OVERCOMING LIMITATIONS OF ORGAN-ON-CHIP (OOC) TECHNOLOGIES TO ADVANCE THE CHARACTERIZATION AND MEDICAL MANAGEMENT OF CHEMICAL AND BIOLOGICAL (CB) THREATS

Bioprinted Functional Human Vasculature To Study Mechanisms Of Vasculitis

Claire Robertson Lawrence Livermore National Lab **William Hynes** Lawrence Livermore National Lab **Jonathan Adorno** Ohio State University **Javier Alvarado** Lawrence Livermore National Lab **Monica Borucki** Lawrence Livermore National Lab **Monica Moya** Lawrence Livermore National Lab

Species differences in viral susceptibility can complicate studying the biological effects of emerging pathogens. For example, SARS-CoV-2 is associated with widespread vascular dysfunction, whereas related viruses such as SARS-CoV-1 and MERS-CoV are relatively lung-tropic. Key models used to study vasculitis, such as k18-Ace2 transgenic mice, lack the human SARS-CoV-2 entry receptor on their endothelia and do not mimic vascular dysfunction. To address these issues, we employed our perfusable bioprinted human vasculature biomimetic model, which we have previously shown models human vascular barrier function, provides an anti-thrombotic endothelial surface, and responds to flow patterns by altering endothelial activation and adhesiveness. We sought to determine whether our bioprinted vessels could be used to model COVID-19 associated vascular damage, study underlying mechanisms, and test potential countermeasures.

To build vascular beds, we printed vascular beds using fugitive inks encased in a fibrin-gelatin hydrogel, then lined these vessels with human cerebral microvascular endothelial cells (hCMEC/d3) and cultured under flow until confluent. Vessels were then treated with either VSV pseudoviruses bearing the sars-cov-2 spike protein on their coats and TNF alpha, or TNF alpha alone. We evaluated and confirmed expression of ACE2 protein in 2D monolayers of hCMEC/D3, an immortalized cell line, as well as in primary endothelial cells from human umbilical cords (2 donors) and human aortic endothelial cells, however, we found no evidence of productive viral infection in any tested line. We were able to detect the circulating viruses using a SARS-CoV-2 spike antibody. We found that viral particles spread throughout the vascular bed and attached to the endothelium with no obvious geometric preference. Pseudovirally treated vessels showed greater surface expression of Vcam-1 especially in disturbed flow regions (i.e., bifurcations), and loss of VE-Cadherin bearing cell-cell junctions. We also noted broad spread abnormalities in nuclear structure in pseudovirally treated vessels, including syncytial formation and micronucleii. To confirm that pseudovirus could alter nuclear morphology in the absence of productive infection, we treated cells in monolayer culture and observed increased median nuclear area, increased NFkB signaling, and decreased cell proliferation, despite lack of productive infection.
In conclusion, we have demonstrated that endothelial cells are not productively infected by SARS-CoV-2, yet exposure of these cells to a pseudovirus with SARS-CoV-2 spike protein on its surface coat take on a profoundly activated cell state with alterations to nuclear morphology, inflammatory pathways, and immune cell receptor activation. This model provides accurate recapitulation of the vascular pathology in an emerging infection, which could accelerate understanding of mechanisms of susceptibility and help develop therapies to reverse vasculitis.

This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344. Funding provided by LLNL LDRD 21-FS-050 and 22-LW-088. LLNL-ABS-837156