The effect of roller head pump on platelet deterioration during the simulated extracorporeal circulation.

Tatsuya Furugaki, B.S.

Osamu Shigeta, M.D., Ph.D.

Yukinori Kozuma, Ph.D.

Toru Tsukada,M.D

Tomomi Nakajima, M.D.

Hiroaki Sakamoto, M.D., Ph.D.

Bryan J. Mathis, Ph.D.

Yuji Hiramatsu, M.D., Ph.D.

Yasuyuki Suzuki, M.D., Ph.D.

Department of Medical Technology, Faculty of Health Science, Kumamoto Health Science University

Department of Cardiovascular Surgery, Faculty of Medicine, University of Tsukuba

Corresponding Author:

Yasuyuki Suzuki, M.D., Ph.D.

Department of Cardiovascular Surgery,

Faculty of Medicine,

University of Tsukuba,

1-1-1 Tennodai, Tsukuba, Ibaraki 305-8575, Japan

TEL & FAX: +81-298-53-3097

E-mail: Ysuzuki@md.tsukuba.ac.jp

Abstract

Roller pumping results in hemolysis and adverse effects on coagulation, but there are few reports on the influence of roller heads on platelets. Here,we evaluate the interaction between roller pumping and platelet function using a simulated extracorporeal circuit incorporating a vinyl chloride tube and roller head pump with 30 minutes recirculation. Platelet aggregation, platelet count, microparticle, P-selectin, Phosphatidylserine (PS) exposure and Ricinus Communis Agglutinin 1(RCA-1) were measured before, 5, 10, 20 and 30 minuets after the recirculation using 100 mL of fresh human blood that had obtained from healthy volunteers (n= 9). Platelet aggregation and platelet count gradually decreased but microparticles significantly increased after the recirculation ($P<0.05$). P-selectin, PS exposure and RCA-1 were measured using flow cytometry. There were no significant differences in the P- selectin and PS exposure expression during recirculation. RCA-1, a platelet apoptosis markers, significantly increased 30 minutes after recirculation ($P<0.05$). We thus conclude that roller pumping induced platelet apoptosis and caused decreases in platelet count and aggregation after the recirculation.

Key words: simulated extracorporeal circuit, microparticle, P-selectin, PS exposure, RCA-1

Introduction

The cardiopulmonary bypass (CPB) circuit is usually comprised of a roller pump, a membrane oxygenator, a cardiotomy reservoir and a connecting tube. As exposure of blood to the oxygenated CPB circuit activates complement, the coagulation system, the inflammatory response, and platelets [1,2], activated clotting time (ACT) is extended to over 500 seconds using anticoagulants [3,4]. Additionally, to reduce whole body basal metabolism and oxygen consumption, core body temperature is lowered during CPB while blood dilution maintains peripheral circulation. However interaction between blood and CPB surfaces, a lowered body temperature and dilution reduce platelet count and function, which causes postoperative bleeding [5].

A roller style pump is a simple structure and inexpensive, but causes hemolysis and adverse effects on coagulation [6,7]. In spite of this, there are few reports on the influence that roller pumps have on the platelets. This study aims to evaluate the interaction of the roller pump and platelet function using measurements of platelet aggregation and surface makers using whole-cell flow cytometry.

Materials and Methods

The simulated extracorporeal circuit incorporated a 60 cm vinyl chloride tube with an inner diameter of 6 mm, a soft bag reservoir (50 mL) and an occlusive roller pump (Stokert 10-10-00 ; Stokert Instrumente GmbH,Munich,Germany). Each circuit was primed with 100 mL of fresh human blood obtained from healthy volunteers (n= 9, race: Asian, age: 30.56 ± 7.70 years old male, BSA: 1.84 ± 0.12 m^2) who were unmedicated at least for 14 days before donation. Blood was drawn directly into the simulated extracorporeal circuit using a 22 G needle (JMS CO., LTD, Hiroshima, Japan) with undifferentiated heparin (4 unit/mL) and recirculated for 30 min at 1000 mL/min with the circuit under a constant 25℃ temperature. Blood samples for each donor were obtained for analysis from the simulated extracorporeal circuit before circulation (time point 0) and after 5,10, 20 and 30 min of recirculation.

This study was conducted with the approval of the Ethics Committee of the University of Tsukuba (approval number 1109)

Blood samples and assays

In vitro, optical platelet aggregation study

Blood was withdrawn from healthy volunteers into tubes containing 1/10 volume of 3.8% sodium citrate and then centrifuged at 180 ×g for 10 min before collection of platelet-rich plasma (PRP). The collected PRP was diluted 200 -fold with 1% oxalic acid, while the remaining blood was centrifuged for a further 10 min at 1600×g to prepare platelet-poor plasma (PPP). Platelet counts were measured by hemocytometer (Improved Neubauer; Nano Tek, Waltham, MA, USA) and an optical microscope (BX 41; OLYMPUS, Japan), before standardization to 3.0×10^5 / platelets/mL with PPP. After 22 μL of agonist (collagen; Takeda Austria GmbH, Austria) was added to 200 μL of adjusted PRP ,an aggregation assay was performed using a laser light-scattering aggregometer (Easy Tracer RT-800; Tokyo Photoelectric Company, Tokyo, Japan) at 37℃, with transmittance of a PPP blank set as 100%. A collagen concentration curve of 0.5 μg / mL, 1.0 μg / mL, 2.0 μg / mL and 4.0 μg / mL determined a maximum aggregation of around 60% and indicated 1.0 μg / mL as an optimal collagen concentration. The percent inhibition at each sample point during the recirculation was normalized to timepoint 0 (before aggregation).

Flow cytometry

To investigate roller pump effects on platelet activation, flow cytometry (FACS Calibur ; Becton and Dickinson, Franklin Lakes, NJ, USA) was used and analysis was performed with Cell Quest software (Becton and Dickinson). Our analysis was based on CD42b staining and intensity of the fluorescent dye CD62P, while Annexin V (AXV) and RCA-1 were used to select for platelet populations.

Samples for flow cytometer measurement (FCM) were prepared by adding 1 mL of 4% paraformaldehyde and 0.3 mL of 3.2% sodium citrate (anticoagulant) in 3 mL of whole blood, then

incubating at room temperature in the dark for 30 minutes. Thereafter, the mixture was centrifuged at 180 ×g for 10 minutes and the supernatant was used as PRP. The remaining material was further centrifuged at 1600 ×g for 10 minutes and the resulting supernatant was designated as PPP. FCM specimens were normalized to 3.0×10^5 / platelets/mL using PRP and PPP then diluted 200-fold with 1% ammonium oxalate before measurements.

AXV (phosphatidylserine [PS] exposure) 2-color measurement

AXV binding buffer (1% fluorescin isothiocyanate plus conjugated anti-human AXV [Sigma]; SIGMA, Saint Louis, MO, USA) that selectively stains exposed PS was prepared by adding 1 μL of CD42b-PECy5 (Becton and Dickinson, San Diego, CA, USA) to the prepared 50 μL sample before mixing up to a total amount of 500 μL with a phosphate-buffered salt solution (PBS: NaCl, 0.2 g / L; Na2HPO4, 1.15 g / L; KCL, 0.2 g / L; KH2PO4, 0.2 g / L; bovine serum albumin, 1 g / L; deionized water, 1 L, pH 7.4) and reacted in the dark at room temperature for 30 minutes, FCM was measured immediately.

CD62P（P-selectin） 2-color measurement

For 50μL of prepared specimen, 1μL PE-conjugated anti-human CD62P (Becton and Dickinson, San Diego, CA, USA) that selectively stains P-selectin and 1μL of CD42b-PECy5 that stains platelets were added and reacted for 30 minutes in the dark at room temperature. Phosphate-buffered salt solution was added up to 500 μL before measurement by FCM.

RCA-1（Desialization）2-color measurement

To 50 μL of the prepared sample was added 2.5 μL of RCA-1 and 1 μL of CD42b-PECy5 to selectively stain the desialylated sugar chain. After reaction for 30 minutes in the dark at room temperature, phosphate- buffered salt solution was added to 500 μL before measurement by FCM.

Platelet derived microparticles (PMPs) 1 -color measurement

To 50 µL of the prepared sample was added 1 µL of CD42b-PECy5 and this was reacted for 30 minutes in the dark at room temperature. Thereafter, phosphate- buffered salt solution was added to 500 µL before measurement by FCM.

Statistical analysis

All data are expressed as means and standard deviation of those means. Data were analyzed using EXCEL software (Microsoft corp., Seattle, WA, USA). The paired Student-T test was used for comparisons between timepoint 0 and subsequent timepoints. A p value of less than 0.05 was considered to be statistically significant.

Result

Whole data are summarized in Table 1.

Data are given as mean and standard deviation of the mean. The included data of the sharp sign (#) show a significant difference as compared with time point 0 ($p<0.05$).

Platelet aggregation and platelet count

Platelet counts gradually decreased during circulation with observed decreases of $34.8\pm8.0\times10^4$/ml before circulation to $17.1\pm8.5\times10^4$/ml after 30 minutes of circulation ($p<0.05$). Microparticles were 3.2±0.3% before circulation and gradually increased to 45.1±11.9% after 30 minutes of circulation ($p<0.001$) (Figure 1). Platelet aggregation was 73.0±14.1% before circulation and gradually decreased to 20.3±4.0% after 30 minutes of circulation ($p<0.001$) (Figure 2).

Flow cytometry

The mean surface PS exposure percentage was 3.4 ± 0.2 % before circulation and rose slightly higher during recirculation, but no significant differences at any timepoint were observed. P-selectin was at 3.3 ± 0.6% before circulation and rose slightly higher but showed no significant differences at any timepoint. RCA-1 expression was 2.9 ± 0.8 % before circulation and gradually increased during the circulation with significant differences after 30 minutes (25.9 ± 12.6%, $p<0.001$) (Figure 3).

Discussion

After cardiac surgery using CPB, non-surgical, iatrogenic post-operative bleeding is a serious issue. Exposure of blood to the CPB circuit and air activates complement, the coagulation system, the inflammatory response, and platelets [1,2] whose count and function are reduced and causes postoperative bleeding. The membrane oxygenator has a large blood contact area and this interaction zone between artificial material and blood, coupled with mechanical shear stress from the roller pumping action, appears to cause major shear stress.

Demirtas and colleagues compared the effects of centrifugal and roller pumping on inflammatory response and oxidant status in patients undergoing coronary artery bypass graft using CPB and found no significant differences in inflammatory response but platelet-activating factor levels after CPB were found to be significantly higher with roller pumping. But comparison of within-group revealed that no significant differences were found in serum PAF enzyme levels before and after CPB in both of study groups. These data were in clinical setting and showed a brief effect of CPB. The data in our experiment using a simulated extracorporeal circuit in consideration of long-time use was different from Demitas's report. We show the effect on platelet of the roller pump precisely except the effect such as systemic other organs. [7]. Halaweish and colleagues reported a retrospective comparison of bleeding complications in extracorporeal membrane oxygenation (ECMO) using centrifugal and roller pumping, finding a higher incidence of nonsurgical bleeding and cryoprecipitates in centrifugal pump patients [8]. They reported that, while mechanisms behind

this phenomenon were multifactorial, higher centrifugal shear stress was causative for acquired vWF deficiency [8]. Murase and colleagues reported that platelet Bax level changes were causative for impaired platelet response to thrombin after CPB with roller pumping possibly due to apoptotic signaling [9], but there was no isolated roller pump effect found. Several reports have compared centrifugal and roller pumping in clinical studies with full cardiopulmonary bypass circuits [10-12], but reports on platelet-specific effects due to roller pumping are scarce.

Our simulated extracorporeal circuit had a shorter than usual connecting tube due to the exclusion of a membrane oxygenator to isolate the effect of the roller pump. Recirculation for 30 min at 1000 ml/min in this circuit was thus equivalent to 9 hours at 2.5L/min recirculation in a clinical setting. In this situation, platelet aggregation decreased gradually after 30 minutes and platelet counts decreased to 50% of baseline while microparticle counts increased to over ten times. This is in line with clinical observations of 40% decreases in platelet counts after CPB. This dramatic drop may have been due to activated platelets being consumed in a hematostatic site or cleared by the reticuloendothelial systems of the liver and spleen [13,14]. During extensive CPB, new platelets formed in the bone marrow would be insufficient for post-surgical bleeding control. We hypothesized that decreased platelet counts and platelet aggregation after recirculation are due to activation and apoptosis after exposure to mechanical stress from the roller pump. We expected that platelet activation markers, such as P selectin and PS exposure [15,16], would increase soon after circulation along with apoptosis markers such as RCA-1 and PMPs [17,18]. However, we observed that platelet activation markers slightly increased during circulation but these changes were not significant. Platelets destroyed by apoptosis were rendered to microparticle size as our circuit could not clear platelets. Lower-than-expected P-selectin and PS exposure expression during circulation, especially in the early stages, indicated that platelets underwent apoptosis as evidenced by increased RCA-1 expression after circulation.

Platelet apoptosis is induced by multiple chemical stimuli and shear stresses. Leytin and colleagues reported that multiple shear instances occurred in platelets within the vascular bed,

causing severe platelet damage, mitochondrial dysfunction, and activation of apoptotic proteolytic machinery[19]. Cell shrinkage and microparticle formation, alterations of platelet surface membranes (including blebbing and filopod extension), P-selectin/PS exposure, and GPIba rearrangements also occurred [19]. Platelets subjected to fluid shear in a cone-plate viscometer abruptly underwent activation above a critical wall shear stress of 8-10 Pa [20]. Although Nascimbene and colleagues reported shear stress in axial flow and centrifugal left ventricular assist devices (LVAD) were 600 and 150-230 Pa [21], respectively, Zhou and colleagues reported the wall shear stress of roller pumping to be from 23.5 to 35.9 Pa [22]. Thus, the much lower shear stress of a roller pump would be expected to damage platelets much less than other pumping methods. This is in line with our results which showed that platelets were not strongly enough activated to significantly increase P-selectin and PS exposure expression. Roller pumping therefore induces intrinsic apoptosis of platelets as shown by RCA-1 increases without platelet activation markers P-selectin and PS exposure.

The major limitations of the present study are a small sample size and non-physiological setting. And, as we did not examine various other pumps, such as centrifugal or axial flow pumps, we could not directly compare results. And oxygenator was not incorporated in this a simulated extracorporeal circuit, influences about oxygenator were unknown. However, our results indicate that the lower shear stress of roller pumping may be superior to other technologies for prevention of platelet activation and fragmentation, but may induced apoptosis and reduced platelet function that leads to post-surgical bleeding.

Conclusion

Our results indicate that roller pumping for a typical CPB duration would be expected to activate intrinsic apoptosis of platelets but at a much lower rate than competing pump styles. Future clinical studies that directly compare roller pumping to other styles of pumps may be useful in determining the best practice for extended CPB.

References

1. Doyle AJ and Hunt BJ. Current Understanding of How Extracorporeal Membrane Oxygenators Activate Haemostasis and Other Blood Components. Frontiers in Medicine 2018; 5: 352-360.
2. Sniecinski RM, Chandler WL. Activation of the Hemostatic System During Cardiopulmonary Bypass. Anesth Analg 2011; 113:1319 –33.
3. Velik-Salchner C, Maier S, Innerhofer P, Kolbitsch C, Streif W, Mittermayer M, Praxmare M, Fries D. An Assessment of cardiopulmonary Bypass-Induced Changes in Platelet Function Using Whole Blood and Classical Light Transmission Aggregometry: The Results of a Pilot Study. Anesthesia Research Socity 2009; 106:1747-1754.
4. Sniecinski RM, Levy JH. Anticoagulation management associated with extracorporeal circulation. Best Practice &Research Clinical Anesthesiology 2015; 29:189-202.
5. Agarwal S. Platelet function testing in cardiac surgery. Transfusion Medicine 2016; 26, 319–329.
6. Kehara H, Takano T, Ohashi N, Terasaki T, Amano J. Pletelet Function During Cardiopulmonary Bypass Using Multiple Electrode Aggregometry: Comparison of Centrifugal and Roller Pump. Artif Organs 2014; 38:924-930.
7. Demirtas H, Iriz E, Demirtas CY, Erer D, Oktar L, Yener A, Arslan M. Investigating the Effects of Two Different Pump Heads (Centrifugal vs.Roller Pump) on Hematological and Immunological Mechanisms. Niger J Clin Pract 2018; 21:847-53.
8. Halaweish I, Cole A, Cooley E, Lynch WR, Haft JW. Roller and Centrifugal pumps: A Retrospective Comparison of Bleeding Complications in Extracorporeal Membrane Oxygenation. ASAIO J 2105; 61:496-501.

9. Murase M, Nakayama Y, Sessler DI, Mukai N, Ogawa S, Nakajima Y. Changes in platelet Bax levels contribute to impaired platelet response to thrombin after cardiopulmonary bypass: prospective observational clinical and laboratory investigations. British Journal of Anaesthesia 2017; 119: 1118–26.
10. Saczkowski R, Maklin M, Mesana T, Boodhwani M, Ruel M. Centrifugal Pump and Roller Pump in Adult Cardiac Surgery:A Meta-Analysis of Randomized Controlled Trials. Artificial Organs 2012; 36: 668–676.
11. Datt B, Nguyen MB, Plancher G, Ruzmetov M, O'Brien M, Kube A, Muro HM, Pourmoghadam KK, DeCamoli WM. The Impact of Roller Pump vs. Centrifugal Pump on Homologous Blood Transfusion in Pediatric Cardiac Surgery. J Extra Corpor Technol. 2017; 49: 36–43.
12. Passaroni AC, Felicio ML, de Campos NLKL, Silva MAM, Yoshida B. Hemolysis and Inflammatory Response to Extracorporeal Circulation during On-Pump CABG: Comparison between Roller and Centrifugal Pump Systems. Braz J Cardiovasc Surg 2018; 33: 64-71.
13. Li R, Hoffmeister KM, Falet H. Glycans and the platelet life cycle. Platelets 2016; 27: 505–511.
14. Lebois M, Josefsson EC. Regulation of platelet lifespan by apoptosis. Platelets 2016; 27: 497–504.
15. Andre´ P. P-selectin in haemostasis. British Journal of Haematology 2004; 126, 298–306.
16. Bevers EM, Williamson PL. Getting to the outer leaflet: Physiology of phosphatidylserine exposure at the plasma membrane. Physiol Rev 2016; 96: 605–645.
17. McArthur K, Chappaz S, Kile BT. Apoptosis in megakaryocytes and platelets: the life and death of a lineage. Blood 2018; 131(6): 605-610.
18. Burnouf T, Goubran HA, Chou ML, Devos D, Radosevic M. Platelet microparticles: Detection and assessment of their paradoxical functional roles in disease and regenerative medicine.Bloos Reviews 2014; 28:155-166.

19. Leytin V. Apoptosis in the anucleate platelet. Blood Reviews 2012; 26: 51–63.
20. Shankaran H, Alexandridis P, Neelamegham S. Aspects of hydrodynamic shear regulating shear-induced platelet activation and selfassociation of von Willebrand factor in suspension. Blood 2003; 101:2637-2645.
21. Nascimbene A, Neelamegham S, Frazier OH, Moake JL, Dong J-F. Acquired von Willebrand syndrome associated with left ventricular assist device. Blood 2016; 127:3133-3141.
22. Zhou X, Liang XM, Zhao G, Su Y, Wang Y. A New Computational Fluid Dynamics Method for In-Depth Investigation of Flow Dynamics in Roller Pump Systems. Artif Organs 2014; 38:E106-117.

Table and Figure legends

Table 1 Whole data are summrized.

Data are given as mean and standard deviation of the mean.

#, $p<0.05$ between before and specific time points (paired Student's t test).

Figure 1

Platelet count and microparticle before (time 0), 5,10,20 and 30 min after the recirculation. White bars indicate platelet counts and black bars indicate microparticle (%). Values are the mean ± standard deviation of the mean. #, $p<0.05$ between before and specific time points (paired Student's t test).

Figure 2

Platelet aggregation before (time 0), 5,10,20 and 30 min after the recirculation. Gray bars indicate platelet aggregation. Values are the mean ± standard deviation of the mean. #, $p<0.05$ between before and specific time points (paired Student's t test).

Figure 3

PS exposure expression, P- selectin expression and RCA-1 expression before (time 0), 5,10,20 and 30 min after the recirculation. White bars indicate PS exposure expression, gray bars indicate P- selectin expression and black bars indicate RCA-1 expression. Values are the mean ± standard deviation of the mean. #, $p<0.05$ between before and specific time points (paired Student's t test).

Table

measurement \ minutes	before(0)	5	10	20	30
Platelet count($\times 10^4/\mu L$)	34.8 ± 8.7	29.9 ± 14.6	26.9 ± 12.9	20.8 ± 10.2 #	17.1 ± 8.5 #
Microparticle($\times 10^4/\mu L$)	3.2 ±0.3	17.4 ±7.3	25.4 ±10.0 #	38.7 ±10.3 #	45.1 ±11.9 #
Platelet aggregation (%)	73.0 ±14.1	50.3 ±27.3	42.8 ±22.8 #	21.8 ±10.4 #	20.3 ±4.0 #
PS-exposure (%)	3.4 ±0.2	18.6 ±20.2	16.1 ±18.0	15.0 ±19.1	5.6 ±7.6
P-Selectin (%)	3.3 ±0.6	6.0 ±3.3	7.0 ±6.3	5.3 ±2.8	7.5 ±6.9
RCA-1 (%)	2.9 ±0.8	4.7 ±3.6	14.8 ±15.3	12.7 ±7.4	25.9± 12.6 #

Figure1

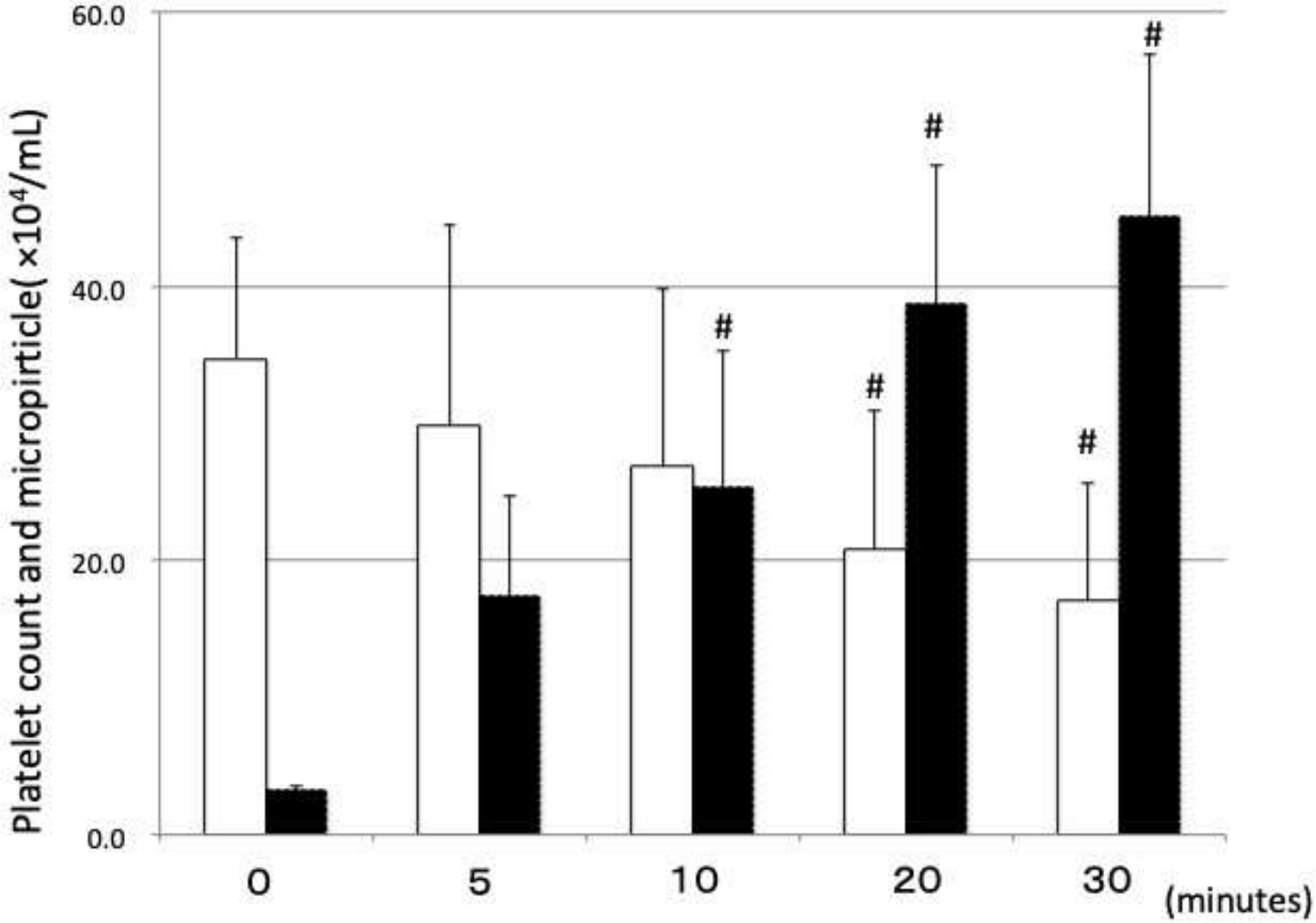

Figure2

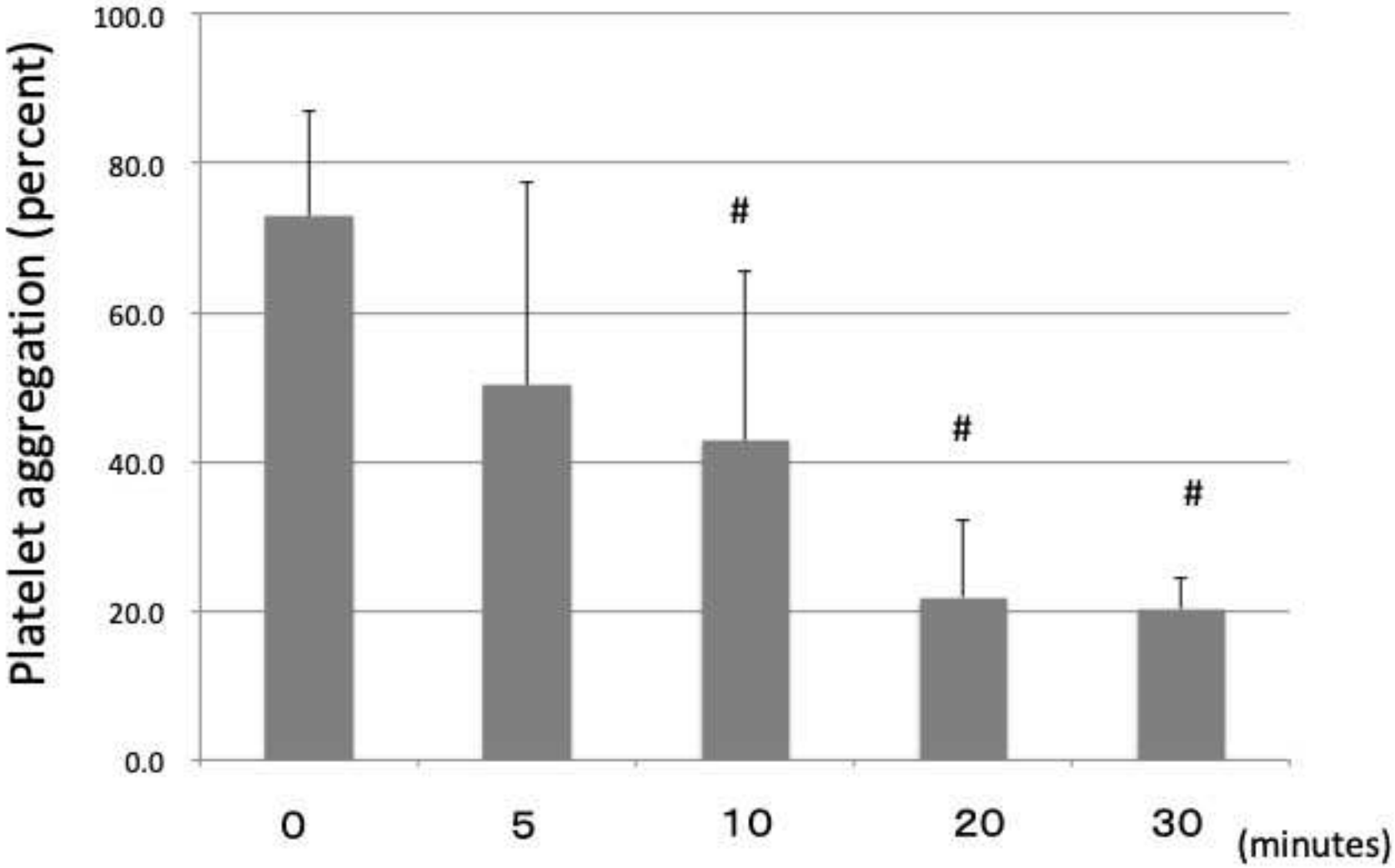

Figure3