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Using Lung-Chip Technology to Characterize Emerging SARS-CoV-2 Variants

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The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorizing documents.

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PREFACE

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EXECUTIVE SUMMARY

This technical report provides viral burden and overall pathogenicity of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) Washington, Delta, and Omicron variants in human lung alveolus organ chips. This work was funded as Defense Threat Reduction Agency (Fort Belvoir, VA) program HDTRA1035724 and was performed in response to the emergence of the Omicron variant in 2021. This study provided U.S. Army Combat Capabilities Development Command Chemical Biological Center (Aberdeen Proving Ground, MD) with valuable information on the use of organ-on-a-chip technology as a model system for viral infections, including the limitations of the systems and what endpoints would be most useful for analysis.

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USING LUNG-CHIP TECHNOLOGY TO CHARACTERIZE EMERGING SARS-COV-2 VARIANTS

1. INTRODUCTION

1.1 Emulate Alveolus Lung Chip

Traditionally, researchers have relied heavily on two-dimensional tissue culture and animal models to make toxicological and pathological predictions.^{1,2} However, *in vivo* work can cost an exorbitant amount of time and money, and tissue cultures grown on dishes and plates often lack physiological relevancy. For these reasons, microphysiological systems (MPSs) have been gaining traction as more accurate and a potentially more cost-effective means for predicting human outcomes in response to exposure to compounds of interest.^{3,4} By providing a three-dimensional architecture composed of a multi-cell type microenvironment complete with fluid flow and other cellular and environmental dynamics present in a functional organ, MPSs are leading the charge for *in vivo* toxicity testing alternatives.⁵

Originally conceptualized at the Wyss Institute for Biologically Inspired Engineering at Harvard University (Boston, MA), the organ-on-a-chip technology (Emulate; Boston, MA) has been applied to multiple organs and biological systems.⁴⁻⁹ The liver chip is Emulate's first MPS. Emulate commercially provides many other systems including the duodenum intestine chip⁸ and the kidney proximal tubule chip.⁶ Unlike the aforementioned organ types, Emulate's lung systems, which recreate either the alveolus or small airway regions of the organ, require that the user purchase and optimize human lung tissues from a third-party vendor. All of Emulate's organ-on-a-chip systems consist of a number of specialized components (Figure 1). The S1 chips are made of polydimethylsiloxane (PDMS) and are composed of a top and bottom channel separated by a porous membrane. The chips are housed in Pod portable modules, which also function to supply fresh medium to the specific cell types. Cells are dosed with drugs and infected with bacteria or viruses through the inlet reservoirs of the Pod, and effluent is collected from the outlet reservoirs for analysis. The flow rate of the medium through the top and bottom channels of the chip is controlled by the Zoë culture module. The Zoë accommodates a pump manifold that is engaged with the chips housed in Pods to produce the medium flow. All gas exchange within the chips is controlled by a device called an Orb hub module.

The top channel of the alveolus lung chip houses human primary alveolus epithelial cells (HPAECs). The bottom layer is seeded with human lung microvasculature endothelial cells (HMVEC-Ls). One of the benefits to Emulate's two-channel chip design is that the multiple cell types can interact in their microenvironment the same way they would in a human lung alveolus. Lung-specific cytoarchitecture and function are maintained. The combination of the microfluidic component of the chips and the pump system designed to control the flow of medium through the chip and over the cells also lends to the physiological relevancy of the system. *In vivo* lung physiology is simulated using three main components: constant flow, air-liquid interface (ALI) culture, and mechanical stretch.¹⁰ These factors allow for a relevant response to an exposure or a bacterial or viral infection that is comparable to that observed *in vivo*.

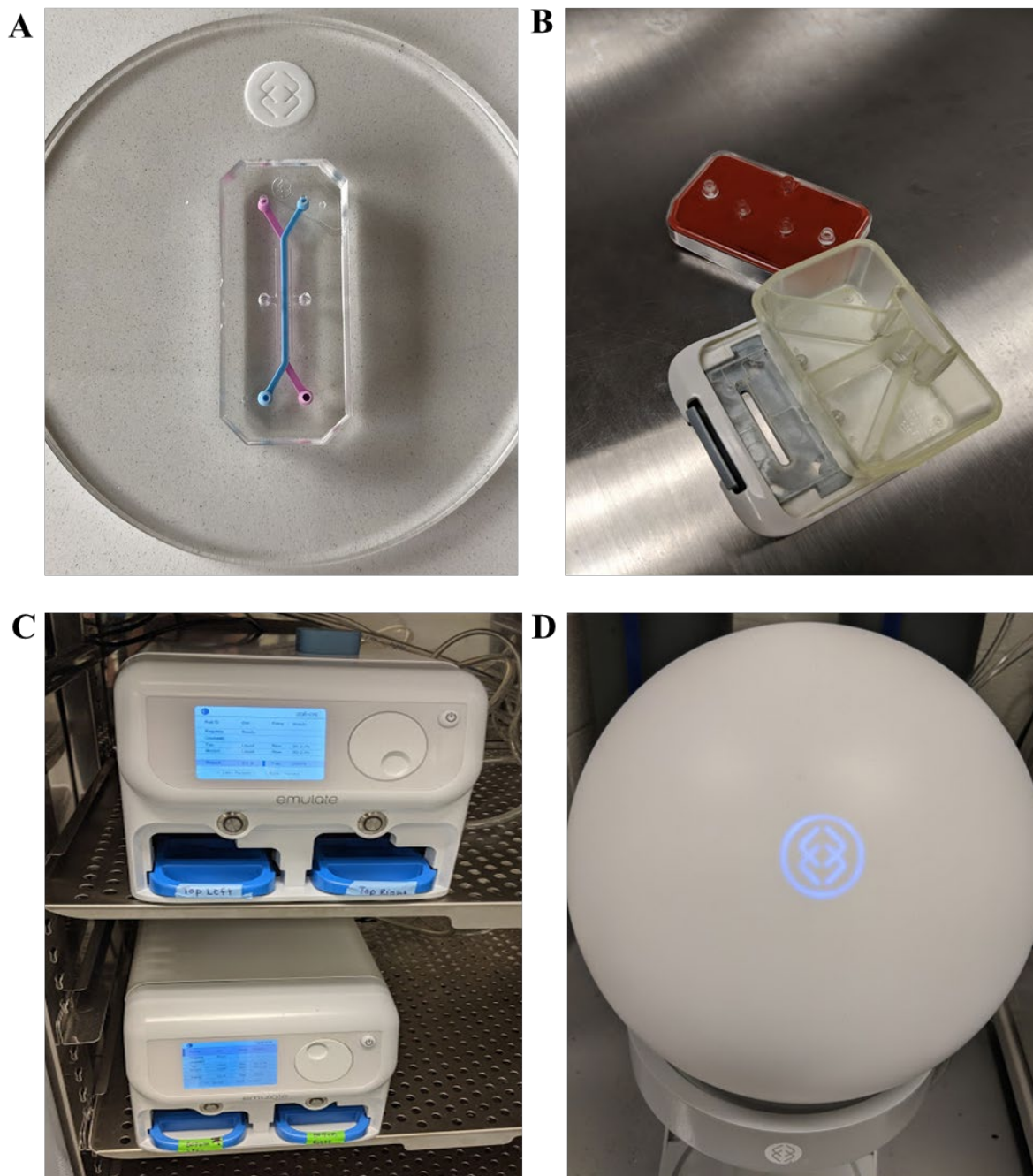


Figure 1. Components of the Emulate MPS. The S1 chip (A) consists of a top channel (blue) and a bottom channel (pink) where the various cells are seeded. The chips are housed in the Pod (B), which contains inlet and outlet reservoirs for medium. The Pods are placed in the Zoë (C), which controls the medium flow. Gas exchange is controlled by the Orb (D).

1.2 SARS-CoV-2

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged at the end of 2019 in Wuhan, China and resulted in an outbreak of a highly transmissible pneumonia known as coronavirus disease 2019 (COVID-19).¹¹ The virus reached pandemic status, wreaking havoc on the world. The spread of SARS-CoV-2 has resulted in over 6 million global deaths,¹² placed enormous strain on emergency medical infrastructure, and caused significant economic fallout from idled supply chains and markets. As the pandemic surged, the fallout from the virus was exacerbated by the emergence of multiple novel SARS-CoV-2 variants, a few of which are considered variants of concern. Some of the hallmarks that set variants of concern apart from other emerging variants are increased transmissibility or virulence, evasion of detection, decrease in therapeutic or vaccination effectiveness, and a reduction in antibody neutralization.

Delta (B.1.617.2 lineage) and Omicron (B.1.1.529 lineage) are two of the more prominent variants of concern that developed throughout the course of the pandemic.¹³ The Delta variant was identified in India in late 2020 and was the fourth SARS-CoV-2 variant of concern. It was first detected in the United States in March 2021 and was responsible for the second large wave of infections that resulted in an uptick in deaths. The Delta variant has increased replication fitness as compared to the Alpha variants, which were the first variants of concern, as well as a reduced sensitivity to neutralizing antibodies derived from infection or vaccination.¹⁴ The Omicron variant was the fifth variant of concern, identified in November 2021 in South Africa. The variant made its way to the United States later the same year and is thought to be nearly three times more infectious than the Delta variant.¹³

1.3 Aims of This Study

As novel variant SARS-CoV-2 cases surged throughout the global population, it became imperative that we use available resources and capabilities to characterize the infection and assess the risk to individuals. U.S. Army Combat Capabilities Development Command Chemical Biological Center (DEVCOM CBC; Aberdeen Proving Ground, MD) recently added Emulate's lung alveolus and small airway chip systems to our predictive toxicology capabilities, which serve as viable models for SARS-CoV-2 infections performed in Biosafety Level 3 laboratories. These systems enable the study of complex human physiology and pathology in an organ-specific context. The systems offer the potential to develop models of human disease that simulate what is observed in vivo. Here, we used Emulate's alveolus lung chips to compare host cell morphological changes, infection rate, and viral burden of the SARS-CoV-2 Omicron, Washington (WA1/2020), and Delta variants.

2. METHODOLOGY

2.1 Chemicals

This list includes the materials and chemicals that were used in this study as well as their manufacturers:

- basic Biokit 2 (Emulate) including 24 S1 chips, 24 Pods, epitope retrieval (ER)-1 solution, and ER-2 solution;
- Dulbecco's phosphate-buffered saline without Ca^{2+} and Mg^{2+} (DPBS; Corning; Corning, NY);
- 10× DPBS without Ca^{2+} and Mg^{2+} (Corning);
- trypan blue (MilliporeSigma; Burlington, MA)
- trypsin-ethylenediaminetetraacetic acid (EDTA; MilliporeSigma);
- small airway epithelial cell growth medium (SAGM; Lonza Bioscience; Basel, Switzerland);
- microvascular endothelial cell growth medium (EGM-2MV; Lonza Bioscience);
- epidermal growth factor (EGF; PromoCell; Heidelberg, Germany);
- vascular endothelial growth factor (VEGF; PromoCell);
- basic fibroblast growth factor (FGF-2 or FGF-b; PromoCell);
- fibronectin (Corning);
- collagen type IV (Sigma-Aldrich; St. Louis, MO);
- laminin (Sigma-Aldrich);
- paraformaldehyde (PFA; Thermo Fisher Scientific; Waltham, MA);
- penicillin–streptomycin (MilliporeSigma);
- GlutaMax supplement (Thermo Fisher Scientific);
- cyclic adenosine monophosphate (cAMP; MilliporeSigma);
- heparin (MilliporeSigma);
- hydrocortisone (MilliporeSigma);
- 3-isobutyl-1-methylxanthine (IBMX; MilliporeSigma);
- keratinocyte growth factor (KGF; Thermo Fisher Scientific);
- medium 199, no phenol red (Thermo Fisher Scientific);
- fetal bovine serum (FBS; MilliporeSigma);
- bovine serum albumin (BSA; Thermo Fisher Scientific);
- HPAECs (Lonza Bioscience);
- HMVEC-Ls (Lonza Bioscience);
- anti-angiotensin-converting enzyme 2 (ACE2) antibody (Abcam; Boston, MA);
- conjugated goat anti-rabbit immunoglobulin G (IgG), heavy and light chains (H&L), Texas Red (Abcam); and
- Hoechst nuclear stain (Thermo Fisher Scientific).

2.2 Cell Storage

HPAECs and HMVEC-Ls were kept frozen in liquid nitrogen storage (vapor phase) until they were cultured.

2.3 Reagent Preparation

All reagents were aliquoted at appropriate volumes prior to use to avoid multiple freeze–thaw cycles. Fibronectin, laminin, and collagen IV were resuspended in cell culture-grade water to a concentration of 1 mg/mL. KGF was resuspended in 1× DPBS with 0.1% BSA to a concentration of 10 µg/mL. cAMP was resuspended in cell culture-grade water to a concentration of 10 mM. EGF was resuspended in cell culture-grade water to a concentration of 1 mg/mL. Basic FGF was resuspended in cell culture-grade water to a concentration of 100 µg/mL. VEGF was resuspended in 50 mM acetic acid with 0.1% BSA to a concentration of 50 µg/mL. Hydrocortisone was resuspended in absolute ethanol and sterile tissue culture medium to a concentration of 50 µg/mL. Heparin was resuspended in cell culture-grade water to a concentration of 50 mg/mL. IBMX was reconstituted in dimethyl sulfoxide (DMSO) to a concentration of 1 M. Dexamethasone was resuspended in cell culture-grade DMSO at a concentration of 1 mM. All reagent aliquots were stored at –20 °C until use.

The following medium preparations were used in this study:

- HPAEC culture medium (500 mL):
475 mL of SABM basal medium, SAGM SingleQuots supplement pack,
25 mL of FBS;
- HMVEC-L culture medium (500 mL):
500 mL of EBM-2 basal medium, EGM-2MV SingleQuots supplement pack;
- HPAEC maintenance medium (500 mL):
488.88 mL of SABM basal medium, SAGM SingleQuots supplement pack,
10 mL of FBS, 500 µL of dexamethasone, 250 µL of KGF, 250 µL of cAMP,
125 µL of IBMX;
- ALI medium (519.53 mL):
500 mL of medium 199, 15 µL of EGF, 500 µL of basic FGF, 1.25 µL of
VEGF, 10 mL of hydrocortisone, 100 µL of heparin, 400 µL of cAMP,
2.5 mL of GlutaMax, 10 µL of dexamethasone, 5 mL of penicillin–
streptomycin, 1 mL of FBS.

2.4 HMVEC-L Culture

HMVEC-Ls were removed from liquid nitrogen storage and quick-thawed by placing the cryovial in a 37 °C water bath. The contents of the vial were transferred to a 15 mL conical tube containing 3 mL of warm HMVEC-L culture medium. The vial was rinsed once with 1 mL of medium, and the conical tube was brought up to a volume of 15 mL of complete HMVEC-L culture medium. The HMVEC-L suspension was added to a T-75 flask and incubated

at 37 °C and 5% CO₂ for 6 h. The HMVEC-L culture medium was aspirated from the T-75 flask and replaced with 15 mL of fresh medium. The cells were incubated at 37 °C and 5% CO₂. HMVEC-Ls were cultured at least two days before chip seeding, and the medium was exchanged every two days.

2.5 HPAEC Culture

A T-25 flask was precoated with gelatin solution and incubated at 37 °C and 5% CO₂ for 10–15 min. HMVEC-Ls were removed from liquid nitrogen storage and quickly thawed by placing the cryovial in a 37 °C water bath. The contents of the vial were transferred to a 15 mL conical tube containing 3 mL of warm HPAEC culture medium. The vial was rinsed once with 1 mL of medium, and the conical tube was brought up to a volume of 15 mL of complete HPAEC culture medium. The cell suspension was centrifuged at 200 ×g for 5 min at room temperature. The supernatant was aspirated and discarded, leaving approximately 100 µL of medium covering the cell pellet. The cell pellet was loosened into the remaining medium by gently flicking the tube. The HPAEC suspension was resuspended in 7 mL of HPAEC culture medium and added to a T-25 flask. The cells were incubated at 37 °C and 5 % CO₂. HPAECs were cultured into a T-75 flask at least two days before chip seeding, and medium was exchanged every two days.

2.6 Chip Activation

ER-1 and ER-2 reagents are both light sensitive, so all manipulations were performed in a biosafety cabinet with the lights turned off. Before preparation of the activation solution, ER-1 and ER-2 reagents were allowed to equilibrate to room temperature. ER-2 reagent (1 mL) was added to the ER-1 reagent vial, and the contents were added directly to a 15 mL conical tube. After an additional 1 mL of ER-2 reagent was added to the ER-1 vial to collect remaining material, the contents were added to the 15 mL conical tube. This process was repeated two more times. Finally, 6 mL of ER-2 reagent was added to the 4 mL of ER-1 solution in the 15 mL conical tube for a final concentration of 0.5 mg/mL. A P200 pipette was used to introduce the ER-1 solution to the top and bottom channels of the S1 chip at volumes of 50 and 20 µL, respectively, being careful not to introduce bubbles. The excess solution was aspirated from the top of the chip. The chips were activated under constant UV light for 20 min. After UV activation, the ER-1 solution was aspirated from both channels, and both channels were washed with 200 µL of ER-2 solution. The top and bottom channels of each chip were washed with 200 µL of 1× DPBS, and cold 1× DPBS was left in the channel (35 µL in the top channel and 25 µL in the bottom). The chips were incubated overnight at 4 °C.

2.7 Extracellular Matrix (ECM) Coating

The ECM was prepared on ice for the top channel by combining collagen IV, laminin, and fibronectin in ice-cold 1× DPBS at concentrations of 200, 5, and 30 µg/mL, respectively. For the bottom channel, collagen IV and fibronectin were added to ice-cold 1× DPBS at concentrations of 200 and 30 µg/mL, respectively. After the ECM solutions were prepared, the cold 1× DPBS was removed from the top and bottom channels of the chips. The appropriate ECM solution was added to each channel using a P200 pipette until small droplets

formed on the channel outlets. Droplets of ECM were placed on all four ports of each chip, and the chips were incubated overnight at 37 °C.

2.8 HPAEC Seeding

The chips were equilibrated to room temperature and washed 3× with 200 µL of HPAEC culture medium. The final wash was left in the top and bottom channels of the chips. The medium from the flask containing HPAECs was aspirated, and the cells were washed with 5 mL of 1× DPBS. The 1× DPBS was aspirated. Trypsin-EDTA (3 mL) was added to the flask and incubated at 37 °C and 5% CO₂ for 10–15 min. The detached cells were resuspended in 10 mL of warm HPAEC culture medium, which was then transferred to a 15 mL conical tube. The cell suspension was centrifuged at 200 ×g for 5 min at room temperature. The supernatant was aspirated, leaving approximately 100 µL of medium above the cell pellet. The pellet was resuspended by gently flicking the tube, and the cell suspension was brought up to a volume of 600 µL in HPAEC culture medium. The HPAECs were counted in trypan blue solution using a hemocytometer and resuspended to a final cell density of 1 × 10⁶ cells/mL in HPAEC culture medium. Cell suspension (50 µL) was carefully added to the top channel of each chip. The chips were incubated at 37 °C with 5% CO₂ for 3 h or until the HPAECs attached. Once the cells were attached, a chip wash was performed on each chip by gently pipetting 200 µL of HPAEC culture medium through the top and bottom channels. The chips were incubated overnight at 37 °C with 5% CO₂.

2.9 Media Replenishment

The next day, 200 µL of warm HPAEC maintenance medium was added to the top and bottom channels of the chips. Droplets of HPAEC maintenance medium were added to the inlet and outlet ports of the top and bottom channels to prevent evaporation from the ports. The chips were then incubated overnight at 37 °C and 5% CO₂.

2.10 HMVEC-L Seeding

The HMVEC-Ls were harvested from the T-75 flask using 3 mL of trypsin-EDTA. Warm HMVEC-L culture medium (9 mL) was added to the cell suspension, and the entire 12 mL was centrifuged in a 15 mL conical tube at 200 ×g for 5 min. The supernatant was aspirated, leaving 100 µL in the tube with which to resuspend the HMVEC-Ls. The cell suspension was brought up to a final volume of 600 µL with additional HMVEC-L culture medium. The HMVEC-Ls were counted in trypan blue solution using a hemocytometer and resuspended to a cell density of 5 × 10⁶ cells/mL in HMVEC-L culture medium. HMVEC-L suspension (20 µL) was added to the bottom channel of each chip. After seeding, the chips were inverted and incubated at 37 °C and 5% CO₂ for 4 h or until the cells attached. Once the cells were attached to the bottom channel, a chip wash was performed with 200 µL of HPAEC maintenance medium for the top channel and HMVEC-L culture medium for the bottom channel. The respective pipette tips were left in the ports to ensure the medium remained inside the channels. The chips were incubated overnight at 37 °C and 5% CO₂.

2.11 Chips to Pods and Pods to Zoë

HPAEC maintenance medium and HMVEC-L culture medium were warmed in a 37 °C water bath for 1 h. To reduce the risk for bubbles in the chips, the medium used for the Pods was gas equilibrated in 50 mL Steriflip conical tubes (Sigma-Aldrich) for 15 min before use. HPAEC maintenance medium (300 µL) was added directly over the microfluidic channel opening, or via, of the top channel outlet reservoir, and 300 µL of HMVEC-L culture medium was added to the bottom channel outlet reservoir in the same manner. The appropriate medium (3 mL) was added to the inlet reservoirs of each Pod. The Pods were inserted into the Zoë and primed. Once primed, the top and bottom channels of the chips were washed with 200 µL of the appropriate medium and were manually connected to each Pod. The Pods with chips were then placed back into the Zoë, and a regulate cycle was run. This was done to stabilize the fluidics of the system. Once the cycle was complete, the flow conditions changed to 30 µL/h for both top and bottom channels. The top and bottom inlet reservoir medium was replenished every two days while the chips were under flow.

2.12 Introduction of ALI

The day after flow was established, or once the epithelial layer was confluent, the chips were removed from the Zoë, and the medium was aspirated from both the inlet and outlet reservoirs of each Pod. The Pods containing the chips were transferred back to the Zoë, and flow was reestablished for 3 min at rates of 1,000 and 0 µL/h for the top and bottom channels, respectively. This step allowed for the remaining medium to be pushed out of the top channel. The Pods were removed, and remaining medium was aspirated from the outlet reservoirs for the top channel. Warm ALI medium (2–4 mL) was added to the bottom channel inlet reservoir of each Pod. ALI medium (1 mL) was pipetted into the top channel inlet reservoir, and an additional 1 mL was immediately pipetted into the top channel outlet reservoir. This equal media distribution of the top channel reservoirs was required to establish static pressure and achieve an air interface with the epithelium. The Pods were transferred back to the Zoë. The top channel was set to the “Air” setting, and the bottom channel flow rate was set to 30 µL/h.

2.13 Initiating Mechanical Stretch

The day after the ALI was established, mechanical stretch was initiated by setting the stretch frequency on the Zoë to 0.20 Hz. This caused the vacuum ports present on each chip to be pulled in and out, simulating the mechanical forces generated by breathing.

2.14 SARS-CoV-2 Infection and Median Tissue Culture Infectious Dose (TCID₅₀) Analysis

Viral working solutions for the SARS-CoV-2 Washington, Delta, and Omicron variants were prepared by diluting viral stocks (American Type Culture Collection; Rockville, MD) in warm ALI medium to a final concentration of 1×10^3 TCID₅₀/mL. The alveolus lung chips were removed from the incubator, and medium was aspirated from the top channel inlet and outlet reservoirs. Viral working solution (2 mL) was added to the top channel inlet reservoirs of each chip, and the chips were returned to the Zoë. The viral solution was inoculated on top of

the HPAECs by running the flow at 400 $\mu\text{L}/\text{h}$ for 30 min. The chips were allowed to incubate statically at 37 °C and 5% CO_2 for 1 h to initiate the viral infection. Medium was removed from the top and bottom inlet and outlet reservoirs, and the chips were placed back under ALI with fresh medium. The infection was allowed to persist under flow for five days. The medium containing virus was collected from the bottom channel outlet reservoir, and 100 μL of the collected medium was added at 10-fold serial dilutions to 48-well plates containing VeroE6 cells at 1×10^6 cells/mL at five days post-infection. Cytopathic effect (CPE) was measured visually after a five-day incubation at 37 °C and 5% CO_2 . The amount of TCID_{50} per milliliter was determined using the Spearman and Kärger method.¹⁵

2.15 Immunofluorescent Staining

The HPAECs were fixed with 4% PFA in $1 \times$ DPBS for 15 min at room temperature. Cells were then permeabilized with 1% saponin in $1 \times$ DPBS for 30 min at room temperature. Blocking was performed by adding 1% BSA and 10% goat serum in $1 \times$ DPBS, which was incubated overnight at 4 °C. The primary antibody solution was made by diluting rabbit anti-ACE2 (1:1000) in blocking buffer, which was added to the top channel of the chips. The chips were incubated overnight at 4 °C. The secondary antibody solution was prepared by diluting goat anti-rabbit IgG H&L conjugation (Texas Red, 1:500) in blocking buffer. Hoechst counterstain was added to the secondary antibody solution at a 1:10,000 dilution. The secondary antibody solution was added to the top channels of the chips, which were then incubated for 2 h at room temperature in the dark. The chips were washed three times with $1 \times$ DPBS, and microscopy images were obtained.

3. RESULTS

3.1 TCID_{50} Analysis

Virus was detected in the medium effluent collected after its passage through the chips for all three variants at five days post-infection. The highest representative titers observed for each variant are reported in Table 1. The Delta variant produced the most virus within the alveolus lung chips after day 5; however, this increase was not significant compared to the other two variants.

Table 1. TCID_{50} Values Five Days Post-Infection

Variant	$\text{TCID}_{50}/\text{mL}$
Washington WA1/2020	6.81
Delta B.1.617.2	14.7
Omicron B.1.1.529	6.81
Medium control	Not detected

3.2 SARS-CoV-2 Lung Chip Pathogenicity

Brightfield and immunofluorescence images of the infected HPAECs were obtained five days post-infection to visualize the CPE. There were no pronounced morphological changes observed to the cells infected with the Washington variant (Figure 2A); however, the monolayer seemed to be disrupted after infection with the Delta and Omicron variants (Figure 2B, 2C). This was most evident in the Delta variant infected HPAECs, which were observed to have less definition to their cellular membranes (Figure 2B). Immunofluorescence staining of the same chips showed fewer ACE2 receptors (needed for initial SARS-CoV-2 host cell entry) in the Delta and Omicron variant infected chips (Figure 2E, 2F) compared to those infected with the Washington variant (Figure 2D). Less ACE2 was detected in host cells after a SARS-CoV-2 infection had fully mounted, potentially suggesting that the Delta and Omicron variants could be more pathogenic than the Washington variant to the alveolus lung chips.

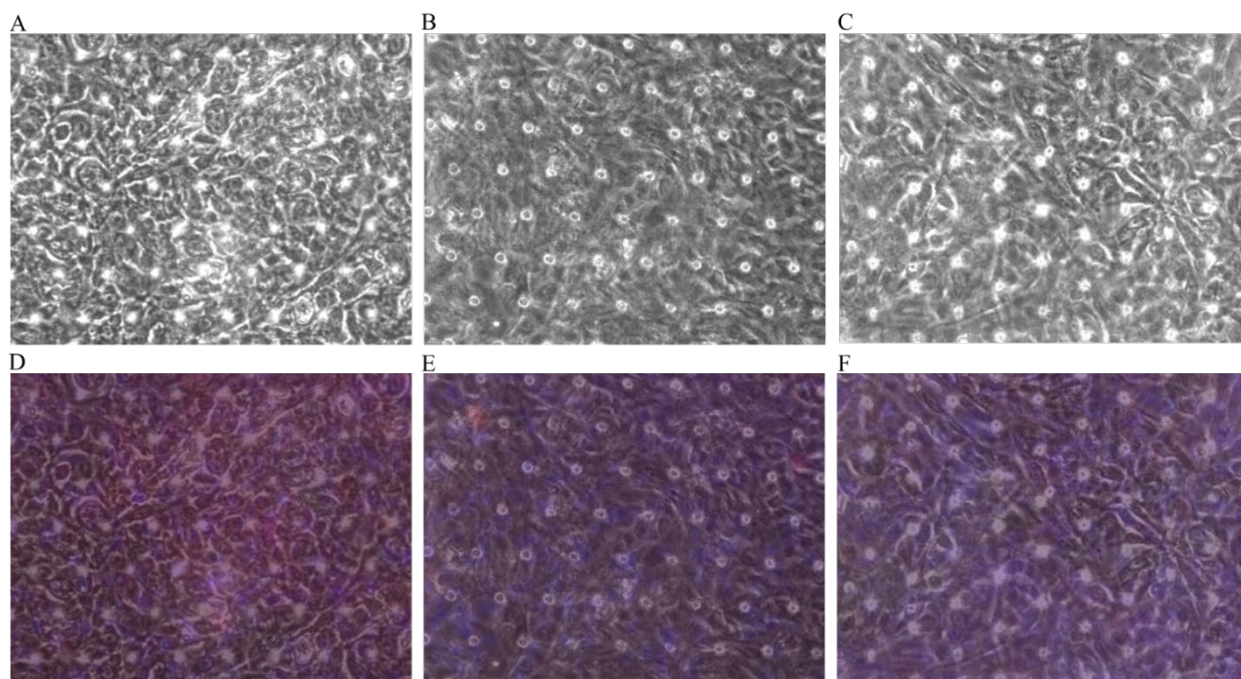


Figure 2. Post-infection alveolus lung chip epithelium. Alveolus lung chip epithelia were stained and imaged five days post-infection with SARS-CoV-2 Washington (A, D), Delta (B, E), or Omicron (C, F) variants. Top row: brightfield images. Bottom row: cells stained with ACE2 (red) and Hoechst nuclear dye (blue) overlaid brightfield.

4. CONCLUSIONS

This report, and the work described herein, provides insight into the utility of microphysiological systems as an effective SARS-CoV-2 infection model. Although the alveolus lung chips were successfully infected by all three SARS-CoV-2 variants, the differences observed in the amounts of TCID₅₀ per milliliter were negligible. This could be a result of low assay sensitivity due to the relatively small cell number in each chip (~50,000 cells per channel)

compared to the volume of medium collected in the outlet reservoir. To adequately quantify differences in viral burden between variants, quantitative polymerase chain reaction should be performed. Small morphological changes were also observed in HPAECs infected with the Delta and Omicron variants compared to the Washington variant, and more ACE2 present on the surface of the Washington variant infected-HPAECs is indicative of lower pathogenicity at day 5 post-infection. However, these changes were not pronounced. A larger viral inoculum, or a longer static incubation of the viral inoculum on the HPAECs, might provide more definite CPE, which would help to better discern the pathogenic differences between the three variants in the context of the alveolus lung chips.

It should also be noted that the viral exposure route used in this study is not the most physiologically accurate. SARS-CoV-2 is commonly transmitted through inhalation of very fine respiratory droplets and aerosol particles.¹⁶ Due to the closed-chip design of the Emulate system, end users are required to perform infections via a liquid interface. The ability to mount infections using aerosol delivery on the organ chips would create a more realistic infection scenario and may result in more relevant data when assessing the effects of microbial infections that typically occur through the aerosol route of exposure. Regardless, this study was successful in establishing an infection model for three separate SARS-CoV-2 variants. This has bolstered the use of organ-on-a-chip technology at DEVCOM CBC as a surrogate model capable of providing valuable data on microbial infections without sole reliance on animal exposures.

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ACRONYMS AND ABBREVIATIONS

ACE2	angiotensin-converting enzyme 2
ALI	air-liquid interface
BSA	bovine serum albumin
cAMP	cyclic adenosine monophosphate
COVID-19	coronavirus disease 2019
CPE	cytopathic effect
DEVCOM CBC	U.S. Army Combat Capabilities Development Command Chemical Biological Center
DMSO	dimethyl sulfoxide
DPBS	Dulbecco's phosphate-buffered saline
ECM	extracellular matrix
EDTA	ethylenediaminetetraacetic acid
EGF	epidermal growth factor
EGM-2 MV	microvascular endothelial cell growth medium
ER	epitope retrieval
FBS	fetal bovine serum
FGF	fibroblast growth factor
H&L	heavy and light chains
HPAEC	human primary alveolus epithelial cell
HMVEC-L	human lung microvasculature endothelial cell
IBMX	3-isobutyl-1-methylxanthine
IgG	immunoglobulin G
KGF	keratinocyte growth factor
MPS	microphysiological systems
PDMS	polydimethylsiloxane
PFA	paraformaldehyde
SAGM	small airway epithelial cell growth medium
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
TCID ₅₀	median tissue culture infectious dose
VEGF	vascular endothelial growth factor

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