). However, FDA has not approved the use of MPS for regulatory use.
    - The drug should not interfere with other drugs – there are many alternatives in place to determine drug-drug interactions which allow for a quick transition to testing in humans.

3. **Quality (batch testing):** Once a safe and efficacious vaccine (or drug) has been found, it needs to be tested for quality and purity. The testing program differs fundamentally from safety and efficacy, as it needs to be applied to each lot of vaccine (or drug) to control for impurities and contaminants. Serological or non-animal batch release tests may be implemented.[40] Rabbit and horseshoe crab tests can be replaced with the Monocyte Activation Test (human blood), and Recombinant Factor C (fFC)[41].

4. **Target discovery**: Drugs can be tested quickly in modern non-animal methods. Some targets may be known from other diseases or research so may provide one of the fastest ways to find suitable drugs (repurposing).

5. **Safe, Sustainable Non-Animal Ingredients for Vaccine Adjuvants:** Some vaccines use adjuvants. Several vaccine candidates for COVID-19 use shark-based squalene in their adjuvant when molecularly identical plant-based options are available.

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