Cyclical depressurization degranulates platelets in an agonist-free mechanism of platelet activation

Aaron J. Velasquez-Mao¹, Mark Velasquez², Moriel H. Vandsburger²

¹ UC Berkeley–UCSF Graduate Program in Bioengineering, Berkeley, CA, United States of America, ² Department of Bioengineering, UC Berkeley, Berkeley, CA, United States of America

Abstract

Activation of circulating platelets by receptor binding and subsequent coagulation events are defined by a well characterized physiological response. However, the growing prevalence of chronic kidney disease (CKD) and implication of platelet-released factors in worsening cardiovascular outcomes with hemodialysis warrant further investigation into the mechanobiology of platelet degranulation. The significant drops in pressure caused by high friction across the hemodialysis flow circuit present an overlooked platelet stimulant not involving immobilization as a driver for cytoskeletal rearrangement. In this study, platelets from healthy and dialysis (pre- and post-treatment) donors were cyclically depressurized in static suspension to measure changes in physiology by integrin αIIbβ3 activation and surface P-selectin expression. The progressive increase in CD62P with no changes in PAC1 over pressure-cycling duration regardless of uremia signifies that hydrostatic depressurization involves a novel agonist-free mechanism leading to platelet degranulation as a unique case in which CD62P and PAC1 do not interchangeably indicate platelet activation. Subsequent stimulation using ADP further suggests that sustained depressurization regimens desensitize integrin αIIbβ3 activation. Variability in platelet response caused by uremia and CKD are observed by elevated baseline PAC1 in pre-dialysis samples, PAC1 retention after ADP exposure, and maximum CD62P with ADP independent of pressure. Theory for hydrostatic pressure-induced degranulation circumventing integrin-initiated signal transduction is here presented based on the Starling Equation.

Introduction

Platelets play a broad role beyond hemostasis as circulating sources of tissue regulatory factors [1]. The contribution to platelet dysfunction by blood filtration therapies like hemodialysis is widely attributed to filter material reactivity and high shear beyond uremia [2, 3]. However, these mechanisms activate platelets by receptor adhesion, whether by defective GPIb and GPIIb/IIIa [4], hydrophobic capture [2], or rolling immobilization to RGDS sites on unfurled vWF [5]. Recent hemodialysis findings link chronically pro-fibrotic circulations to...
increasingly differential platelet granule secretory kinetics \[6\], amplifying the need to interro-
gate the drivers and mechanisms of platelet degranulation.

Examining dialysis, the use of hollow microtubes and ultrafiltration produces a flow circuit
categorized by rapid and repetitive pressure drops. While receptor-driven signal transduc-
tion is well mapped, the effects of non-native pressure cycling present a potential internally-
driven alternative that enhances pathological degranulation. Ronco et al measured frictional
pressure drops across 200\(\mu\)m diameter hollow fiber dialyzers of up to 200mmHg at a 500mL/
min blood flow rate \[7\]. Interestingly, existing studies on platelets subjected to pressure-based
stimulation in isolation are limited. In decompression sickness platelets have heightened reac-
tivity linked to sudden circulatory decompression \[8\], while the role of platelets in high altitude
thrombus formation has long been a topic of debate \[9, 10\]. Hydrostatic decompression
induces platelet aggregation during reductions from both atmospheric pressure and high pres-
surization irrespective of oxygen partial pressure \[11\], while positive pressure inhibits aggrega-
tion \[12\]. Elsewhere, hydrostatic pressure directly regulates eukaryotic cell volume, cortical
tension, and intracellular ion concentration \[13\]. The application of positive pressure inhibits
exocytosis in patch-clamped nerve cells \[14\], induces volumetric contraction by cytoskeletal
rearrangements \[15\], and causes ion transport \[16\] including by effects on volume-regulated
channels \[13\]. For platelets, whose behavior is intricately linked to geometry, sphericalization
and cytoskeletal expansion characterize the first steps of activation preceding coagulation \[17\].
Thus, expansive depressurization, particularly of a cyclical nature, may drive platelet activation
by influencing the cytoskeleton, ion transport, and exocytotic surface expansion.

In these experiments, platelets are subjected to a more intense depressurization of
460mmHg to establish an upper bound of effects from this isolated stimulus. To exaggerate
the repeated acclimation and stimulation characteristic of dialytic recirculation, chamber depres-
surization occurred periodically over 0.7s at maximum pump evacuation followed by instanta-
neous re-pressurization to atmospheric levels and 0.7s of rest. Platelet activation was measured
by detection of activated GPIIb/IIIa (PAC1) and P-Selectin (CD62P), which is localized to
internal granules and externally presented upon degranulation. Because of differences mea-
sured in these historically interchangeable biomarkers of platelet activation \[18\], expression
was also measured as a function of ADP exposure for pressure-stimulated and unstimulated (0
minute) samples. Experiments were repeated on platelets from dialysis patient donors before
and after hemodialysis to measure differences in clinical settings. Common expression trends
reveal a novel and universal basis for pressure-driven degranulation distinct from shear-based
activation, while contrasting population baselines substantiate response variability owing to
uremic dysfunction. Depending on the proteomic and transcriptomic contents of circulating
platelets, this could exacerbate platelet contributions to fibrotic pathologies and cardiovascular
dysregulation in vulnerable patient demographics \[6\].

**Methods**

**Blood collection & platelet isolation**

Written informed consent was obtained from all participants under protocol number 2017-
04-9810 approved by the IRB at University of California, Berkeley. Hemodialysis blood sam-
ple set were collected immediately before vascular access and after tubing removal from four
patients at DaVita Oakland. All patients were dialyzed using NIPRO single-use, hollow-fiber
ELISIO filters. Four control blood samples with no history of smoking, drug abuse, current
medications, or comorbidities were collected at the University of California, Berkeley Tang
Center. **Table 1** summarizes subject demographics. All samples were collected in ACD-A
Vacutainers and processed immediately by centrifugation at 200g for 10min to collect the top
90% of platelet-rich plasma (PRP). Collected PRP was centrifuged again for 5min at 200g to remove remaining erythrocyte and leukocyte contamination and gently pipetted into a 60mL plastic syringe in a BSL2 biosafety cabinet for immediate use. Participants provided informed consent under protocols approved by the U.C. Berkeley IRB and in compliance with the Declaration of Helsinki.

### Cyclical depressurization & sample collection

The PRP-loaded syringe was evacuated isochorically by 460mmHg using a rigid spacer and 12V/12W diaphragm vacuum pump under constant maximum operation (Fig 1A). The chamber was re-pressurized to atmospheric using a 12V solenoid valve given an electrical cycle of 700ms impulse followed by 700ms release (Fig 1B). Chamber pressure was measured by a vacuum transducer (Omega, PX141) elevated to water-level. Valve, pump, sensor, and stepper motor-operated spacer were controlled by NI myDAQ using LabVIEW. PRP was collected by gentle extrusion from the syringe at 0min (no stimulation) through 180min of cyclical depressurization into sterile polypropylene and left at dark room-temperature (RT) until final time-point collection.

### Flow cytometry & controls

Upon final collection, sub-samples were siphoned and exposed to ADP at $2.5 \times 10^{-4}$M for 5min at RT. Then, antibodies for CD41 (Thermo, MA180666), PAC1 (Thermo, BDB340507), and CD62P (Thermo, 12-0626-82) were added 1:2 to 5μL PRP at RT for 15min in a 96U plate, followed by addition of paraformaldehyde in calcium-free saline to 1%. Arg-Gly-Asp-Ser

| Table 1. Subject demographics. |
|--------------------------------|
|                                |
| Control demographics (n = 4)    |
| Age, years—median (range)      | 25 (25–26) |
| Gender ratio—[male/female]     | 4/0        |
| Patient demographics (n = 4)    |
| Age, years—median (range)      | 69 (46–79) |
| Gender ratio—[male/female]     | 3/1        |
| Ethnicity                      |
| African American               | 2          |
| Asian American                 | 1          |
| Caucasian                      | 1          |
| Prior time on dialysis, months—median (range) | 24 (3–96) |
| Cause of renal failure         |
| Hypertension                   | 3          |
| Diabetes mellitus              | 1          |
| Comorbidities                  |
| Anemia                         | 2          |
| Hyperlipidemia                 | 1          |
| CHF                            | 2          |
| Hypothyroidism                 | 1          |
| Medication                     |
| Lipid-lowering drugs           | 2          |
| Platelet inhibitor (non-P2Y12) | 2          |
| RAS Inhibitors                 | 1          |
| None                           | 0          |

https://doi.org/10.1371/journal.pone.0274178.t001
(RGDS) (Sigma, A9041) was added to a separate PAC1 stain mixture to $2 \times 10^{-2}$M prior to platelet labeling as a competitive inhibitor to subtract nonspecific binding (Fig 1D). Isotype controls for FITC (Thermo, 11-4714-81) and PE (Thermo, 12-4714-82) were used for each sample to subtract nonspecific binding. Single-plex fluorescence was measured on an Attune NxT Flow Cytometer immediately after staining. The same fluorescence gates were used across all trials.

**Fig 1. Experimental setup.** (A) Machine for static depressurization of platelets suspended in plasma within a syringe. (B) Hydrostatic pressure profile experienced by platelets involving linear evacuation of 460mmHg from atmospheric pressure over 700ms followed by instantaneous release at 50% duty cycle. (C) CD41 positive gating using FITC isotype control indicates $>99\%$ pure platelet populations across samples. (D) Flow cytometry measurement example of PAC1 by subtraction of RGDS control.

https://doi.org/10.1371/journal.pone.0274178.g001

(RGDS) (Sigma, A9041) was added to a separate PAC1 stain mixture to $2 \times 10^{-2}$M prior to platelet labeling as a competitive inhibitor to subtract nonspecific binding (Fig 1D). Isotype controls for FITC (Thermo, 11-4714-81) and PE (Thermo, 12-4714-82) were used for each sample to subtract nonspecific binding. Single-plex fluorescence was measured on an Attune NxT Flow Cytometer immediately after staining. The same fluorescence gates were used across all trials.

**Statistics**

Significantly different means were identified amongst test groups by Friedman’s ANOVA followed by Dunn & Sidák’s multiple comparisons test. Measurement distributions on figures were compared by two-sample t test, assuming unequal variance and two tails. Linear
dependencies were assessed by Pearson correlation. Significance in all cases was defined by $p \leq 0.05$. Figure error bars represent 95% confidence intervals.

**Results**

Pure platelet populations across trials were confirmed by unimodal forward-scatter (FSC) against side-scatter (SSC) density and > 99% CD41 expression (Fig 1C). Cylindrical depressurization alone yielded no response in platelet PAC1 expression (Fig 2). No linear trends were observed within healthy, pre-dialysis, and post-dialysis test groups (Fig 2A). No differences were found between any two test groups at any time point (Fig 2B). A possible transient difference in receptor rate of change is observed by significantly elevated PAC1 expression at 60min compared to 0min and 180min in healthy trials (Fig 2A). However, no time point had a mean significantly different from any other time point for healthy ($p = 0.062$), pre-dialysis ($p = 0.35$), or post-dialysis ($p = 0.90$) trials by Friedman’s ANOVA. In the absence of a universal PAC1 trend observed as a function of pressure-cycling duration, expression data was aggregated by test group across all time points to assess baseline differences attributable to population (Fig 3). Healthy and post-dialysis samples had nearly identical ranges ($2.1 \pm 1.9$ vs $2.1 \pm 1.8$, mean $\pm$SD), while pre-dialysis measurements were significantly elevated and significantly more varied ($SD = 3.9$). Friedman’s ANOVA identified a significantly different group mean ($p = 2.7E-5$), while Dunn & Sidák’s multiple comparisons test pinpointed pre-dialysis measurements as significantly different from healthy ($p = 9.0E-5$) and post-dialysis measurements ($p = 2.2E-4$). Thus, overall PAC1 elevation in pre-dialysis samples may be attributed to uremia, while

![Fig 2. Platelet PAC1 expression.](https://doi.org/10.1371/journal.pone.0274178.g002)
baseline expression levels in the same dialysis patients are comparable to a healthy range after uremic clearance.

When subsequently stimulated with ADP, platelets reliably decreased in PAC1 expression with increasing duration of pressure-stimulation. Negative linear correlations were detected in all test groups (Fig 4A). However, healthy platelets demonstrated a greater reduction in expression overall \( R = -0.92 \) compared to pre-dialysis \( R = -0.78 \) and post-dialysis \( R = -0.79 \) platelets. In the healthy test group, platelets at 60min \( p = 0.015 \), 120min \( p = 0.014 \), and 180min \( p = 2.4E-5 \) expressed PAC1 at significantly lower levels compared to pressure-unstimulated platelets at 0min. In comparison, pre-dialysis \( p = 0.013 \) and post-dialysis \( p = 0.022 \) platelets expressed significantly lower levels of PAC1 only after 180min of pressure cycling. The more rapid reduction in PAC1 expression in healthy platelets with longer pressure-cycling duration preceding identical exposure to ADP compared to dialysis platelets is further reflected in significantly lower expression at 30min and 60min compared to the post-dialysis population (Fig 4B).

Unlike PAC1, CD62P unilaterally increased in expression with cyclical depressurization. Positive linear trends were detected in all test groups (Fig 5A), with no differences measured between test groups at any time point (Fig 5B). These trend consistencies led to the aggregation of expression data across all test groups to measure overall CD62P behavior as a function of pressure-cycling duration (Fig 6). Increased statistical strength \( p = 6.9E-11 \) signifies that CD62P increases in expression with pressure stimulation as a universal platelet response that is agnostic to uremia. Because CD62P is originally stored in differentiated granules within platelets, these data support that repetitive depressurization induces degranulation.
When subsequently stimulated with ADP, platelets expressed CD62P in greater proportion, with behavioral discrepancies observed between healthy and dialysis samples. A positive correlation was observed in the healthy test group, while no significant trend could be fitted to predialysis or post-dialysis measurements (Fig 7A). However, pressure-and-ADP-stimulated platelets expressed greater CD62P at all durations of pressure stimulation compared to pressure-only stimulated platelets from the same test group (Fig 7B). Thus, while for healthy platelets ADP and depressurization may have additive effects on degranulation, dialysis patient platelets may have heightened sensitivity to both pressure and ADP. The latter is supported by significantly higher expression levels in ADP-only (0 minute) stimulated platelets for both predialysis (p = 0.0098) and post-dialysis (p = 0.0097) samples. Furthermore, significant elevation in middle time points for post-dialysis samples may corroborate additive interactions between pressure and ADP on degranulation, while the absence of significant differences between any time points in pre-dialysis samples may indicate a dependency in sensitivity on uremic state. Comparing test group aggregates confirms elevated baseline expression levels in dialysis samples (Fig 7C). Friedman’s ANOVA identified a significantly different group mean (p = 2.1E-9), while Dunn & Sidák’s multiple comparisons test pinpointed healthy measurements as significantly different from pre-dialysis (p = 3.3E-8) and post-dialysis measurements (p = 4.0E-7).

**Discussion**

Cyclical depressurization induced platelet degranulation without activating the GPIIib/IIIa integrin complex in a unique and unexplored mechanism of platelet stimulation. While
CD62P increased linearly with duration of pressure cycling, PAC1 expression remained at base unstimulated rates. In comparison, when stimulating platelets by shear stress, both CD62P and PAC1 interchangeably predict platelet activation [18]. Interestingly, co-stimulation with ADP additively enhanced CD62P expression while decreasing PAC1 expression. When stimulated using ADP alone, platelets have been shown to retain PAC1 expression for up to 8hr [18]. Taken together, these data suggest that depressurization circumvents receptor signal transduction to induce exocytosis and further that this mechanism desensitizes platelets to chemical stimuli. In hemodialysis, this may contribute to bleeding disorders [2] while still exacerbating the circulation of platelet factors [6].

While CD62P profiles were universal, population-level differences observed between healthy, pre-dialysis, and post-dialysis PAC1 measurements indicate effects attributable to uremia and chronic kidney disease (CKD). Pre-dialysis platelets exhibited overall PAC1 elevation compared to healthy and post-dialysis platelets whose profiles were equivalent, indicating a baseline state of hyperactivity in dialysis patients that is corrected with uremic clearance and platelet renewal. Further, the significant early increase in paired pre-dialysis platelet PAC1 expression could signify a heightened sensitivity to pressure shock. However, the “acclimation” observed by a significant negative rate of change at 60min to average unstimulated levels may imply that perturbations on PAC1 caused by depressurization are not sustained, regardless of uremic state. Examining the combinatorial effects of ADP on top of pressure, both pre- and post-dialysis platelets showed a slower decline in PAC1 expression over time compared to healthy platelets considering comparable ranges at 0 minutes of pressure stimulation between

**Fig 5. CD62P expression.** (A) Expression correlated positively to pressure-cycling duration in all test groups. (B) No significant differences observed in percent expression between test groups at any time point.

https://doi.org/10.1371/journal.pone.0274178.g005
all groups. As pressure-inhibition of GPIIb/IIIa may be beneficial in preventing capillary bed plugging [19, 20], inclined retention of the activated integrin after agonist exposure demonstrates a troubling adaptive possibility in which CKD patient platelets remain preferentially adherent for longer.

Study limitations include low donor count, higher pressure drop used than experienced across hemodialysis, the use of a single pressure profile, number of platelet activation reporters, and additional agonists used. The experimental pressure drop of 480mmHg is over two-fold higher than that experienced by platelets during hemodialysis, though remains relevant as a benchmark for the progression of blood ultrafiltration technology [7]. Additionally, recruitment protocols at University Health Services and DaVita restricted accessible demographics, resulting in an age mismatch between healthy and dialysis donors. Future studies should probe the effects of varying pressure drop rate and frequency to establish bounds of safe usage.

Further investigation of depressurization effects on phosphatidylserine and clotting factor binding would better characterize whether the same downstream procoagulant phenotype as from shear is produced [19, 21]. Similarly, sensitivity to other agonists including thrombin would round out the resulting coagulative profile [1, 17].
Theory

The Starling Equation describes an outward volumetric flux, $J$, across a membrane driven by hydrostatic pressure $P$ and osmotic pressure $\pi$.

$$J_v = L_pS(P_{in} - P_{out}) - \sigma(\pi_{in} - \pi_{out}),$$

where $L_p$, $S$, and $\sigma$ are constants.

A sudden reduction in $P_{out}$ by external hydrostatic depressurization would generate a solvent convective force outwards unless met with a net ion influx, increasing $\pi_{in}$ thereby expanding cortical volume. In these experiments, no population-level changes in platelet diameter were evident by forward scatter distributions, so any potential volumetric changes to pressure shock were not sustained after stimulation. Thus, either absorptive ion influx was
followed by equivalent contracting ion outflux upon vacuum release, or filtrative solvent outflow caused localized forces at the platelet membrane followed by reabsorption. In both cases, the cytoskeleton is strained, and both geometric rearrangements are evident in the classic clotting response. In fact, in response to agonist-induced activation, platelets first become spherical by the influx of calcium and secondly hydraulically generate podia [17]. While neither platelet shape nor volume were actively tracked throughout the applied pressure cycle, the feasibility of inducing these same swelling and protruding forces without a surface receptor agonist may explain the here-mentioned observations of downstream degranulation by CD62P presentation without initiation by integrin signal transduction measured by PAC1.

The theorized cortical forces across platelet membranes may be symptomatic of other non-contact platelet stimuli beyond the repetitive frictional pressure shock characteristic of hemodialytic filtration. Taking the other driver of flux from the Starling Equation, osmotic shock likely also induces platelet degranulation by the same principles. Early studies reported platelet morphological expansion [22], aggregation [23], and free ion flux [24] in both hyper- and hypotonic solutions with implications on blood storage. Considering the drastic shifts in osmolarity experienced in the renal medulla and procoagulant platelet phenotypes observed in representative hyperosmotic solutions [21], agonist-free platelet degranulation may contribute to worsening CKD in dialysis patients exhibiting pro-fibrotic platelet disposition [6].

Supporting information
S1 Graphical abstract.
(TIF)

Acknowledgments
Access to flow cytometry was provided by Dr. Mary West in the UC Berkeley Stem Cell Core Facility. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation.

Author Contributions
Conceptualization: Aaron J. Velasquez-Mao, Moriel H. Vandsburger.
Data curation: Mark Velasquez.
Formal analysis: Aaron J. Velasquez-Mao, Mark Velasquez.
Funding acquisition: Moriel H. Vandsburger.
Investigation: Aaron J. Velasquez-Mao, Mark Velasquez.
Methodology: Aaron J. Velasquez-Mao.
Project administration: Aaron J. Velasquez-Mao, Mark Velasquez.
Software: Aaron J. Velasquez-Mao.
Supervision: Moriel H. Vandsburger.
Validation: Moriel H. Vandsburger.
Writing – original draft: Aaron J. Velasquez-Mao.
Writing – review & editing: Moriel H. Vandsburger.
References

1. Sánchez­González DJ, Méndez-Bolaina E, Trejo-Bahena NI. Platelet-rich plasma peptides: Key for regeneration. Int J Pept. 2012;2012. https://doi.org/10.1155/2012/532519 PMID: 22518192

2. Daugirdas JT, Bernardo AA. Hemodialysis effect on platelet count and function and hemodialysis-associated thrombocytopenia. Kidney Int. 2012; 82: 147–157. https://doi.org/10.1038/ki.2012.130 PMID: 22592187

3. Ding J, Chen Z, Niu S, Zhang J, Mondal NK, Griffith BP, et al. Quantification of Shear-Induced Platelet Activation: High Shear Stresses for Short Exposure Time. Artif Organs. 2015; 39: 576–583. https://doi.org/10.1111/aor.12438 PMID: 25808300

4. Nomura S, Hamamoto K, Kawakatsu T, Kido H, Yamaguchi K, Fukuroi T, et al. Analysis of platelet abnormalities in uremia with and without Glanzmann’s thrombasthenia. Nephron. 1994; 68: 442–448. https://doi.org/10.1159/000188935 PMID: 7870229

5. Tsun Wong AK. Platelet biology: The role of shear. Expert Rev Hematol. 2013; 6: 205–212. https://doi.org/10.1586/ehm.13.5 PMID: 23547868

6. Velasquez-Mao AJ, Velasquez MA, Hui Z, Armas-Ayon D, Wang J, Vandsburger MH. Hemodialysis exacerbates proteolytic imbalance and pro-fibrotic platelet dysfunction. Sci Rep. 2021; 11: 1–14. https://doi.org/10.1038/s41598-021-91416-8 PMID: 34083719

7. Pickles DM, Ogston D, Macdonald AG. Effects of hydrostatic pressure and inert gases on platelet aggregation in vitro. J Appl Physiol. 1990; 69: 2239–2247. https://doi.org/10.1152/jappl.1990.69.6.2239 PMID: 2077022

8. Liu S, Tao R, Wang M, Tian J, Genin GM, Lu TJ, et al. Regulation of cell behavior by hydrostatic pressure. Appl Mech Rev. 2019; 71: 1–13. https://doi.org/10.1065/amp.e07-05-0026 PMID: 31700195

9. Heinemann SH, Conti F, Stühmer W, Neher E. Effects of hydrostatic pressure on membrane processes: Sodium channels, calcium channels, and exocytosis. Journal of General Physiology. 1987; 90: 765–778. https://doi.org/10.1085/jgp.90.6.765 PMID: 2450167

10. Myers KA, Rattner JB, Shrive NG, Hart DA, Myers KA, Rattner JB, et al. Hydrostatic pressure sensation in cells: Integration into the tensegrity model. Biochemistry and Cell Biology. 2007; 85: 543–551. https://doi.org/10.1139/o07-108 PMID: 17901896

11. Hui TH, Zhou ZL, Qian J, Lin Y, Ngan AHW, Gao H. Volumetric deformation of live cells induced by pressure-activated cross-membrane ion transport. Phys Rev Lett. 2014; 113: 1–5. https://doi.org/10.1103/PhysRevLett.113.118101 PMID: 25260007

12. Shin EK, Park H, Noh JY, Lim KM, Chung JH. Platelet shape changes and cytoskeleton dynamics as novel therapeutic targets for anti-thrombotic drugs. Biomol Ther (Seoul). 2017; 25: 223–230. https://doi.org/10.4062/biomother.2016.138 PMID: 27871158

13. Lu Q, Malinauskas RA. Comparison of Two Platelet Activation Markers Using Flow Cytometry After In Vitro Shear Stress Exposure of Whole Human Blood. Artif Organs. 2011; 35: 137–144. https://doi.org/10.1111/j.1525-1594.2010.01051.x PMID: 20946295

14. Cheng H, Yan R, Li S, Yuan Y, Liu J, Ruan C, et al. Shear-induced interaction of platelets with von Willebrand factor results in glycoprotein Ibα shedding. Am J Physiol Heart Circ Physiol. 2009; 297: 2128–2135. https://doi.org/10.1152/ajpheart.00107.2009 PMID: 19820200

15. Tymk K. Critical Role for Oxidative Stress, Platelets, and Coagulation in Capillary Blood Flow Impairment in Sepsis. Microcirculation. 2011; 18: 152–162. https://doi.org/10.1111/j.1549-8719.2010.00080.x PMID: 21199094
21. Gatidis S, Borst O, Föllner M, Lang F. Effect of osmotic shock and urea on phosphatidylinerine scrambling in thrombocyte cell membranes. Am J Physiol Cell Physiol. 2010; 299: 111–118. https://doi.org/10.1152/ajpcell.00477.2009 PMID: 20237147

22. Law P. The tolerance of human platelets to osmotic stress. Exp Hematol. 1983; 11: 351–357. PMID: 6852145

23. Fantl P. Osmotic stability of blood platelets. J Physiol. 1968; 198: 1–16. https://doi.org/10.1113/jphysiol.1968.sp008590 PMID: 5677030

24. Lundberg A, Meryman H, Estwick N. of human platelets media to at 37 and -SC and hypotonic. American Journal of Physiology-Legacy Content. 2021; 222: 1100–1106.