SARS-CoV-2 variant lung-on-a-chip infection differences in microphysiological systems and static models.

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BACKGROUND

- The coronavirus disease 2019 (Covid-19) pandemic had an immense impact on human health and the economy[1].
- Traditional *in vitro* immortalised cell culture models are unable to effectively replicate complex tissues and human physiological environments[2].
- Animal models are expensive and fundamental differences to the human host prevent direct comparisons to be made. This is particularly evident for SARS-CoV-2, as viral adaptations may have allowed more effective infection in humans compared to rodents[3].
- Dynamic microphysiological systems (MPS) have the potential to bridge the knowledge gap between traditional *in vitro* models and human clinical trials to enable us to understand SARS-CoV-2 infection and identify effective therapeutics.
- We have therefore undertaken experiments to compare SARS-CoV-2 variant infection in a MPS (CN-Bio's PhysioMimix™) lung-on-a-chip (LOC) and 'static' primary lung cells grown without MPS.

Methods

- Human bronchial epithelial cells (HBEC) and human pulmonary microvascular endothelial cells (HPMEC) were grown in Transwell® inserts with air-liquid interface (ALI) to create an upper airway co-culture, LOC model (Figure 1.).
- LOC's were cultured for 12 days in traditional static conditions or under perfusion in the PhysioMimix™ OOC MPS prior to infection with SARS-CoV-2.
- After 12 days, LOC's were infected with SARS-CoV-2 variants (Pre-Alpha, Delta and Omicron) at a high and low multiplicity of infection (MOI) of 1 or 0.01, respectively, on the apical surface for 2 hrs.
- Samples were collected from the apical surface on day 0, 2, 4 and 7 post-infection. Cells were also harvested for gene expression analysis or fixed for IF imaging at the experimental end-point.
- SARS-CoV-2 infection was analysed by plaque assay in Vero E6 cells to determine live-viral load by plaque forming units (PFU), as well as SARS-CoV-2 RNA expression using genesig® COVID-19 real-time PCR assay.

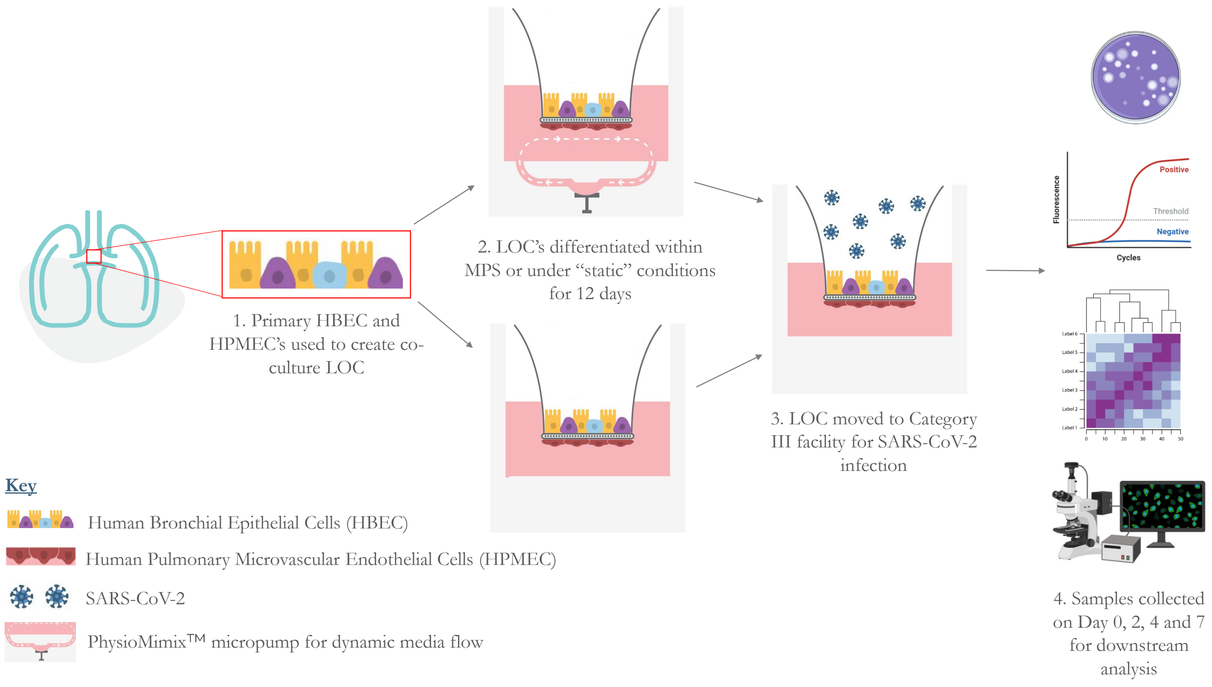

Figure 1. Schematic of upper airway LOC's using PhysioMimix™ OOC MPS or "static" conditions and infection of SARS-CoV-2 infection. *1. Primary human bronchial epithelial cells (HBEC) and human pulmonary microvascular endothelial cells (HPMEC) were expanded in tissue culture.* ***2.*** *The apical surface of Transwell® inserts were seeded with HBEC and HPMEC seeded on the basolateral side to create a coculture, LOC model. LOC's were either grown in static culture or CN-Bio Barrier (MPS-12) plates within the PhysioMimix™ system with a dynamic flow of media and allowed to differentiate for 12 days at ALI.* ***3.*** *After 12 days, static and MPS LOC's were moved into the category III laboratory where they were infected with SARS-CoV-2 variants.* ***4.*** *On days 0, 2, 4 and 7 post-infection, samples were collected from the apical surface to be used for quantification of SARS-CoV-2 virus via plaque assay and qPCR and cytokine panel. Cell pellets were also retained for Nanostring gene analysis or fixed with 4% PFA for IF imaging.*

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Results

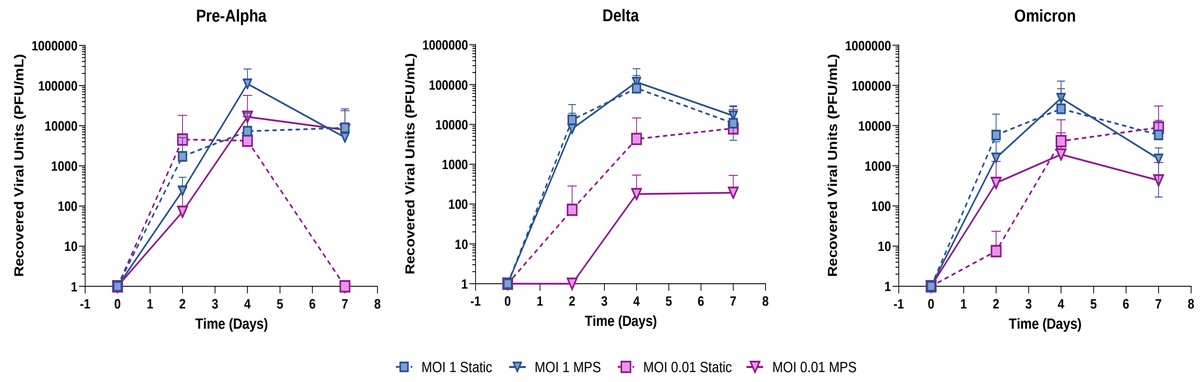

Figure 2. Upper airway LOC's SARS-CoV-2 variant infection at high and low MOI, in static and MPS conditions. *Upper airway LOC's were infected with SARS-CoV-2 variants, pre-Alpha, Delta and Omicron at an MOI of 1 or 0.01 for 2 hrs. Apical surface samples were collected on day 0, 2, 4 and 7. Live viral load was quantified by plaque assay in Vero E6 cells and expressed at plaque forming units (PFU)/mL. Data from n = 6 – 9 LOC's. all downstream experiments were conducted in triplicate.*

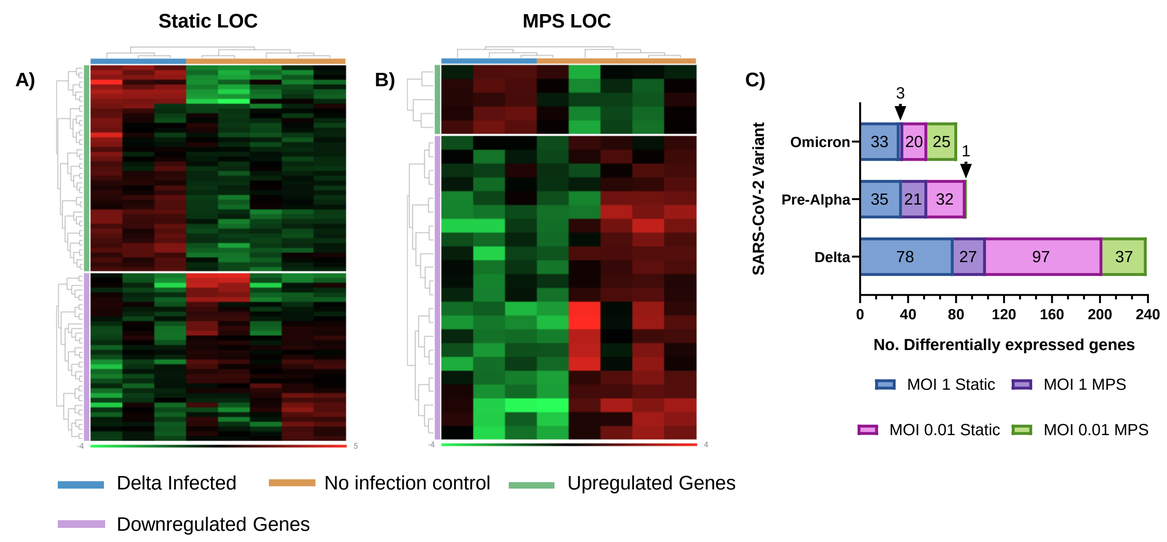

Figure 3. Gene expression analysis of SARS-CoV-2 infected MPS and static LOC's. *Cell lysates were collected, and RNA extracted on day 7, post-infection of SARS-CoV-2 variants (pre-Alpha, Delta and Omicron). Gene expression analysis was conducted using the NanoString nCounter Host Response Panel. Expression data were analysed using Rosalind® software, heatmaps display genes up or downregulated by greater than 2-fold change with a p value ≤0.05 compared to uninfected controls. Intensity score is shown as Log2 of fold-change, with green signifying reduced expression and red representing increased expression. A) Significant gene changes from static LOC's infected with Delta at an MOI of 1. B) Significant gene changes from MPS LOC's infected with Delta at an MOI od 1. C) Total differentially expressed genes from static and MPS LOC's infected with pre-Alpha, Delta and Omicron at an MOI of 0.01 and 1.*

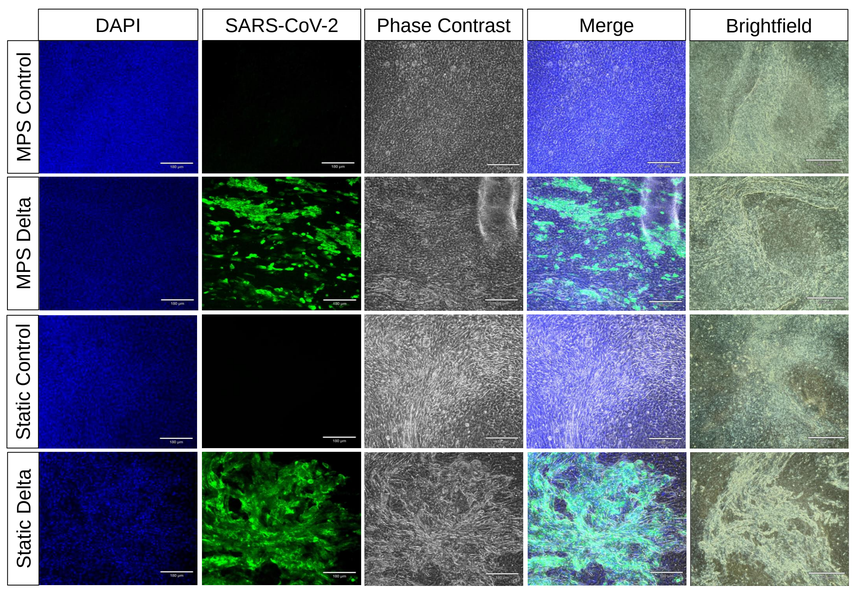

Figure 4. Comparison of Morphology and SARS-CoV-2 Delta infection in Static and MPS LOC. *On day 7, post-infection of SARS-CoV-2 Delta LOC's were fixed with 4% PFA and stained with Anti-SARS-CoV-2 nucleocapsid protein (Abcam) and Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) secondary (Abcam) and DAPI. Representative images shown were taken using ECHO Revolve IF microscope at 20x objective, brightfield images were taken at 10x objective.*

Conclusions

- The rate of infection was similar across all variants investigated, and in both the static and MPS LOC models.
- Overall, the static LOC showed the most gene expression changes following infection with SARS-CoV-2 compared to the MPS model.
- Delta resulted in the most gene expression changes in either model compared to pre-Alpha and Omicron. This corresponds to clinical observations that delta was a more virulent strain[4].
- Delta causes greater morphological changes and damage in the static LOC, compared to MPS – different morphology in MPS may give tissues more protection from SARS-CoV-2 cell damage.
- These LOC models have the utility to be implemented to understand the pathogenesis of numerous infectious diseases beyond SARS-CoV-2. As well as being used to identify novel therapeutics against emerging and current pathogens of concern.

Further Work

- Further analysis of gene expression data is underway to identify specific genes/pathways which are involved in SARS-CoV-2 infection in a static vs MPS LOC.
- Cytokine panel analysis will also be conducted to understand the LOC's host response to infection.