SARS-CoV-2 Spike Protein-Induced Toxicity in 3D Engineered Vascular Networks

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Statement of Purpose: Endothelial dysfunction has been implicated in progression of COVID-19 due to high ACE2 expression of endothelial cells[1]. When infected, these cells release coagulation proteins as well as inflammatory cytokines that result in disruption of vessel networks[2]. To model this effect and determine the effectiveness of candidate drugs, we propose the use of induced pluripotent stem cell-derived endothelial progenitor cells encapsulated in collagen hydrogels. To simulate viral infection, we added SARS-CoV-2 spike protein to the cells at different time points and used a computational pipeline to quantitatively determine the effects of the protein on vessel networks[3].

Materials and Methods: Induced pluripotent stem cell-derived endothelial progenitor cells (iPSC-EPs) were differentiated using an established 7-day protocol[2]. To isolate endothelial progenitor cells, iPSC-EPs were stained using an anti-CD34 antibody and sorted using fluorescence activated cell sorting (FACS), Sorted cells were encapsulated in a 2.5 mg/mL type 1 collagen hydrogel. iPSC-EPs were cultured in endothelial growth medium supplemented with 50 ng/mL vascular endothelial growth factor for 7 days. Media was replaced daily. At select time points, either one or five days after encapsulation, 0.1 µg of SARS-CoV-2 spike protein (CSP) was added to simulate viral infection. The cells were exposed to CSP for 24 hours. After 7 days of culture, hydrogels were fixed, stained with rhodamine phalloidin, and imaged using a confocal microscope to visualize vascular network formation. An open-source computational pipeline was used to quantify vessel network formation[3].

Results: Addition of CSP to iPSC-EPs in collagen hydrogels resulted in significant reduction of vascular network formation, as measured by the computational pipeline. This effect was dependent on the time of CSP treatment, with CSP addition at later time points resulting in a greater change in network formation. Specifically, for both time points, there was a statistically significant decrease in the number of end points measured, which indicates a decrease in the number of viable iPSC-EPs. There was also a decrease in vessel connectivity as a result of CSP addition, since the largest vascular network that formed was made up of a smaller percentage of the total number of vessels observed. When CSP was added five days after encapsulation, in addition to the changes in cell viability and vascular network connectivity, there was also a decrease in the number of vessels observed. This endothelial dysfunction is also seen in patients affected by COVID-19[2]. Signaling from SARS-CoV-2 binding to ACE2 on endothelial cells induces a proinflammatory state, and one of the effects of this is a loss of VE-cadherin and detachment from the basement membrane[2]. The more significant change in network formation when CSP is added later is likely due to the iPSC-EPs' maturing over multiple days of culture. This would result in an increase in ACE2 expression and therefore their responsiveness to CSP.

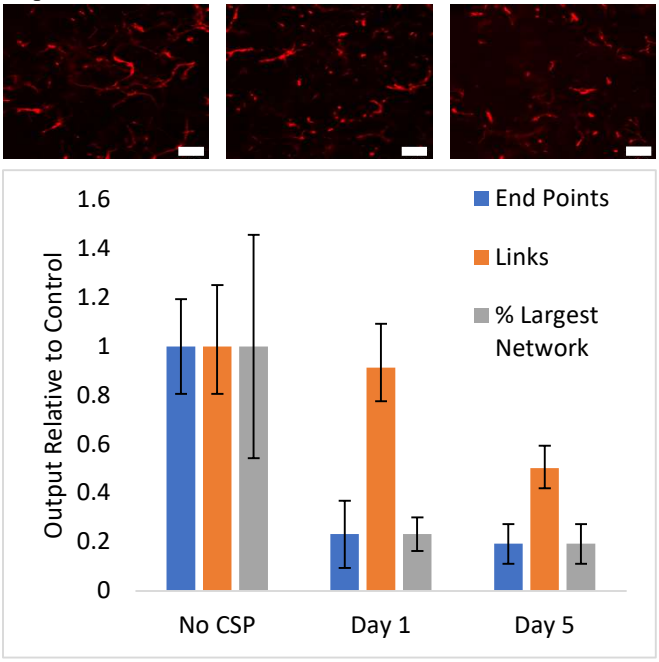

Figure 1: Impact of CSP on hiPSC-EP-derived vascular networks. Representative images of vascular networks after 7 days of culture and treated with (a) no CSP, (b) 0.1µg of CSP on day 1, and (c) 0.1µg of CSP on day 5. Scale bar = 200µm (d) Output from the computational pipeline showing a reduction in number of end points, links, and vessel connectivity.

Conclusion: Addition of SARS-CoV-2 spike protein to induced pluripotent stem cell-derived endothelial progenitors results in disruption of vasculature that mimics what is observed *in vivo*. Future work will include performing an indirect coculture with macrophages to verify immune cell activation upon CSP binding to iPSC-EPs.

References:

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[2] Libby, P. European Heart Journal (2020); 41(32)
[3] Crosby, C. Tissue Engineering A. 2019; 25(9)
[4] Lian, X. Stem Cell Reports. 2014; 3(5)