COVID-19-ASSOCIATED CYTOKINE STORM IN MECHANICAL CIRCULATORY SUPPORT: AN IN-VITRO STUDY

By

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Table of Contents

Abstract

Catheter-based mechanical circulatory support (MCS) systems are increasingly utilized for therapy in advanced heart failure patients. The need for MCS has grown in parallel with COVID-19-associated heart dysfunction. Following COVID-19 infection, there is a rapid increase in levels of circulating pro-inflammatory cytokines referred to as a “cytokine storm”. This “cytokine storm” has been shown to disrupt the immune system drastically. Proteins (e.g. cytokines) are sensitive to their biochemical environment, undergoing conformational changes that can ultimately affect biological function. It is well recognized that MCS impart shear stress on the blood and circulating components. However, there remains a lack of understanding as to the impact of MCS devices on cytokines, particularly COVID-19-associated cytokines. Here, we utilized an Impella5.5 to circulate a cytokine mixture (IL-6, IL-8, TNFα, IL-1β) representative of the COVID-19 “cytokine storm”. We hypothesize that MCS-induced flow alterations - i.e. turbulence and shear stress, will alter cytokine structure and binding ability as indicated by gel electrophoresis and ELISA binding. We found a significant increase in cytokine-antibody binding via ELISA after 1 hour of shear exposure, compared to the resting sample. Gel electrophoresis of samples allowed for evaluation of molecular mass against protein standard (2-25 kDa). Notably, single bands of IL-6 and TNFα were visible in the resting samples; however, sheared samples showed double bands at the same location, indicating influence of shear on a portion of IL-6 and TNFα population size. Our findings suggest MCS play a role in cytokine function and ultimately inflammation in a wide range of diseases. With further translation and defining mechanisms involved, these findings could help to inform improved MCS therapy.

Introduction

Mechanical circulatory support (MCS) devices have been increasingly used to treat patients with advanced stages of heart failure.[1] Such devices are unique because they can continue circulating blood in the system for a varied amount of time as patients often await heart transplantation.[2] In order to accomplish such a feat, specific MCS devices called Ventricular Assist Devices (VADs) can be inserted into the left ventricle or can just take blood from the atrium and circulate it back to the aorta. Those that are inserted into the left ventricle are catheter-based systems. The goal of both forms is to continue to oxygenate our tissues, however, these VADs do not mimic the functions of the heart as they impart higher physical forces than does the normal heart.[3] As such, investigating these devices remains pertinent to understanding the interactions they carry with the cellular systems within the heart.

In previous studies by our research group, a high-speed micro-axial, catheter-based MCS device, the Impella 5.5 (ABIOMED, Danvers, MA), was tested against a centrifugal MCS device, the CentriMag.[4] It is important to note that neither device rotates at physiological levels but not native hemodynamics. The CentriMag device has been previously demonstrated to indicate low hemolysis rates via computational fluid dynamics.[5] This foreign rotation rate produces an inherent caution when utilizing these devices and was a significant driver in this comparison. The Impella device at its highest setting (P-9) can rotate at roughly 46,000 rpm, which maintains a flow rate of 5L/min, which is seen physiologically.[6] However, we sought to investigate this rotational speed of the rotor and precisely what its effects were on different cellular markers as related to platelets and thrombosis. We demonstrated that both systems had a limited effect on

hemolysis (breaking of the red blood cells) and did not cause platelet granulation which can often lead to harmful clotting in these vessel-based systems. Furthermore, the Impella 5.5, compared to the centrifugal device, demonstrated lower microparticle generation, which can similarly lead to less clotting in the system. These results affirmed that the new Impella 5.5 device was a good substitute and potential replacement for the previously used standard for these MCS devices. Regardless, both devices still impart a level of shear stress on the surrounding environments that can affect the way that the system functions.

Shear stress is an important concept when identifying the interactions of the MCS devices on the surrounding cellular environments.[7] Defined as a force leading to the deformation of a material (often platelets in previous studies) along a plane due to imposed stress, Shear stress begins to play a significant role when the imposed centrifugal force or acceleration due to a linear flow rate begins to interact with platelets in a cellular environment.[8] Frequently these levels of shear stress lead to thrombosis or whole blood clotting and can lead to further complications with blocked blood vessels and ultimately ischemia.[9] The laboratory has done a significant amount of work to characterize this role of shear stress, and the comparison noted above was the next step in characterizing how the MCS devices' non-physiological shear stress affects the cellular envrironment.[10]

Recently, with the introduction of COVID-19, there has been a significant increase in heart failure due to complications with the virus.[11] In a setting like the emergency department, quick stabilization methods are often utilized to stabilize the patient for future treatment, as such acute MCS devices have been increasingly used by

physicians and nurses with patients undergoing COVID-19 cardiac complications.[12] Similarly, it has been noted that in patients with just COVID-19, there is a significant increase in the number of cytokines, which are pro-inflammatory proteins, in the bloodstream. However, in patients undergoing both cardiac complications, often heart failure, and COVID-19 infection, there is an even more significant increase in the concentration of these cytokines in the bloodstream, often denoted the "cytokine storm".[13] Here, we investigated specific cytokines that were found in the cytokine storm, interleukin-6, interleukin-8, interleukin-1beta, tumor necrosis factor-alpha. A baseline study demonstrated that in ordinarily healthy adults, cytokine concentrations in serum plasma were 2.91, 23.9, 2.04, and 3.21 pg/mL for IL-6, IL-8, IL-1B, and TNFa.[14] A following study demonstrated that following severe SARS infections, median levels of cytokines increased to 45, 27, 5, and 10 pg/mL.[15] It has been noted that proteins (i.e., cytokine) are sensitive to their physical and biochemical environments.[16] They often respond quickly to sudden changes in their environments, which can be conformational and affect their long-term physiological function.[17] These cytokines play a significant role in our immune response signaling leukocytes and other cell types to fight infection.[18] If their ability to accomplish this is compromised due to their destruction physiologically, patients can be more susceptible to infections and may be unable to fight current infections (i.e., COVID-19), leading to morbidity.[19] Ultimately, our project aims to discern the effects of the shear stress imposed by the Impella device on the cytokine's structure and binding ability. We hypothesize that the MCS-induced flow alterations - i.e., turbulence and shear stress, will alter cytokine structure and binding ability.

Materials and Methods

Endpoint 1A: Effect of Shear and Turbulent Flow on Cytokine Structure

Preparation of the Samples and Solutions

To examine the effect of the MCS system on the structure of cytokines, a modified Impella 5.5 system setup was created. A flint glass tube was filled with 15mL of Phosphate-Buffered Saline (PBS), the line marked, and the PBS was disposed of. In parallel, frozen human cytokines of IL-6, IL-8, TNFa (R&D Systems) at a concentration of 25ug, and IL-1B [10ug] were dissolved in 500uL of 1XPBS and inverted several times to ensure the cytokine stocks were fully dissolved. The final concentration of these stock cytokine solutions was 50ug/mL for IL-6, IL-8, TNFa, and 20ug/mL for IL-1B. Once completed, 6.2mL of 1X PBS was added to the empty flint glass tube, and 250uL of each cytokine stock solution was added to the flint glass tube for a final volume of 8mL. After each cytokine addition, the solution was mixed repeatedly via pipetting to ensure proper mixing of the cytokines into the 1X PBS. After mixing, the final concentration of cytokines in the 8mL flint glass tube solution was 2.81ug/mL for IL-6, IL-8, TNFa, and 1.13ug/mL for IL-1B. This solution was then chilled on ice, and the flint glass tube was covered. Resting samples were also prepared, wherein 800uL of 1XPBS solution was added to 1, 1.5mL microcentrifuge tubes. 45uL of the respective cytokines were added to one tube and then mixed by pipetting for the same final concentration as what was found in the 8mL flint glass tube solution. These tubes were then closed and placed once for proper chilling.

MCS run and sample collection

A temperature probe was submerged into the flint glass solution, and the temperature was recorded. The Impella 5.5 was then submerged into the solution and fixed 1.5inches from the bottom of the flint glass tube via a silicone stopper that encapsulated the area of the cylindrical circle and created a closed loop system except for the catheter that is attached to the Impella 5.5 and allows for the addition of purge solution into the system.

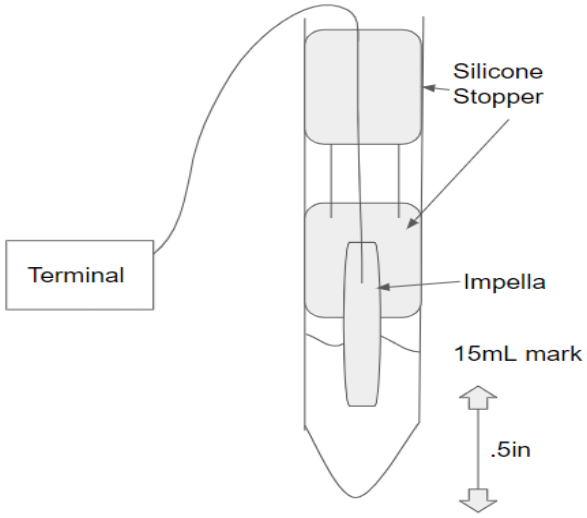

Figure 1. Modified Impella setup indicating Impella placement, connection to terminal, purge system, and dimensions.

The purge solution of 1XPBS was prepared simultaneously. First, the purge system was primed via a de-airing system as offered by the Impella interface, and the 1XPBS entered into the purge solution. After the preparation of the setup, the Impella 5.5 was run at P-1, equating to 12000 rpm for a total time of 1 hour or until the flint glass

tube had reached the 15mL mark previously set due to an influx of purge solution throughout the run, whichever of the two came first. After the one-hour time point, the Impella 5.5 was removed from the system, and the temperature was rerecorded via the temperature probe. Immediately following the temperature recording, the final purge flow record in L/min was also recorded, and the remaining volume in the tube was measured if below the 15mL mark. After the volume was measured, a proportional amount of 1XPBS solution was added to the resting sample to mimic the purge solution added to the running sample. Samples were then prepared by taking two 400uL samples of the remaining volume in the flint glass tube. As a quick note, each 400uL sample should have contained 1.13ug of IL-6, IL-8, TNFa, and 0.45ug of IL-1B. For the resting samples, two 40uL samples were aliquoted into microcentrifuge tubes and placed in ice. Finally, two, 200uL resting samples were taken and placed into 1.5mL microcentrifuge tubes, and similarly, two 200uL running samples were taken and placed into 1.5mL microcentrifuge tubes for BCA testing later. These samples were then frozen in addition to the leftover running and resting solutions and were placed into a 15mL centrifuge tube and frozen in the -80℃ freezer.

Acetone Precipitation

160uL of chilled acetone (chilled in the -20℃ freezer) was added to each of the 2, 40uL resting microcentrifuge tubes for a final volume of 200uL. Similarly, 1600uL of chilled acetone was added to the two 400uL running samples for a final volume of 2mL. These samples were mixed via vortexing for 30 seconds and incubated for 60 minutes at -20℃. Following incubation, the samples were vortexed again for 30 seconds and then centrifuged at 20000xg for 30 minutes. If a pellet was not found after the initial

centrifugation process, the samples were recentrifuged for another 30 minutes at 200000xg. If a pellet was located visually, the acetone was discarded, and the supernatant was decanted. The pellets were then allowed to dry for 30 minutes. After the final 30-minute drying period, the samples were considered ready for gel electrophoresis preparation once all the acetone had evaporated.

Gel electrophoresis

Sample buffer was prepared by aliquoting 450uL of tricine sample buffer solution into a 1.5mL microcentrifuge tube. Next, 10uL of beta-mercaptoethanol was added to the tricine sample buffer and mixed well by pipetting. Previously prepared pellets were resuspended in 60uL of prepared sample buffer and mixed via pipetting. Samples were then heated in a water bath at 70℃ for 10 minutes. In a 16% denaturing Tris/Tricine gel, 5uL of protein ladder was added into lane 3, and 25uL of each of the four samples (2 running, and two resting) were added respectively in the order of one resting, two running, and one resting in lanes 3-7. The gel was then run for 2 hours at 150V. After two hours, the glassware was cleaned with 50% nitric acid and rinsed with high-quality deionized water to fix the gels.

Development accelerator solution was then prepared by placing a 1 L Erlenmeyer flask on a magnetic stir plate and adding a stir bar. 950mL of DI water was added to the flask, and 50g of development accelerator agent was slowly added. DI water was again added until the 1L fill mark. The solution was then allowed to stir until the accelerator agent had fully dissolved. In parallel, the fixative enhancer solution was prepared in a small glass beaker by adding 400mL of reagent-grade methanol, 80mL of

reagent-grade acetic acid, 80mL fixative enhancer concentrate, and 240mL of DI water for a total solution volume of 800mL. The gel was then removed from the electrophoresis tank and submerged into a fixative solution for 20 minutes with gentle agitation. Once complete, the fixative enhancer solution was discarded, and the gel was rinsed in DI water and submerged in DI water for 40 minutes post-fixing. The water was then discarded, and the gel re-rinsed and re-submerged in DI water for another 40-minute incubation period. Again, the water was discarded. Immediately before use, the prep staining solution was prepared by adding 35mL of DI water, 5mL of complex silver solution, 5mL of reduction moderator solution, and 5mL of image development reagent into a small Erlenmeyer flask with a PTFE-coated stir bar. Finally, 50mL of room temperature development accelerator was added to the flask and allowed to stir briefly. The gel was then submerged in the staining solution for 20 minutes with gentle agitation. The staining solution was discarded, and the gel was submerged in a 5% acetic acid stop solution for 15 minutes. The acetic acid stop solution was discarded, and the gel was rinsed for 5 minutes in DI water. Upon completion of the rinse, the gels were either prepared for drying and refrigeration or for immediate photography.

BCA Protein Quantification

A BCA protein quantification assay was run to verify and quantify the amount of protein in the solution. After the loop run, the samples put away for BCA were allowed to dethaw on ice. While dethawing, Serial dilutions of the stock albumin standard were taken to achieve our standard solutions. 15ul of the stock 2.0mg/mL albumin ampule was added to 735uL of 1XPBS in a 1.5mL microcentrifuge tube for a final concentration of 40ug/mL in standard A. 375uL of standard A was added to 375uL of 1XPBS for

standard B at a final concentration of 20ug/mL. The steps were repeated until all stocks were created at 40ug/mL, 20ug/mL, 10ug/mL, 5ug/mL, 2.5ug/mL, 1ug/mL, 0.5ug/mL, and 0ug/mL. The stock solutions were then placed in ice until further use. The Micro BCA working reagent was prepared by combining 2.5mL of reagent A, 2.4mL of reagent B, and 0.1mL of reagent C in a 5mL centrifuge tube and then mixed by pipetting. A 96-well plate was used, and duplicate sets ran for both standards and samples. 150uL of each standard was added in duplicates, and similarly, 150uL of each sample was added in duplicates. To each well with a standard or a sample, 150uL of working reagent was added, and the sealing cover was used to cover the well plate. The plate was then incubated at 37℃ for 2 hours and, once complete, allowed to cool to room temperature. A spectrophotometer was set to 562nm, and the endpoint was measured for the area designated with standards and samples. The average absorbance was taken for all the standards between the duplicated and a standard curve created for the absorbance reading vs. the concentration of the standard. This curve was then used to determine the protein concentration of each unknown sample.

Endpoint 1B: Effect of shear stress and turbulent flow on cytokine intactness

Blood Collection and Cytokine Preparation

Fresh porcine whole blood was obtained from the University of Arizona campus agricultural center. Blood was collected into an ACD-A solution (85mM trisodium citrate, 78mM citric acid, 111mM dextrose) to a final concentration of 10% v/v ACD-A in blood. Platelet-rich Plasma was obtained via centrifugation of the ACD-A whole blood at 300g for 15 minutes.

Stock cytokines were prepared at concentrations of IL-6 (4.5ug/mL), IL-8 (4.5ug/mL) , TNFα (4.5ug/mL) and IL-1 (1.8ug/mL) and added at the relative concentration to the PRP collected above. The PRP was then mixed via a 30cc syringe, and a resting sample collected.

Loop run and sample collection

An Impella 5.5 was affixed within a closed loop in-vitro system made of Tygon hemocompatible tubing. The setup was created so that the inflow and outflow canula were separated and the device can mitigate retrograde flow into the inflow canula. The closed loop system featured two lure-connectors that allowed for sample collection. Clamps were then added around the outside of the tubing to set the system at 60 mmHg and this was pressure tested prior to use via two Omega pressure transducers. 270mL of cytokine porcine PRP was loaded into the loop via 30cc syringes through the lure connector. Bubbles were removed from the system via the addition of cytokine PRP and the intake of the bubbles through a syringe in the opposing port. Once there were no remaining bubbles in the system, the purge system was rid of air via the de-airing method on the Abiomed controller. A 5% glucose solution was created by diluting 5 grams of glucose into 100mL of distilled water and mixed. The purge system was then primed via the glucose solution. Two 5cc syringes were filled with remaining cytokine PRP and screwed into the lure-connectors to mitigate pressure flow upon sample taking. The loop was then set to P-9 (33,000 RPM) and run for a total of four hours. Samples were collected at 0 minutes, 5 minutes, 1 hour, and 4 hours, with a resting sample taken at each. These were then frozen at -80℃ prior to further use.

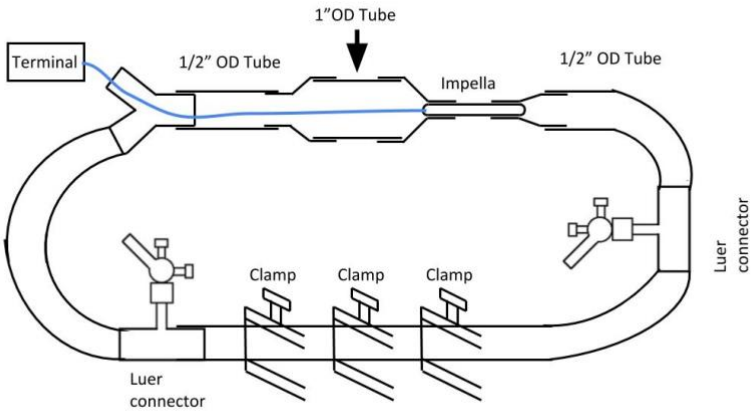

Figure 2. Closed loop Impella setup indicating Impella placement, connection to terminal, purge system, pressure clamps, lure connectors and dimensions.

ELISA Assay

To determine the antibody binding ability of the cytokines post-interaction with the Mechanical Circulatory Support device, an ELISA assay specific to porcine cytokines was conducted for IL-6, IL-8, IL-1B, and TNFa. 1X Wash buffer was prepared by diluting 20mL of 20x room temperature wash buffer concentrate into 380mL of DI water. Diluent was then prepared by spinning down biotin conjugate and adding 100uL of 1X assay diluent into a glass tube with the biotin conjugate. The vial was then mixed gently by pipetting and labeled as 1X assay diluent. PRP with cytokines was diluted two-fold prior to use. Standards were then diluted by spinning down the initial vial of lyophilized standard. 400uL of 1X assay diluent was added to the lyophilized standard to prepare a 50ng/mL solution, which was dissolved by gentle mixing. 100uL of cytokine

standard solution from the vial of reconstituted standard was added into a new tube and mixed with 400uL of 1X assay diluent to create a 10,000pg/mL standard solution. Using the 10,000pg/mL standard solution, serial dilutions were created by adding 200uL of the preceding concentration and 300uL of 1X assay diluent into each tube to create standard that were at 4000, 1600, 640, 256, 102.4, 40.96, and 0pg/mL. Upon completion and 15 minutes prior to use a 1X streptavidin-HRP solution was created by spinning down the streptavidin-HRP and mixing by pipetting. The streptavidin-HRP stock was then diluted 320-fold with 1X assay diluent.

Using a 96 well plate, 100uL of each standard were added to the appropriate wells, and 100uL of dilute samples were added to their appropriate well. The samples were allowed to incubate at room temperature for 2.5 hours. The solution was then discarded, and wells washed 4 times with 1X wash buffer. 300uL of 1X wash buffer was added to each well and removed via pipetting. After the last wash, remaining wash buffer was removed via decanting and the plate cleaned by blotting paper towels against it. 100uL of prepared biotin conjugate was then added to each well and incubated for 1 hour at room temperature with gentle shaking. The solution was then discarded, and the wells was again 4 times with 1X wash buffer and dried. 100uL of streptavidin-HRP was added to each well, and the plate incubated for 45 minutes at room temperature with gentle shaking. The solution was then discarded and washed again via the same procedural steps as before. 100uL of TMB substrate was then added to each well, and the plate allowed to incubate for 30 minutes at room temperature in the dark. Finally, 50uL of stop solution was added to each well and the side of the plate was tapped to gently mix.

The plate was then read in a spectrophotometer at 450nm, and a standard curve of absorbance vs cytokine concentration was created. The unknown samples were then compared to the standard curve to identify the corresponding absorbance and binding ability of our unknown samples.

Aggregation Assay

Finally, to determine platelet aggregation when exposed to the cytokine storm, a light transmission aggregometer was utilized. Porcine PRP was collected from the Lure ports at 0, 5min, 60min, and 240min from the running loop setup. Platelet Poor Plasma (PPP) was then obtained by centrifuging the 600uL of PRP at 5000xg for 5 minutes. 300uL of corresponding supernatant PPP was aged into glass tubes used for blanking the aggregometer. 300uL of PRP was then added into each glass tube, and the COVID cocktail added into their respective tubes with 1X PBS acting as our control sample. The glass tubes were then incubated at 37℃ for 15 minutes, stir bars were added and finally the agonists (12uM Calcium Ionophore, and 5ug/mL Collagen, and 20uM ADP) were added into the tubes and aggregation was run. Area under the generated curve as measured as platelet aggregation.

Results

Binding ability and structure of cytokines

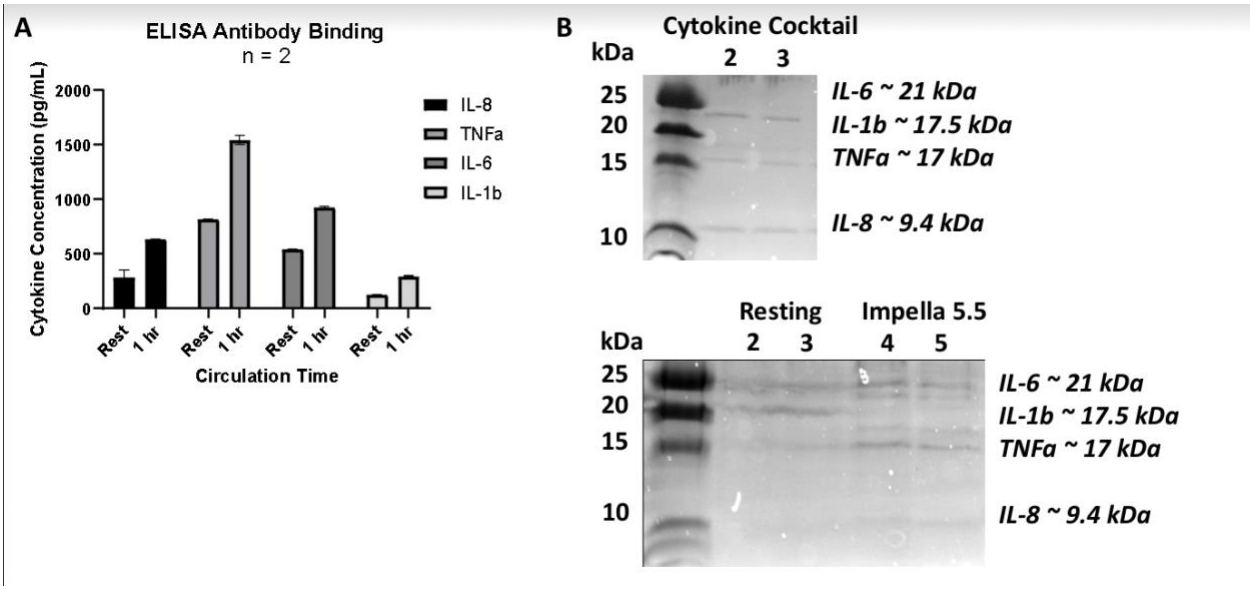

Figure 3. Effect of shear flow and turbulence pattern on cytokine binding ability and structure. (A) IL-6, IL-8, TNFa, and IL-1B (in PBS) were quantified via antibody binding in fluorescent-based ELISA after 1 hr circulation through a closed loop system with an Impella 5.5 (P9 flow level). (B) Cytokines were combined into a “COVID-19 Cocktail” and samples were taken before and after exposure to Impella 5.5 flow for 1hr (P1 flow level) and loaded into a 16.5% Tris-Tricine gel for electrophoresis.

The binding ability of our cytokines post exposure to the Impella rotor at 12,000 RPM was measured by our ELISA antibody assay in figure 1A demonstrating concentration vs circulation time. Similarly, covid-cocktail cytokines were via gel electrophoresis and individual bands were identified at the respective molecular weights of each cytokine in the resting samples (figure 1B), however duplicate bands were

identified at surrounding the molecular weights of the cytokines in samples exposed to the Impella 5.5 at 12,000 RPM.

Platelet Aggregation

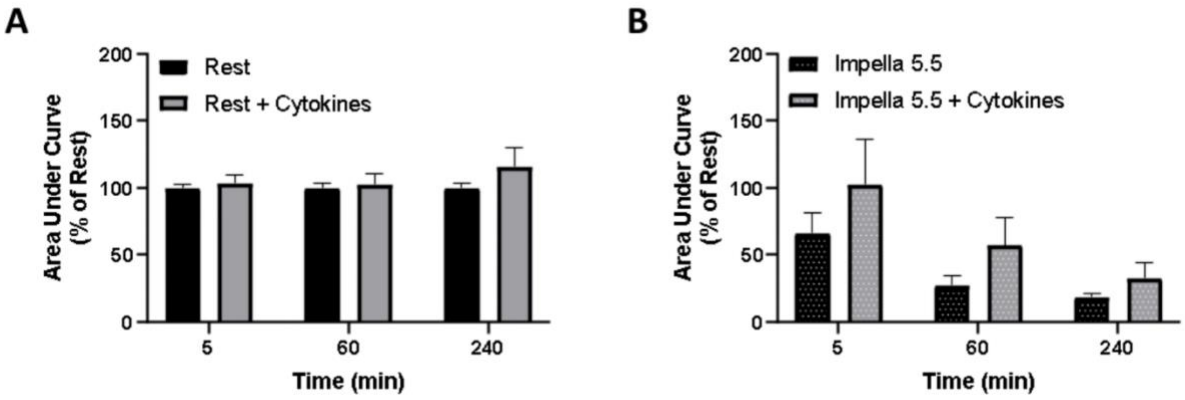

Figure 4. ADP-Mediated Platelet Aggregation. Porcine platelet-rich plasma (PRP) alone or incubated with "COVID-Cocktail" cytokines were stimulated to aggregate via addition of 1mM CaCl2 and 20uM ADP. Data are reported as area under the curve of platelet aggregation signal and are relative to aggregation seen on platelets at rest during corresponding time points. Aggregation was measured after 5, 60, and 240 minutes of incubation under (A) resting conditions; (B) circulation via Impella 5.5. All values are displayed as mean ± SEM of data collected from N ≥ 4 pigs.

To evaluate platelet aggregability post exposure to cytokines and the Impella device, platelet aggregation was stimulated with Calcium Ionophore, Collagen, and ADP. ADP induced aggregation acts as an agonist of P2Y1 and P2Y12 receptors, where we observed a decrease in aggregation when exposed to the Impella device as compared to resting samples, and a further decrease in aggregation between Impella + Cytokine samples, as compared to just Impella samples alone (Figure 4). However,

ultimately these changes were not statistically significant between resting samples and those of the Impella.

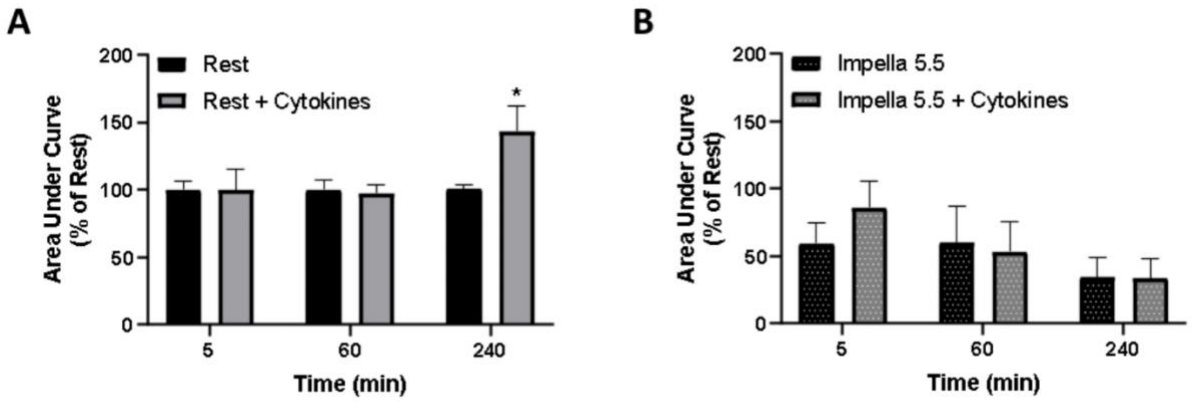

Figure 5. Collagen-Mediated Platelet Aggregation. Porcine platelet-rich plasma (PRP) alone or incubated with "COVID-Cocktail" cytokines were stimulated to aggregate via addition of 1mM CaCl2 and 5uM Collagen. Data are reported as area under the curve of platelet aggregation signal and are relative to aggregation seen on platelets at rest during corresponding time points. Aggregation was measured after 5, 60, and 240 minutes of incubation under (A) resting conditions; (B) circulation via Impella 5.5. All values are displayed as mean ± SEM of data collected from N ≥ 4 pigs; * indicates p-value < 0.05.

Collagen platelet aggregation is dependent upon integrins, or proteins that attach the extra cellular matrix to the cytoskeleton. Collagen platelet aggregation demonstrated a significant difference in resting PRP exposed to cytokines at the t = 240 minute time point. Additionally, a similar trend of decreased activation when exposed to the Impella as compared to resting samples at all time points were noted, yet not similar trend of additional decrease when exposed to cytokines for the Impella samples. (Figure 5). Ultimately again, these changes were not statistically significant in the variations of aggregations.

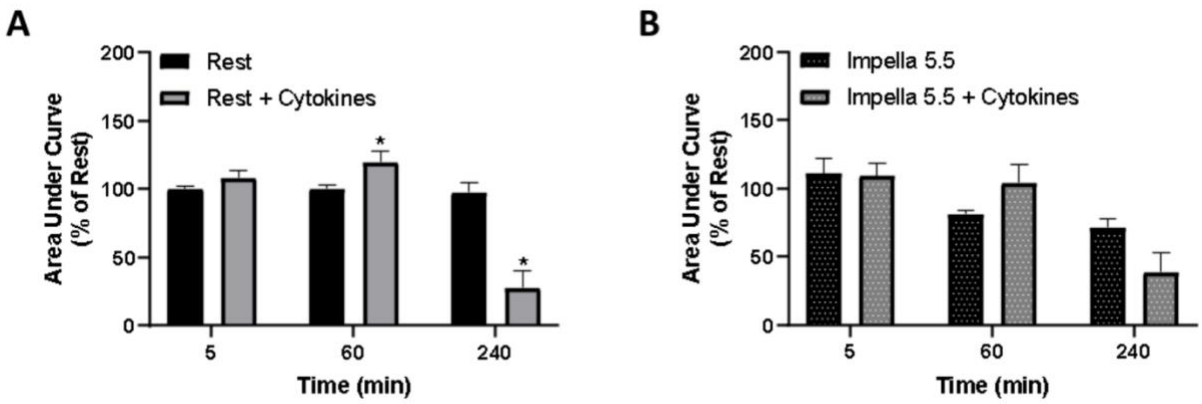

Figure 5. Collagen-Mediated Platelet Aggregation. Porcine platelet-rich plasma (PRP) alone or incubated with "COVID-Cocktail" cytokines were stimulated to aggregate via addition of 1mM CaCl2 and 5uM calcium ionophore. Data are reported as area under the curve of platelet aggregation signal and are relative to aggregation seen on platelets at rest during corresponding time points. Aggregation was measured after 5, 60, and 240 minutes of incubation under (A) resting conditions; (B) circulation via Impella 5.5. All values are displayed as mean ± SEM of data collected from N ≥ 4 pigs; * indicates p-value < 0.05.

Finally, resting, COVID samples exposed to calcium-ionophore demonstrated statistically significant differences in platelet aggregation at the t = 60 min point which indicated a significant increase in aggregation as compared to resting PRP alone, and a significant decrease in aggregation at t = 240 min when compared to resting PRP alone. For samples exposed to the Impella 5.5 there were no significant differences compared to resting samples, and within the Impella 5.5 group there was a decrease in aggregation from t = 5 min to t = 240 min (figure 6).

Discussion

Mechanical Circulatory Support Devices continue to emerge as the new standard of care for acute heart failure patients, stemming from their ability to stabilize a patient and allow for additional treatment moving forward.[20] However, the variations in design across MCS devices and their non-physiological flow rate can affect the surrounding cellular environment. Although there has been previous research evaluating the effect of these devices' shear stress and turbulent flow components on erythrocytes and thrombocytes, more research needs to be done to elucidate the effects on proteins and their downstream biochemical interactions.[21] As such, our study noting an exciting combination of COVID-19 infection and acute heart failure in patients that leads to an increase in the level of pro-inflammatory cytokines in the body sought to elucidate such an effect of the MCS devices of the structure, binding ability, and interaction with platelets when exposed to a clinically used MCS device. Cytokine binding ability was found to be insignificant, yet the overall trend identified that after exposure to the MCS device, the binding ability of the cytokines increased. Overall, our results suggest that the MCS device is altering the binding ability of the cytokines in a manner that deems that there is more surface area to bind to the corresponding antibodies of the assay.

These observations offer insight into what might be occurring within the physical environment of the protein, wherein the shear stress and turbulent flow might be breaking the cytokines into smaller pieces, thereby increasing their surface area of the binding and leading to an increased binding concentration. Furthermore, identifying a lack of single and even doublet bands in certain circumstances when analyzing the gel indicates that, at some level, the molecular weight of the cytokines post-interaction with

the MCS device is changing. This presents an interesting question moving forward: Can the actual change in structure be identified and does this change depending on the type of flow each device utilizes. As previously mentioned, there are multiple methods of continuing to circulate the blood, whether it be centrifugal or axial. As this project moves forward, identify how these two flow patterns affect. Specifically, proteins could be interesting in examining the corresponding change in function that these proteins take on in the respective signaling pathways and within their biochemical environment. All in all, additional work needs to be completed to characterize the corresponding effect on function as there have been issues with validating the levels of cytokines when running the gel electrophoresis assay and locating the respective bands since the molecular weight of the cytokines on our gels is towards to bottom. Ultimately, the next step in this specific aim would be to then characterize the change in function of the cytokines by utilizing an assay that can identify the respective roles of the cytokines, for example, in signaling the release of lymphocytes when an infection has been signaled and comparing resting cytokine lymphocyte concentrations as compared to those exposed to the Impella.

As we know, the cellular environment we are talking about is not in a vacuum. Previous work has demonstrated the effect of interactions of platelets and MCS devices, but also the reactiveness of platelets to small changes in their cellular environments, leading to thrombosis.[22,23] Our data demonstrated that for each of the three agonists, ADP, Collagen, and Calcium Ionophore, all of which work towards the mechanisms of platelet activation in different ways, demonstrated a slight decrease in the effect of cytokines in the resting sample to cytokines, PRP, and the running of the Impella.[24,25]

This data suggests that there may be a mechanism in which that the cytokines could be preventing platelet clotting when stimulated by the MCS device. Although the data was non-significant, this could pose an interesting thought when understanding when to use the MCS device acutely, as it could shift depending on what other infection a patient may have. As such, a table could be created in the future demonstrating that when patients have heart failure and X disease, these are the effects of the MCS devices on clotting and erythrocyte changes in a means of understanding the best length of use, rotational speed, and purge solution to use with specific patients.

Conclusions

Ultimately, there are two main avenues to pursue moving forward – identifying the effect of the MCS device on other proteins and their corresponding functions. This is done while simultaneously characterizing the differences in function depending on the type of rotational flow each device utilizes. The second further illustrates the effects of the MCS devices with co-infections and allows for informing best practices surrounding clinical uses of the device. Both avenues work harmoniously between the basic sciences and the clinical setting to improve patient care.

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References:

1. Dhruva, S. S., Ross, J. S., Mortazavi, B. J., Hurley, N. C., Krumholz, H. M., Curtis, J. P., ... & Desai, N. R. (2021). Use of mechanical circulatory support devices among patients with acute myocardial infarction complicated by cardiogenic shock. *JAMA network open*, *4*(2), e2037748-e2037748
2. Milano, C. A., & Simeone, A. A. (2013). Mechanical circulatory support: devices, outcomes and complications. *Heart failure reviews*, *18*, 35-53.
3. Birks, E. J. (2010). Left ventricular assist devices. *Heart*, *96*(1), 63-71.
4. Roka-Moiia, Y., Li, M., Ivich, A., Muslmani, S., Kern, K. B., & Slepian, M. J. (2020). Impella 5.5 versus Centrimag: a head-to-head comparison of device hemocompatibility. *Asaio Journal*, *66*(10), 1142.
5. Zhang, J., Gellman, B., Koert, A., Dasse, K. A., Gilbert, R. J., Griffith, B. P., & Wu, Z. J. (2006). Computational and experimental evaluation of the fluid dynamics and hemocompatibility of the CentriMag blood pump. *Artificial organs*, *30*(3), 168-177.
6. Anderson, M., Smith, D., Kane, P., Lee, R., Khalpey, Z., & Williams, J. (2021). Impella 5.5 direct aortic implant and explant techniques. *The Annals of Thoracic Surgery*, *111*(5), e373-e375.
7. Papaioannou, T. G., & Stefanadis, C. (2005). Vascular wall shear stress: basic principles and methods. *Hellenic J Cardiol*, *46*(1), 9-15.
8. Resnick, N., Yahav, H., Shay-Salit, A., Shushy, M., Schubert, S., Zilberman, L. C. M., & Wofovitz, E. (2003). Fluid shear stress and the vascular endothelium: for

better and for worse. *Progress in biophysics and molecular biology*, *81*(3), 177-199.

9. Koskinas, K. C., Chatzizisis, Y. S., Antoniadis, A. P., & Giannoglou, G. D. (2012). Role of endothelial shear stress in stent restenosis and thrombosis: pathophysiologic mechanisms and implications for clinical translation. *Journal of the American College of Cardiology*, *59*(15), 1337-1349.
10. Roka-Moiia, Y., Walk, R., Palomares, D. E., Ammann, K. R., Dimasi, A., Italiano, J. E., ... & Slepian, M. J. (2020). Platelet activation via shear stress exposure induces a differing pattern of biomarkers of activation versus biochemical agonists. *Thrombosis and haemostasis*, *120*(05), 776-792.
11. Vasudeva, R., Challa, A., Al Rifai, M., Polana, T., Duran, B., Vindhyal, M., & Lewis, E. F. (2022). Prevalence of cardiovascular diseases in COVID-19 related mortality in the United States. *Progress in Cardiovascular Diseases*.
12. Storrow, A. B., Jenkins, C. A., Self, W. H., Alexander, P. T., Barrett, T. W., Han, J. H., ... & Collins, S. P. (2014). The burden of acute heart failure on US emergency departments. *JACC: Heart Failure*, *2*(3), 269-277.
13. Ragab, D., Salah Eldin, H., Taeimah, M., Khattab, R., & Salem, R. (2020). The COVID-19 cytokine storm; what we know so far. *Frontiers in immunology*, 1446.
14. Kim, H. O., Kim, H. S., Youn, J. C., Shin, E. C., & Park, S. (2011). Serum cytokine profiles in healthy young and elderly population assessed using multiplexed bead-based immunoassays. *Journal of translational medicine*, *9*, 113. https://doi.org/10.1186/1479-5876-9-113

15. Bülow Anderberg, S., Luther, T., Berglund, M., Larsson, R., Rubertsson, S., Lipcsey, M., Larsson, A., Frithiof, R., & Hultström, M. (2021). Increased levels of plasma cytokines and correlations to organ failure and 30-day mortality in critically ill Covid-19 patients. *Cytokine*, *138*, 155389. https://doi.org/10.1016/j.cyto.2020.155389
16. Tanford, C. (1968). Protein denaturation. *Advances in protein chemistry*, *23*, 121-282.
17. Moulin, A. M., O'shea, S. J., Badley, R. A., Doyle, P., & Welland, M. E. (1999). Measuring surface-induced conformational changes in proteins. *Langmuir*, *15*(26), 8776-8779.
18. Foster, J. R. (2001). The functions of cytokines and their uses in toxicology. *International journal of experimental pathology*, *82*(3), 171-192.
19. de Lucena, T. M. C., da Silva Santos, A. F., de Lima, B. R., de Albuquerque Borborema, M. E., & de Azevêdo Silva, J. (2020). Mechanism of inflammatory response in associated comorbidities in COVID-19. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*, *14*(4), 597-600.
20. Schramm, R., Morshuis, M., Schoenbrodt, M., Boergermann, J., Hakim-Meibodi, K., Hata, M., & Gummert, J. F. (2019). Current perspectives on mechanical circulatory support. *European Journal of Cardio-Thoracic Surgery*, *55*(Supplement_1), i31-i37.
21. Girdhar, G., Xenos, M., Alemu, Y., Chiu, W. C., Lynch, B. E., Jesty, J., ... & Bluestein, D. (2012). Device thrombogenicity emulation: a novel method for

optimizing mechanical circulatory support device thromboresistance. *PloS one*, *7*(3), e32463.

22. Noble, D. (2006). Systems biology and the heart. *Biosystems*, *83*(2-3), 75-80.
23. Esmon, C. T. (2003). Inflammation and thrombosis. *Journal of Thrombosis and haemostasis*, *1*(7), 1343-1348.
24. Sangkuhl, K., Shuldiner, A. R., Klein, T. E., & Altman, R. B. (2011). Platelet aggregation pathway. *Pharmacogenetics and genomics*, *21*(8), 516.
25. Savage, B., Cattaneo, M., & Ruggeri, Z. M. (2001). Mechanisms of platelet aggregation. *Current opinion in hematology*, *8*(5), 270-276.