347 - Infection of human alveolar MPS at high containment as a model for pandemic pathogens and assessment of medical countermeasures

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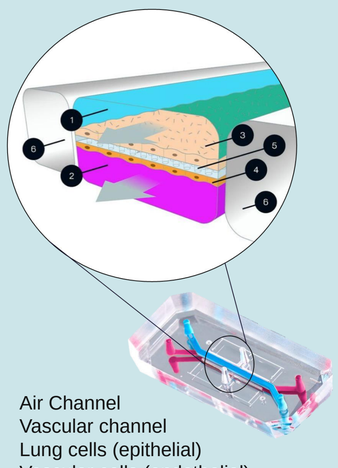
INTRODUCTION

The SARS-CoV-2 pandemic caused significant worldwide disruption to economies and healthcare systems. It is predicted that the frequency at which new pandemics occur in the future will increase due to factors such as climate change, global population increase, intensification of agriculture and closer contact with wildlife. All but one of the pathogens on the current WHO R&D emergency context priority disease list are viruses which require work to be performed at containment level 3 or 4 (BSL3 or BSL4) [1]. There is a need for physiologically relevant *in vitro* systems to assist pre-clinical research in preparedness for the next pandemic. For example, Disease X which by definition will be a human disease, may not infect current cell lines or animal models commonly used in virus research.

Here we present the results from infections with SARS-CoV-2, SARS-CoV-1 and MERS-CoV of an alveolar model using the commercially available Emulate system (Figure 1) at BSL3. We also describe the results from antiviral assessment in this model.



1. Air Channel
2. Vascular channel
3. Lung cells (epithelial)
4. Vascular cells (endothelial)
5. Porous membrane
6. Vacuum channels

Figure 1. Schematic of the Emulate lung chip

METHODS

Set up of Emulate alveolar co-culture chips
Alveolar epithelial (HPAEC) and endothelial (HMVEC) cell co-culture chips were set up as per Emulate Inc. SOP EP180 with stretch (5%, 0.2 Hz) and airflow (30µl/hr). The steroid dexamethasone was omitted from the cell culture medium prior to virus infection.

Set up of alveolar mono-culture transwells
12mm transwells were seeded with $1x10^5$ HPAEC per well and taken to air-liquid interface (ALI) after three days using the same media components and timeline as Emulate Inc. SOP EP180. Following eight days at ALI, transwells were transferred to BSL3 for infectious work

Infection of chips with Ancestral SARS-CoV-2 (pre-D614G), SARS-CoV (HKU-39849) or MERS-CoV (England1)
Seven days post establishment of ALI in the alveolar model, chips were transferred to BSL3. Chips were mock-infected or infected with SARS-CoV-2 at an MOI of 1. ALI and stretch were then re-established. For antiviral assessment, EIDD-1931 (active metabolite of Molnupiravir) or Nirmatrelvir (antiviral component of Paxlovid) was introduced into the basal channel at 1µM following virus adsorption and was refreshed daily.

Readouts post-infection
- Immunofluorescence microscopy for the viral nucleocapsid, ZO-1, type 2 alveolar cells (HT280) was performed. Nuclei were counterstained with DAPI
- Quantification of viable virus by focus forming assay or virus shedding by RT-qPCR for nucleocapsid gene.

Containment system in UKHSA BSL3 laboratories
A custom-made flexible film isolator (FFI), complete with half-suit, was designed and built by PFI Systems. The design allows for sufficient space for a full-size CO_2 incubator and microscopy equipment for complete containment of the system and is readily transferrable to BSL4.

RESULTS

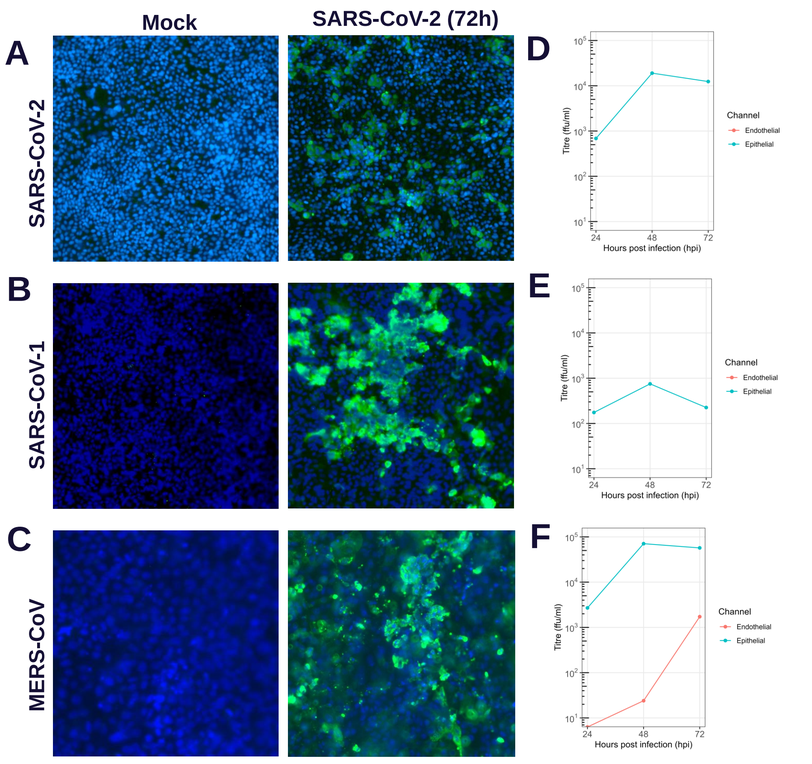


Figure 2. Infection of Emulate human alveolar chips with SARS-CoV-2, SARS-CoV-1 and MERS-CoV results in a productive infection over the course of 72 hours

Alveolar epithelial cells from Emulate chips infected with Ancestral SARS-CoV-2 **(A)**, SARS-CoV-1 **(B)** or MERS-CoV **(C)**. Cells were fixed at 72hpi and stained for virus nucleocapsid (green). Nuclei were counterstained with DAPI (blue). Quantification of viable virus from epithelial and endothelial compartments of the same Emulate alveolar chips infected with Ancestral SARS-CoV-2 **(D)**, SARS-CoV-1 **(E)** or MERS-CoV **(F)** was performed by focus forming assay.

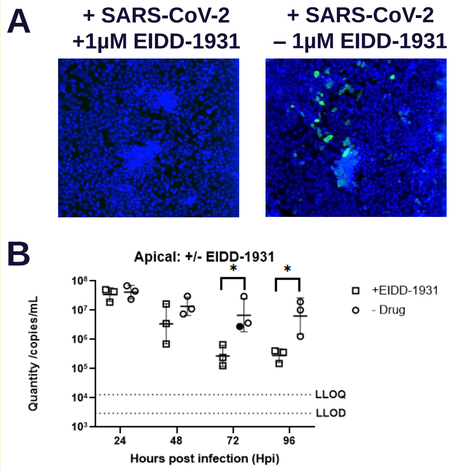


Figure 3. Treatment of SARS-CoV-2 in Emulate human alveolar chips with EIDD-1931 (Molnupiravir) significantly reduces viral shedding from the epithelium and incidence of cellular infection

(A) Alveolar epithelial cells from Emulate chips infected with Ancestral SARS-CoV-2 +/- treatment with 1 µM EIDD-1931 and fixed at 96 hpi. Cells were stained for SARS-CoV-2 nucleocapsid (green) and nuclei counterstained with DAPI (blue). Phase contrast images from the same field of view were also taken.

(B) Quantification of virus shedding by RT-qPCR from apical washes from Emulate alveolar chips infected with Ancestral SARS-CoV-2 with or without EIDD-1931 treatment.* = p<0.05 (two-tailed students t-test with Welch's correction).

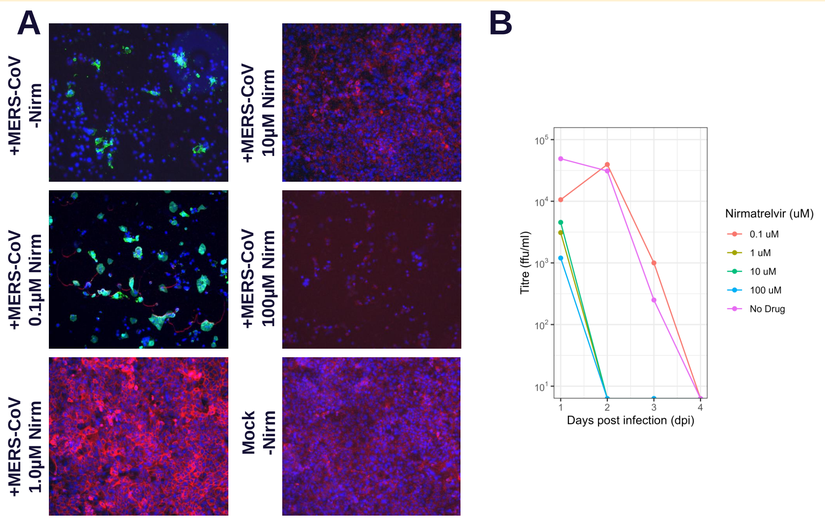


Figure 4. Response to treatment of MERS-CoV in human alveolar transwells with Nirmatrelvir (Paxlovid) is dose-dependent

Alveolar epithelial cells seeded on transwells infected with MERS-CoV and treated with increasing concentrations of Nirmatrelvir (0.1 – 100 µM). **(A)** Cells fixed at 96hpi and stained for virus nucleocapsid (green), ZO-1 (red), DAPI (blue). **(B)** Quantification of viable virus from epithelial cell washes was performed by focus forming assay.

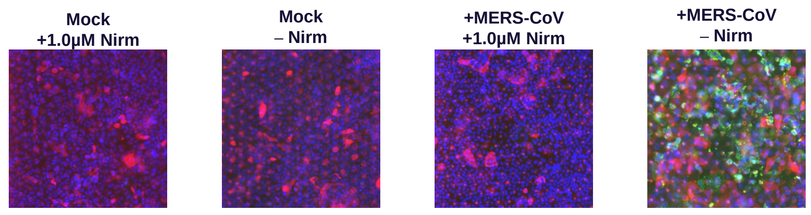


Figure 5. Treatment of MERS-CoV in Emulate human alveolar chips with Nirmatrelvir (Paxlovid) is effective

Alveolar epithelial cells from Emulate chips infected with MERS-CoV +/- treatment with 1 µM Nirmatrelvir and fixed at 96 hpi. Cells fixed at 96hpi and stained for virus nucleocapsid (green), type 2 alveolar cells HT280 (red) and, DAPI (blue).

DISCUSSION

The Emulate system was used to set up alveolar chips which were infected with Ancestral SARS-CoV-2 [2], SARS-CoV-1 or MERS-CoV at 1 MOI then monitored over 72 hours. Evidence of viral replication and shedding was present in the cells of the alveolar epithelia by IF and FFA for all viruses. In MERS-CoV infected chips, evidence of virus shedding into the basal channel was observed by FFA, however there was no positive staining by IF.

The antivirals, Molnupiravir and Paxlovid, have been demonstrated to inhibit SARS-CoV-2 replication and are used to treat COVID-19 patients. To demonstrate our model could effectively replicate real-world results, we assessed one of these countermeasures by treating infected alveolar chips with the active metabolite of Molnupiravir (EIDD-1931) [3]. We saw effective inhibition of SARS-CoV-2 with this physiologically relevant dose of EIDD-1931. Initial work in alveolar transwells also demonstrated this drug to be effective against SARS-CoV.

To investigate efficacy of SARS-CoV-2 anti-virals against other members of the coronavirus family we treated alveolar chips infected with MERS-CoV with the antiviral component of Paxlovid (Nirmatrelvir) [4]. Virus replication was inhibited demonstrating cross-protection to other coronaviruses with existing therapeutics.

We demonstrate that the alveolar Emulate model is a physiologically relevant system for studying human virus infections and their countermeasures. This is relevant to current efforts to develop models to study Disease X which may not infect current cell lines or animal models commonly used in virus research.

CONCLUSIONS

- The human alveolar Emulate model is susceptible to infection with severe Coronaviruses; SARS-CoV-2, SARS-CoV-1 and MERS-CoV.
- The active metabolite of the antiviral drugs, Molnupiravir and Paxlovid, effectively inhibited SARS-CoV-2 and MERS-CoV replication, respectively, in the human alveolar model at physiologically relevant doses.

Future work:

- UKHSA have isolated and propagated all SARS-CoV-2 variants of concern. These could be used to infect these models and compare or detect differences in virus kinetics and cytopathogenicity.
- Use these models for studying other pandemic BSL3 pathogens, such as SARS or MERS. This work is transferrable to preparing for Disease X.
- Assess additional countermeasures
- Investigate additional organ models e.g. gut for studying virus infections.
- Develop organ chip models for other species e.g. NHP to provide a tool for screening compounds prior to animal studies, thus reducing the use of animals in research.

ACKNOWLEDGEMENTS

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The set-up and infection of the alveolar Emulate model in Figure 2A was performed at QIB (Norwich, UK).

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