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**Authors**

Govender, Krianthan
Munoz, Carlos J
Williams, Alexander T
et al.

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# NEGATIVE PRESSURE INCREASES MICROVASCULAR PERFUSION DURING SEVERE HEMORRHAGIC SHOCK

Krianthan Govender[a], Carlos J. Munoz[a], Alexander T. Williams[a], Pedro Cabrales[a,*]
[a]Department of Bioengineering, University of California San Diego, La Jolla, CA, 92093

## Abstract

Hemorrhagic shock (HS) is a severe life-threatening condition characterized by loss of blood volume and a lack of oxygen ($O_2$) delivery to tissues. The objective of this study was to examine the impact of manipulating Starling forces in the microcirculation during HS to increase microvascular perfusion without restoring blood volume or increasing $O_2$ carrying capacity. To decrease interstitial tissue pressure, we developed a non-contact system to locally apply negative pressure and manipulate the pressure balance in capillaries, while allowing for visualization of the microcirculation. Golden Syrian hamsters were instrumented with dorsal window chambers and subjected to a controlled hemorrhaged of 50% of the animal's blood volume without any fluid resuscitation. A negative pressure chamber was attached to the dorsal window chamber and a constant negative pressure was applied. Hemodynamic parameters (including microvascular diameter, blood flow, and functional capillary density [FCD]) were measured before and during the four hours following the hemorrhage, with and without applied negative pressure. Blood flow significantly increased in arterioles during negative pressure. The increase in flow through arterioles also improved microvascular perfusion as reflected by increased FCD. These results indicate that negative pressure increases flow in the microcirculation when fluid resuscitation is not available, thus restoring blood flow, oxygen delivery, and preventing the accumulation of metabolic waste. Applying negative pressure might allow for control of microvascular blood flow and oxygen delivery to specific tissue areas.

## Keywords

reabsorption; Starling forces; tissue perfusion; intravital microscopy

*Correspondence to: Pedro Cabrales, Ph.D., University of California, San Diego, Department of Bioengineering, 0412 9500 Gilman Dr. La Jolla, CA 92093-0412, Fax: (858) 822-4830, Telephone: (858) 534-5847, pcabrales@ucsd.edu.

## INTRODUCTION

According to the Center for Disease Control (CDC) and the World Health Organization (WHO), 5.8 million people die from unintentional violence annually[1]. A significant portion of these traumatic events involve hemorrhagic injuries. There are four different classes of hemorrhagic shock (HS), with the most severe resulting from a loss of more than 40% of total blood volume (classified as a Class IV hemorrhage)[1]. This hypovolemia causes a cascade of changes systemically, the most immediate being reduced blood pressure, reduced cardiac output, increased heart rate (HR), and systemic vasoconstriction[2], [3]. The reduced blood volume and vasoconstriction resulting from the hemorrhage impairs the microcirculation significantly, which presents as a complete collapse of microvessels and decreased functional capillary density (FCD)[4], [5]. FCD provides the number of capillaries perfused with red blood cells and serves as a basic indicator of microcirculation function[6], [7].

One of the most effective compensatory mechanisms leveraged by the body to alleviate the consequences of HS is fluid reabsorption from interstitial tissues. Along the length of capillaries, varying pressure differentials across the endothelium drive fluid exchange between the interstitial space and lumen, as described by the Starling Equation[2], [8]:

$$J_v = k([P_c - P_i] - \sigma[\pi_p - \pi_i]) \quad \begin{aligned} J_v &= fluid\ flux\ across\ endothelium \\ k &= filtration\ constant \\ P_c &= hydrostatic\ pressure\ within\ capillaries \\ P_i &= hydrostatic\ pressure\ of\ interstial\ fluid \\ \pi_p &= oncotic\ pressure\ of\ plasma \\ \pi_i &= oncotic\ pressure\ of\ interstitial\ fluid \\ \sigma &= reflection \end{aligned} \quad (1)$$

$coefficient$

As blood volume is reduced and arterioles vasoconstrict to preserve blood pressure after hemorrhage, the hydrostatic pressure within the arterial side of capillaries decreases, decreasing the hydrostatic pressure differential between the interstitial space and the capillary, lowering water filtration from the capillary into the interstitial space, leading to a net increase in plasma volume to help resolve hypovolemia.

One of the earliest uses of negative pressure as a therapeutic for improving blood flow is the practice of cupping therapy[9]. Ischemia forms within the region of skin in contact with the rim of the cup[10], which is reperfused when removed. Therefore, cupping may increase blood flow due to the local response to the ischemia reperfusion via reactive hyperemia[11], rather than a direct effect of the applied negative pressure. Negative pressure has been used for therapeutic application in space travel, such as the gravity suits that astronauts wear[12] to redistribute fluid volume towards the lower limbs[13]. Studies have shown that the application of negative pressure to the lower body reduces intracranial pressure[13], [14] as well as internal jugular vein cross sectional area[14], [15], demonstrating that this therapy prevents adverse blood volume distribution during zero-gravity. Normally on Earth, the heart must pump against the force of gravity in the upper body, but this additional force is not present in space. The lower body negative pressure creates a net proximal-to-distal force on

the lower body, simulating the additional body force induced by height change from the heart to the lower extremities, which assist in increasing flow to the lower extremities. As such, lower negative pressure body suits directly impact the surface microcirculation, which translates to significant effects systemically. However, current studies do not analyze the implications of negative pressure in the microcirculation.

The goal of our study was to utilize a non-contact negative pressure system to control the negative pressure without creating ischemia, and to study the effect of changing tissue interstitial pressure to prevent reduction in microvascular flow during reduced capillary pressure in HS. The non-contact system is imperative, since any mechanical contact could act as a tourniquet, resulting in ischemia-reperfusion conditions that could confound the results. In this context, the data derived from this new setup will help distinguish the effects of mechanical contact from the effects of decreased interstitial pressure due to application of negative pressure. This study aims to investigate if negative pressure improves microvascular flow during HS, whether any changes induced from negative pressure persist after normalizing interstitial pressure, and whether any microvascular changes persist through multiple applications of negative pressure.

## METHODS

### Animal Preparation.

Male Golden Syrian hamsters were purchased from Charles River Laboratories and were provided free access to water and ENVIGO Teklad 8406 rodent diet. Hamsters between 55 g and 70 g were fitted with dorsal windows and catheters in the left carotid artery and right jugular vein as previously described[16]. Briefly, hamsters were anesthetized via intraperitoneal ketamine-xylazine (200 mg/kg and 10 mg/kg). A titanium dorsal window with a 12mm opening was secured to the dorsal skin flap. After recovery for 48 hours, animals were re-anesthetized with ketamine/xylazine, and a PE-50 catheter with a PE-10 tip was implanted in the right carotid artery, secured with 5–0 silk sutures, exteriorized dorsally, and secured to the window chamber. Hamsters were housed individually post-surgery and allowed to recover for at least one day before subjected to experimental protocols. All hamsters were cared for in accordance with the NIH Guide for the Care and Use of Laboratory Animals[17], and all experimental protocols were approved by the University of California San Diego (UCSD) Institute for Animal Care and Use Committee (IACUC). During studies, hamsters were restrained in an acrylic tube, and studied in the awake, unanesthetized state. After completion of experimental protocols, hamsters were euthanized via sodium pentobarbital overdose (300 mg/kg).

### Systemic Parameters.

Mean arterial pressure (MAP) was measured continuously by connecting the carotid artery catheter to a pressure transducer (MP150, Biopac Systems Inc., Santa Barbara, CA). Heart rate (HR) was determined from the blood pressure traces. Blood gases, oximetry, and chemistry were measured with a microcapillary tube of blood (ABL90 Flex, Radiometer, Brea, CA). Hematocrit (Hct) was measured via centrifugation from arterial blood collected into heparinized capillary tubes.

### Microvascular Diameter, Flow, and Perfusion.

Intravital microscopy was used to determine microcirculatory blood flow. Velocity and diameter measurements using intravital microscopy were performed as previously described[18]–[22]. Briefly, light is shone through the vessel, and RBC shadows passing are captured by two photodetectors separated by a known distance. A correlogram calculates the cross-correlation between the two signals and outputs a voltage based on the phase shift, which corresponds to a velocity. Diameter was determined via image shearing as explained previously[23], [24]. Flow was then calculated as the velocity multiplied by the cross-sectional area for each vessel. Five venules and five arterioles with diameter greater than 60 μm were selected at baseline and followed through the experiment to increase statistical power. In additional to vessel flow parameters, functional capillary density (FCD) was also quantified by measuring the number of capillaries with RBC flow in an area of the window chamber (totaling 0.46 cm$^2$) to quantify capillary flow.

### Acceptance Criteria.

All measurements were taken at least one day after surgery, while the hamsters were unanesthetized. Each hamster was considered viable at baseline if MAP was 100–130 mmHg, HR was 350–500 BPM, Hct was greater than 40%, and microscopic examination of the window chamber at 650$^x$ revealed no signs of edema or inflammation.

### Attaching the Negative Pressure Chamber.

Once baseline measurements were taken, the negative pressure chamber was applied, as illustrated in Figure 1. Briefly, silicone grease was applied around the rim (3) of the negative pressure chamber in order to provide an adequate seal around the skin of the dorsal skin preparation. The hole in the side of the negative pressure chamber (2) was connected to a pneumatic line and a three-way Luer stopcock. One end of the stopcock was connected to a syringe filled with saline, while the other end was connected to a pressure transducer to monitor the negative pressure applied. Then, the slit of the negative pressure chamber (1) was slid underneath the top bolt (4) of the window chamber preparation, and the negative pressure chamber was then lowered until the silicone was in contact with the dorsal skin flap. The bolt was then tightened slightly to prevent the negative pressure chamber from moving.

### Inducing Hemorrhagic Shock.

Once the negative pressure chamber was applied, a Grade IV hemorrhage was induced by withdrawal of 50% of the animal's total blood volume (estimated as 7% of the animal's body weight) over 10 minutes. The catheter was then flushed with heparinized saline (30 IU/mL) to maintain patency and the animal was then allowed to stabilize for a minimum of 10 minutes before measurements for the shock timepoint were taken.

### Negative Pressure Cycling.

After the shock measurement was taken, two cycles of negative pressure were applied, with each cycle consisting of 1-hour periods of −5 mmHg and 0 mmHg applied pressure. The pressure was adjusted via the saline-filled syringe connected to the three-way Luer and was

monitored by the AcqKnowledge software. The applied pressure stabilized for 10 minutes before any measurements were taken, and the pressure was removed only after all measurements for that particular time point were taken. An overview of the entire experimental procedure is provided in Figure 2.

### Statistical Analysis.

Before experiments were initiated, sample sizes were calculated based on $\alpha = 0.05$, and power $= 0.9$ to detect differences between primary end points (FCD, arteriole flow, venule flow). Results are presented as Tukey box-and-whisker plots. All animals included in the study passed Grubbs' test for outliers to confirm closeness for all parameters at baseline. Microcirculation flow and FCD data are presented as the percentage change from baseline. Statistical significance was analyzed using a Kruskal Wallis test between experimental groups and timepoints, with a Dunn's multiple comparison test performed *ad hoc*. All statistical analyses were performed using GraphPad Prism 6 software. Results were considered statistically significant if $p < 0.05$.

## RESULTS

A total of 14 hamsters were used for this study and were randomly assigned to two groups: Shock Control (CTRL) (n = 7) and Shock and Negative Pressure Cycles (NP) (n = 7). The CTRL group was hemorrhaged but did not receive the negative pressure cycles, while the NP group was hemorrhaged and negative pressure cycles were applied. All animals used for the study survived the entire experimental protocol.

### Microvascular Hemodynamics.

Changes in microvascular hemodynamics are shown in Figure 3. There were no significant differences between groups at the Shock timepoint for either arterioles or venules. However, during the first cycle of negative pressure, the NP group had statistically significantly higher RBC velocity and volumetric flow rate compared to the CTRL group. The significant increase in velocity and flow did not diminish when the negative pressure was removed. Interestingly, there was no statistically significant differences in diameter for arterioles at any timepoint. There were no differences in diameter, velocity, or flow in the venules, either between timepoints or between groups.

### Functional Capillary Density.

Functional capillary density decreased significantly from baseline after shock and did not recover for either group. Changes in FCD from baseline are presented in Figure 4. There were no differences between groups at Shock before application of the negative pressure. However, the NP had significant recovery in FCD compared to the CTRL group when the negative pressure was applied.

### Systemic Hemodynamics.

MAP and HR measurements are shown in Figure 5. Hemorrhage significantly decreased MAP for both groups compared to baseline, and there were no differences in MAP or HR

between groups at any time point. Application of the negative pressure did not have an effect on MAP or HR.

### Comparison of the 1st Cycle to the 2nd Cycle.

Changes in arteriolar velocity and flow, and FCD between the first and second cycle of application of negative pressure are presented in Figure 6. There were no statistically significant differences within groups between the first and the second negative pressure cycle. However, the effects of negative pressure seem to be at least partially diminished during the second application, as there were no significant differences observed between the groups during the second cycle.

## DISCUSSION

The purpose of this study was to analyze the effects of negative pressure on the microcirculation during HS using a novel non-contact approach. The principle finding of this study is that FCD increased following HS when negative pressure was applied, which suggests that the application of negative pressure to tissues under low perfusion can improve microcirculatory blood flow. There was a significant increase in velocity and flow of arterioles following application of negative pressure, but no changes in diameter were observed. Interestingly, the increase in blood flow in arterioles persisted even after the applied negative pressure was removed during the first cycle. However, there were no significant differences between the application of the first and second cycles of negative pressure. We attribute this lack of significant differences during the second cycle to reaching an upper bound of additional fluid that can be pulled from the tissues into the bloodstream by negative pressure, or overall decreased cardiovascular function because of the length of shock without resuscitation.

Based on the observed results, there are two possible mechanisms that can explain the shift in microcirculatory blood flow after application of negative pressure. The first possible mechanism is that changes in the microcirculation from negative pressure are induced purely via mechanical stress applied to the vessels. The varying viscoelastic properties of arterioles, venules, and capillaries lead to different strain behaviors exhibited in these vessels. Specifically, the skin microvasculature exhibits arcade geometry[7], in which the distensibility increases from venules to capillaries to arterioles[25]. Mechanically, the application of negative pressure could prevent capillary collapse by decreasing interstitial pressure, as capillaries contain very few structural elements. The lack of differences in cycle 2 could be attributed to mechanical preconditioning of the tissue, which could significantly reduce the response of the dorsal skin flap to the negative pressure. The second possible mechanism is that the changes that improved microcirculatory flow following negative-pressure application arose from biochemical factors. However, further studies with different experimental designs are needed to elucidate the specific mechanism responsible for these observed changes.

In this study, we show that negative pressure significantly improves microvascular perfusion, which could be used in several therapeutic areas. Negative pressure application could help with treatment of acute compartment syndrome, as it could be used to reduce tissue

interstitial pressure and promote the removal of excess fluid, thus mitigating edema in the extremities due to trauma[26]. Negative pressure application may help with HS resuscitation efforts, as we saw a significant improvement in microcirculatory flow, which is a key factor in preventing multiple organ dysfunction syndrome[27]. Pulling fluid into the bloodstream from the surrounding tissues may alleviate the shunting of blood away from non-essential parts of the body that occurs during severe HS. This may help ensure that metabolic washout happens gradually before resuscitation fluids are introduced to restore subjects to euvolemia. However, it should be noted that the pressures in these therapies would need to be highly studied before clinical implementation.

There were some limitations with the methodology used for this study. The most important limitation is that proper application of the silicone grease is required to maintain a complete seal during the experiment. The efficacy of this seal not only prevents pressure drifting during the cycles, but also prevents the possibility of the negative pressure chamber pressing on the dorsal skin flap, which could induce an ischemia condition[28]. If the silicone grease was also not applied properly, it could obscure some of the window chamber, which is also a limitation of the negative pressure chamber. The pressure chamber itself was made of clear acrylic, but with each layer added to the intravital microscope setup, less light can reach the eyepiece of the microscope. This makes implementation of the technique more difficult, and the method itself should be refined before large-scale dissemination to other microcirculatory researchers.

Additionally, it should be noted that the microvascular setup in this experiment is of the skin. In order to maintain vital organ function during hypovolemic shock, blood is shunted from non-vital organs such as the integumentary system and the periphery. Therefore, the changes observed through the dorsal skin flap underestimates the changes that could be observed if the negative pressure was applied to different tissues. Additionally, there could be a more pronounced systemic change if the negative pressure was applied over a larger surface area of the hamster. This could increase the available volume of fluid that could be pulled into the blood stream, which may result in observable changes in MAP and HR.

## CONCLUSIONS

In this paper, we present a novel method of negative pressure application to the dorsal skin flap model to look at the effects on microvascular flow. By introducing this non-contact system, we minimize any potential ischemia in the skin fold, and ensure that the microcirculatory changes observed are due to the application of negative pressure, rather than ischemia-reperfusion injury. The flow through arterioles increased during the entire first cycle of negative pressure. FCD increased when the −5 mmHg of negative pressure was applied during the first cycle. Both these results indicate that the application of negative pressure increased microvascular perfusion after Grade IV hemorrhage, despite the lack of resuscitation fluid. Future work needs to be conducted to isolate the mechanism causing these changes, so that it can be leveraged for various fluid imbalance impairments, such as improving the outcomes from HS resuscitation.

## ACKNOWLEDGMENTS

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**Highlights:**

- Developed a non-contact system for applying localized negative pressure.

- Localized negative pressure without compression improved functional capillary density and arteriolar flow.

- Changes in microvascular hemodynamics were only observed during the first negative pressure cycle; additional cycles showed less hemodynamic changes.
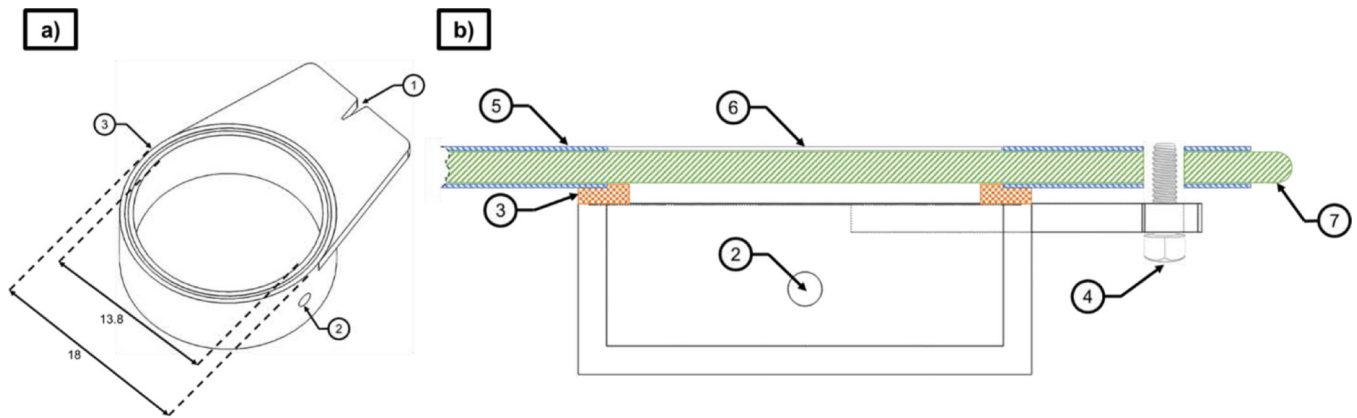
**Figure 1.**
Schematics of a) Isometric view of the negative pressure chamber with dimensions in millimeters and b) Sectional view of the negative pressure chamber attached to the dorsal skin flap window chamber for intravital microscopy. Note that the rest of the hamster is restrained in an acrylic tube on the left (not depicted). 1) slit for the bolt to secure the negative pressure chamber to the window chamber; 2) female connector for the pneumatic line; 3) rim of the negative pressure chamber where silicone grease was applied; 4) bolt from the window chamber preparation; 5) titanium plates for the window chamber; 6) glass cover to allow for intravital microscopy; 7) dorsal skin flap of the hamster.
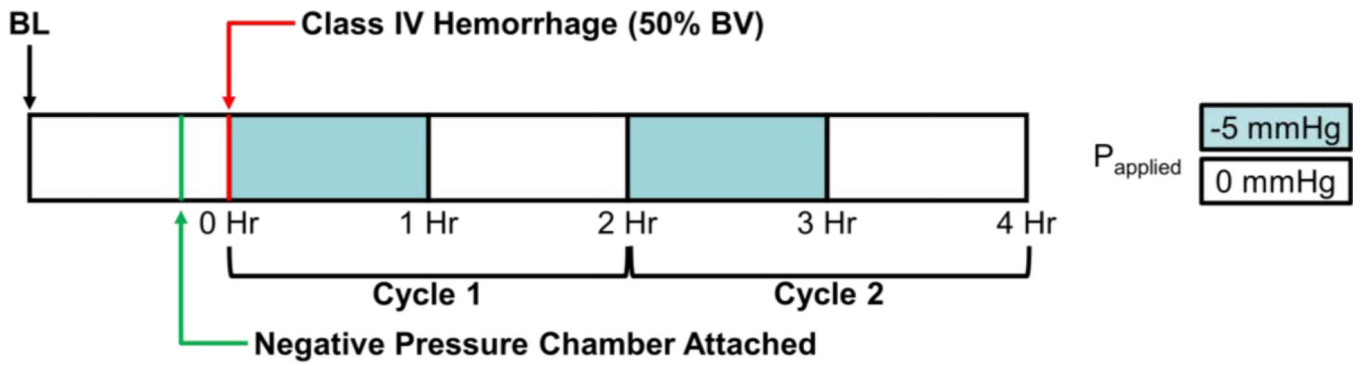
**Figure 2.**
Overview of the experimental procedure. Note that the different colors correspond to the applied negative pressure. BL = baseline, BV = blood volume.
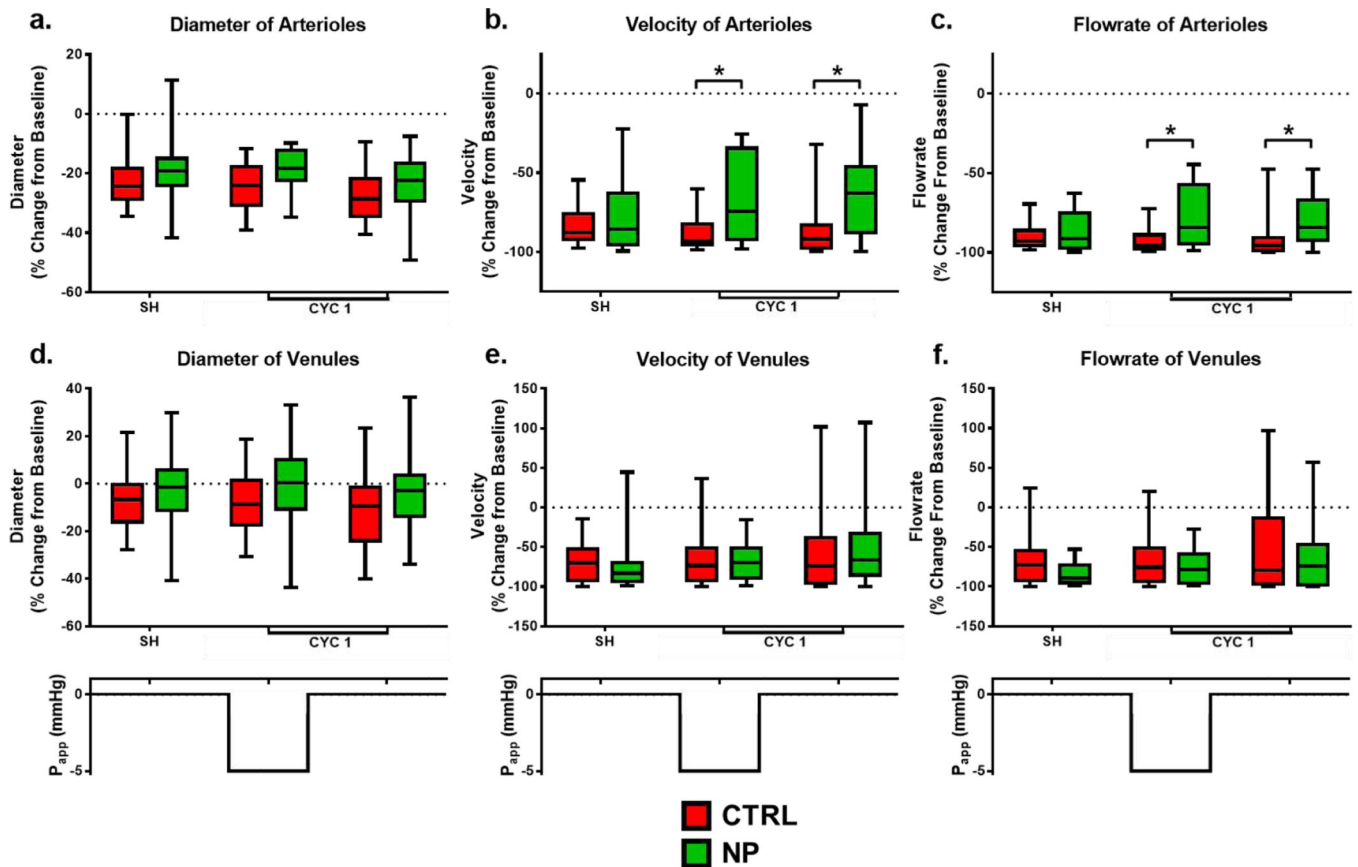
**Figure 3.**
Normalized microcirculation flow of arterioles and venules during shock and the first cycle of negative pressure: a) arteriole diameter, b) arteriole velocity, c) arteriole flowrate, d) venule diameter, e) venule diameter, f) venule flowrate. Baseline values are: a) CTRL (75 µm) and NP (86 µm), b) CTRL (5.4 mm/s) and NP (4.4 mm/s), c) CTRL (25 nL/s) and NP (28 nL/s), d) CTRL (90 µm) and NP (85 µm), e) CTRL (1.3 mm/s) and NP (1.3 mm/s), f) CTRL (9 nL/s) and NP (10 nL/s). The pressure applied to the skin fold for each measurement is shown in the bottom row of the figure. *$P<0.05$ between groups, at the same timepoint.
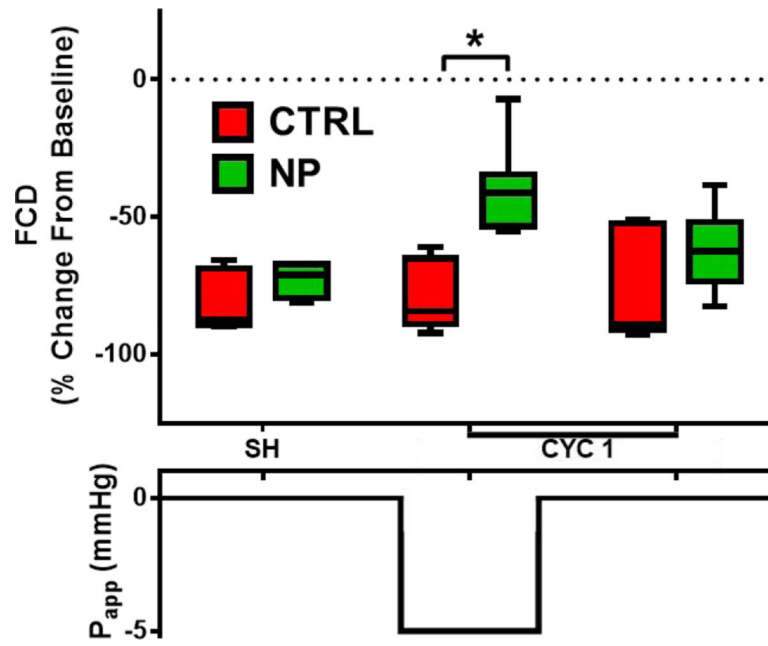
**Figure 4.**
Normalized functional capillary density during shock and the first negative pressure cycle. Baseline values are: CTRL (299) and NP (236). The pressure applied to the skin fold for each measurement is shown in the bottom row of each graph. *P<0.05 between groups, at the same timepoint.
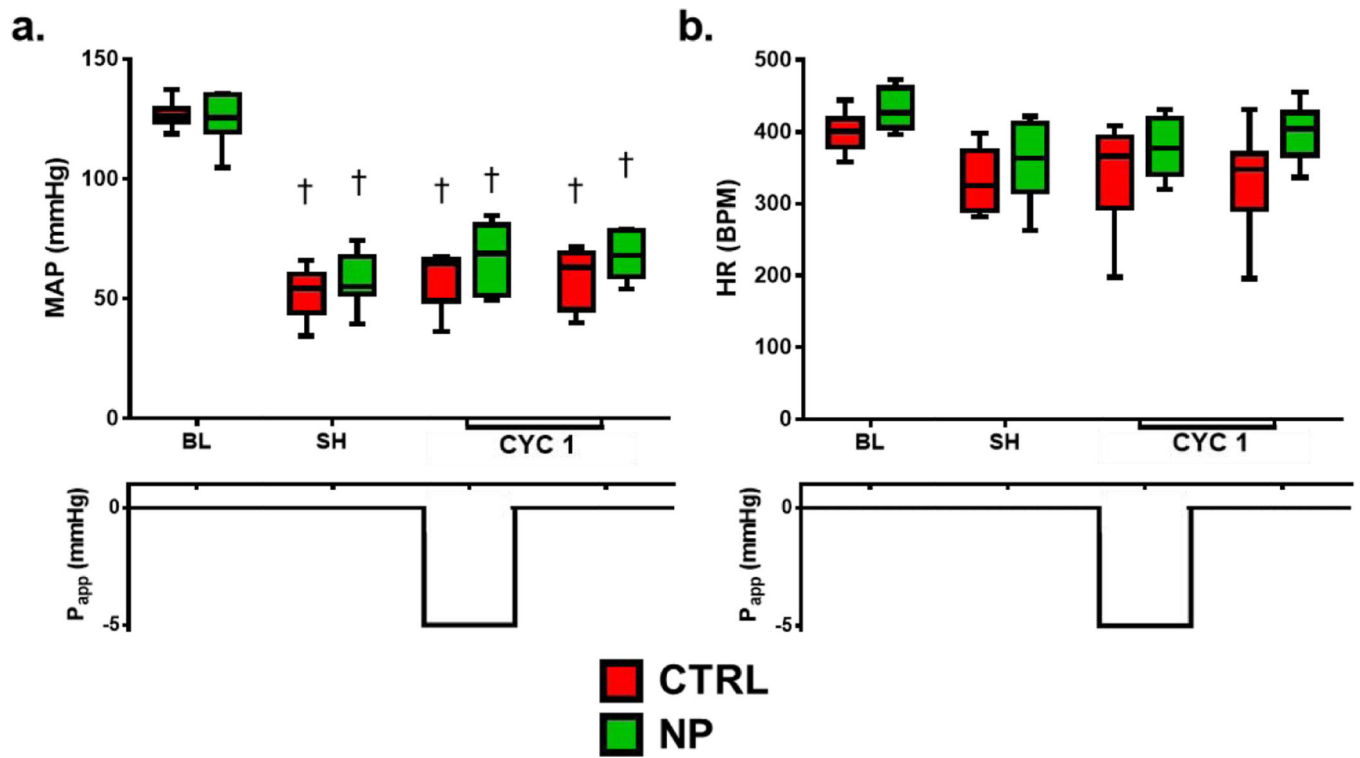
**Figure 5.**
Systemic cardiovascular parameters: a) MAP, b) HR. The pressure applied to the skin fold for each measurement is shown in the bottom row of each graph. †P < 0.05 vs BL.
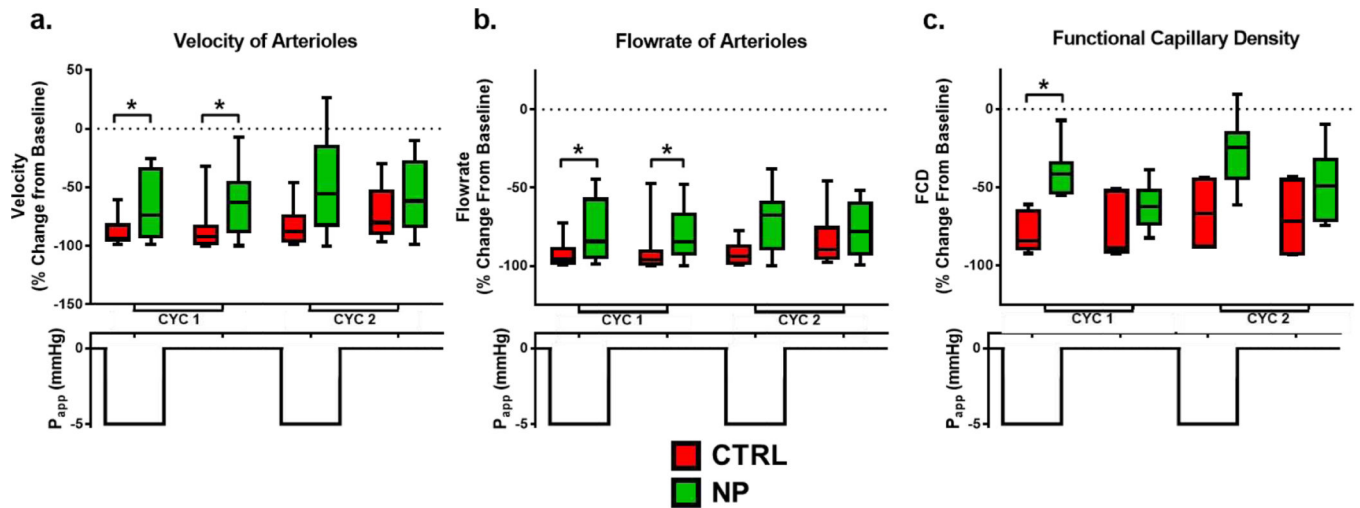
**Figure 6.**
Effects of applying a second cycle of negative pressure for a) arteriole velocity, b) arteriole flow rate, c) FCD. Baseline values are: a) CTRL (5.4 mm/s) and NP (4.4 mm/s), b) CTRL (25 nL/s) and NP (28 nL/s), c) CTRL (299) and NP (236). The pressure applied to the skin fold for each measurement is shown in the bottom row of each graph. *P<0.05 between groups, at the same timepoint.