The Fibrin-derived Peptide FX06 Protects Human Pulmonary Endothelial Cells Against the COVID-19-Triggered Cytokine Storm

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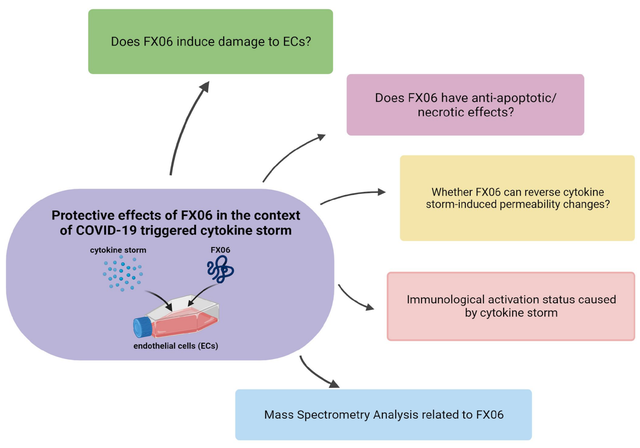
BACKGROUND

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has been a major health emergency since 2019. Endothelial dysfunction is a hallmark of COVID-19, leading to severe illness, i.e. multi-organ failure, coagulopathy, and death[1]. FX06, a fibrin-derived natural peptide, formerly known as $B\beta_{15-42}$, has shown beneficial effects not only for ischemia/reperfusion injury IRI in animal models, but also for numerous diseases (such as Ebola infection, COVID-19-induced respiratory distress syndrome)[2]. Therefore, it is a promising therapeutic candidate for endothelial complications such as capillary leak in COVID-19 and other infectious diseases. The aim of this project is to investigate whether FX06 can help to prevent COVID-19 progression *in vitro*.

AIM



METHODS

- Flow Cytometry Analysis
- Immunofluorescence
- Collagen-based Transendothelial Migration (TEM) Assay Under Static And Shear Stress Conditions
- Confocal Microscope based-High Content Screening

EXPERIMENT DESIGN

Mimic inflammatory status of COVID-19 infection

Treating endothelial cells with cytokine cocktails

Cytokine cocktails		
Cytokines	Mild	Severe
	Concentration, ng/ml	
IL-1α	20	20
IL-1β	10	10
IL-6	10	50
IL-18	50	50
TNF-α	100	100
IFN-γ	50	50
IL-1ra	20	50
IL-8	20	20
IL-10	10	20
IP-10	50	150

in the presence or absence of FX06

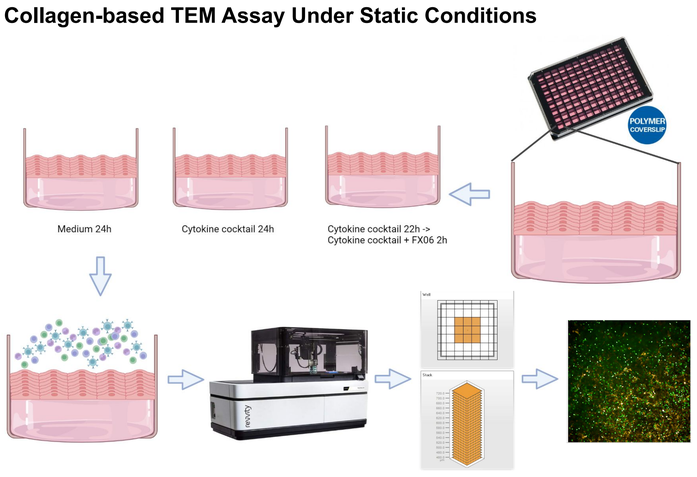
Endothelial cell assessment

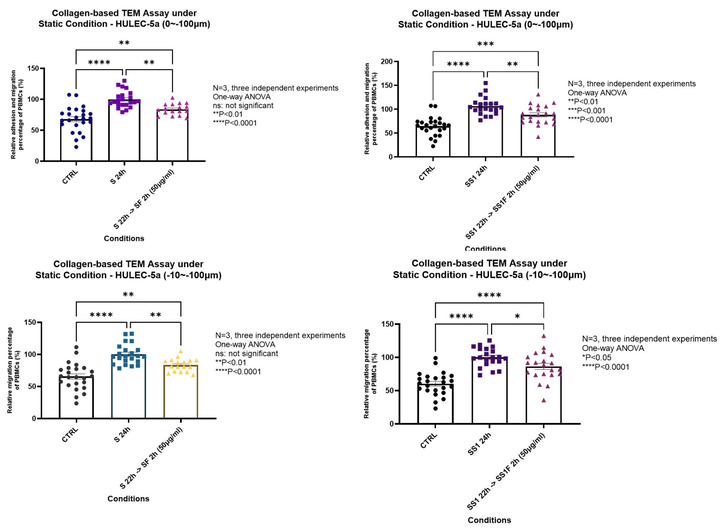
- Viability (apoptosis)
- Immunological activation (i.e. CD54, CD106, CD144, CD31, HLA-A,B,C, etc)
- Functional changes (transendothelial migration of PBMCs)

RESULTS

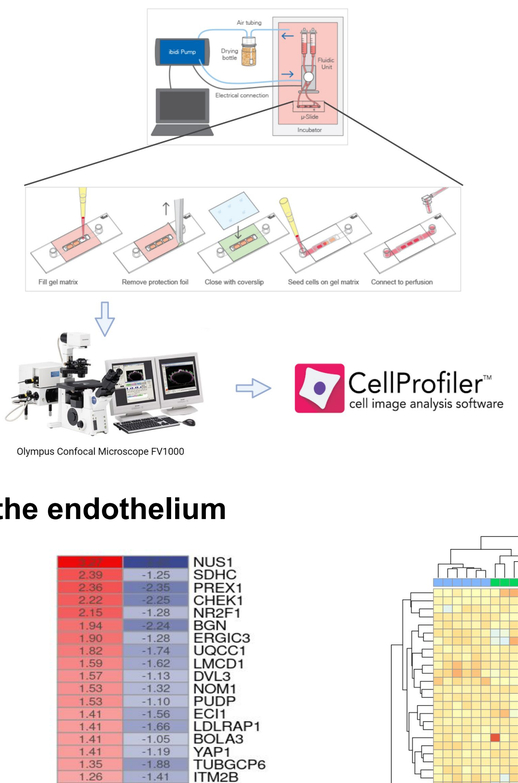
FX06 prevents TEM of PBMCs under static and shear stress conditions

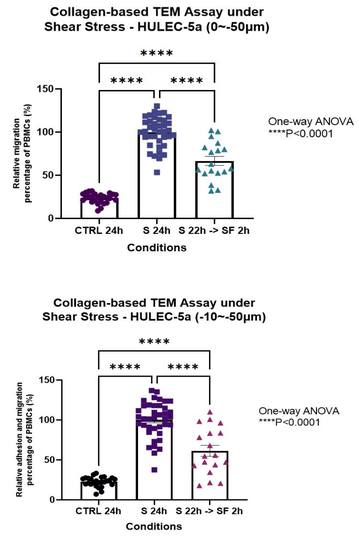
Collagen-based TEM Assay Under Static Conditions



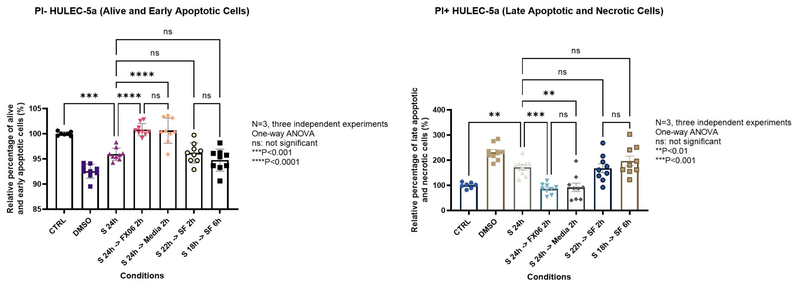


Collagen-based TEM Assay Under Shear Stress

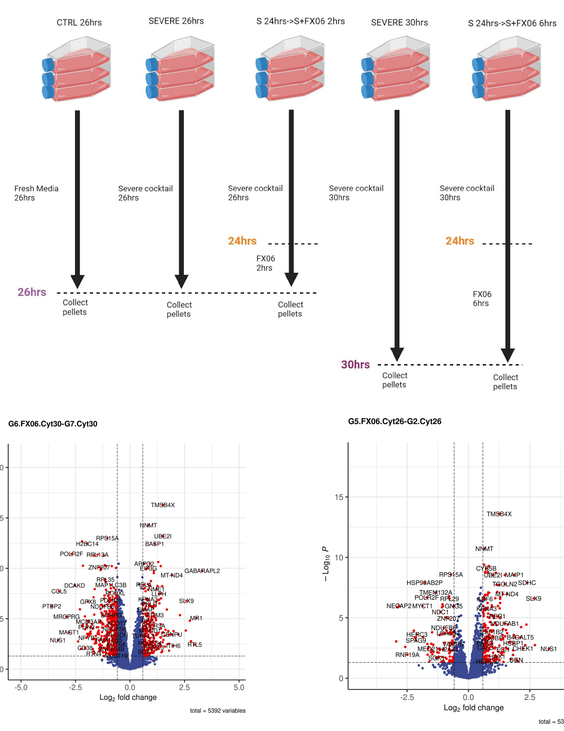


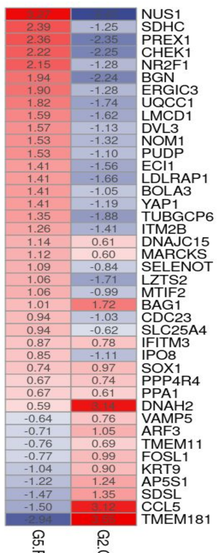


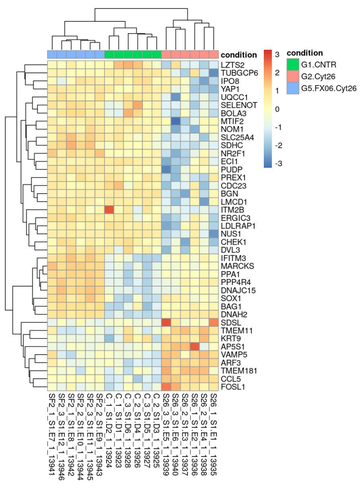
FX06 does not protect the endothelium from cytokine-induced cell death



FX06 alters protein expression to protect the endothelium





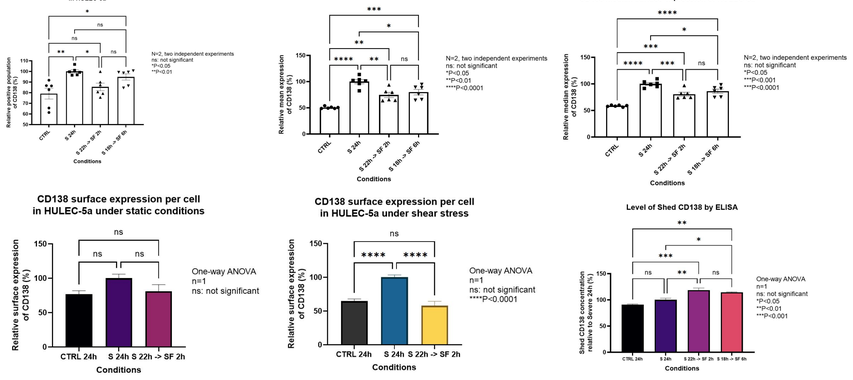


Conclusion:
Several interesting proteins found to have altered expression in the presence of FX06 after severe cytokine treatment.

Downregulated in FX06 treatment
- ROCK1: effector for small GTPase RhoA, role in control and maintenance of endothelial cytoskeleton;
- LGALS3: Involved in acute inflammatory responses including neutrophil activation and adhesion, chemoattraction of monocytes macrophages, opsonization of apoptotic neutrophils, and activation of mast cells;

Upregulated in FX06 treatment
- NUS 1: encodes Nogo-B receptor, loss of Nogo-B negatively impacts cell adhesion, reduced levels of Rho-A and F actin containing filaments

CD138 – A potential marker for therapeutic response?



FUTURE PLANS

- Collagen-based TEM assay under shear stress regarding cytokine storm co-incubated with FX06;
- Comprehensive flow cytometric analysis of pulmonary EC phenotype;
- Identification of secondary target structures/signal transduction pathways in EC responsible for the protective effect of FX06 on TEM of PBMC, focusing on cytoskeletal glycocalyx changes (e.g, F-actin, ROCK, heparanase, surface and shed syndecan-1);
- Further investigation in terms of SARS-CoV-2 Spike Protein S1 combined with cytokine storm.

REFERENCES

1. Norooznezhad, A. H., & Mansouri, K. (2021). Endothelial cell dysfunction, coagulation, and angiogenesis in coronavirus disease 2019 (COVID-19). Microvascular research, 137, 104188.

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