

## Intracellular origin and ultrastructure of platelet-derived microparticles

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### Abstract

© 2017 International Society on Thrombosis and Haemostasis Essentials Platelet microparticles play a major role in pathologies, including hemostasis and thrombosis. Platelet microparticles have been analyzed and classified based on their ultrastructure. The structure and intracellular origin of microparticles depend on the cell-activating stimulus. Thrombin-treated platelets fall apart and form microparticles that contain cellular organelles. Summary: Background Platelet-derived microparticles comprise the major population of circulating blood microparticles that play an important role in hemostasis and thrombosis. Despite numerous studies on the (patho)physiological roles of platelet-derived microparticles, mechanisms of their formation and structural details remain largely unknown. Objectives Here we studied the formation, ultrastructure and composition of platelet-derived microparticles from isolated human platelets, either quiescent or stimulated with one of the following activators: arachidonic acid, ADP, collagen, thrombin or calcium ionophore A23187. Methods Using flow cytometry, transmission and scanning electron microscopy, we analyzed the intracellular origin, structural diversity and size distributions of the subcellular particles released from platelets. Results The structure, dimensions and intracellular origin of microparticles depend on the cell-activating stimulus. The main structural groups include a vesicle surrounded by one thin membrane or multivesicular structures. Thrombin, unlike other stimuli, induced formation of microparticles not only from the platelet plasma membrane and cytoplasm but also from intracellular structures. A fraction of these vesicular particles having an intracellular origin contained organelles, such as mitochondria, glycogen granules and vacuoles. The size of platelet-derived microparticles depended on the nature of the cell-activating stimulus. Conclusion The results obtained provide a structural basis for the qualitative differences of various platelet activators, for specific physiological and pathological effects of microparticles, and for development of advanced assays.

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### Keywords

blood microparticles, cellular microvesicles, electron microscopy, platelet activation, platelets

### References

- [1] Boudreau LH, Duchez A-C, Cloutier N, Soulet D, Martin N, Bollinger J, Pare A, Rousseau M, Naika GS, Levesque T, Laflamme C, Marcoux G, Lambeau G, Farndale RW, Pouliot M, Hamzeh-Cognasse H, Cognasse F, Garraud O, Nigrovic PA, Guderley H, et al. Platelets release mitochondria serving as substrate for bactericidal group IIA-secreted phospholipase A2 to promote inflammation. *Blood* 2014; 14: 2173-83.
- [2] Kelton JG. The pathophysiology of heparin-induced thrombocytopenia. Biological basis for treatment. *Chest* 2005; 127: 9-20.
- [3] Nomura S, Ozaki Y, Ikeda Y. Function and role of microparticles in various clinical settings. *Thromb Res* 2008; 123: 8-23.
- [4] Varon D, Shai E. Platelets and their microparticles as key players in pathophysiological responses. *J Thromb Haemost* 2015; 13: 40-6.
- [5] Aatonen M, Grönholm M, Siljander PR. Platelet-derived microvesicles: multitasking participants in intercellular communication. *Semin Thromb Hemost* 2012; 38: 102-13.
- [6] Horstman LL, Ahn YS. Platelet microparticles: a wide-angle perspective. *Crit Rev Oncol Hematol* 1999; 30: 111-42.
- [7] Nomura S, Shimizu M. Clinical significance of procoagulant microparticles. *J Intensive Care* 2015; 3: 1-11.
- [8] Thushara RM, Hemshekhar M, Basappa, Kemparaju K, Rangappa KS, Girish KS. Biologicals, platelet apoptosis and human diseases: an outlook. *Crit Rev Oncol Hematol* 2015; 93: 149-58.
- [9] Burnouf T, Goubran HA, Chou M-L, Devos D, Radosevic M. Platelet microparticles: detection and assessment of their paradoxical functional roles in disease and regenerative medicine. *Blood Rev* 2014; 28: 155-66.
- [10] van der Pol E, Böing AN, Harrison P, Sturk A, Nieuwland R. Classification functions, and clinical relevance of extracellular vesicles. *Pharmacol Rev* 2012; 64: 677-705.
- [11] Mezouar S, Mege D, Darbousset R, Farge D, Deboureau P, Dignat-George F, Panicot-Dubois L, Dubois C. Involvement of platelet-derived microparticles in tumor progression and thrombosis. *Semin Oncol* 2014; 41: 346-58.
- [12] Owens AP, Mackman N. Microparticles in hemostasis and thrombosis. *Circ Res* 2011; 108: 1284-97.
- [13] Ayers L, Harrison P, Kohler M, Ferry B. Procoagulant and platelet-derived microvesicle absolute counts determined by flow cytometry correlates with a measurement of their functional capacity. *J Extracell Vesicles* 2014; 3: 25348.
- [14] Nieuwland R, van der Pol E, Gardiner C, Sturk A. Platelet-Derived Microparticles. In: Michelson AD, ed. *Platelets*, 3rd edn. San Diego, CA, USA: Academic Press, 2013: 453-67.
- [15] Aatonen MT, Öhman T, Nyman TA, Laitinen S, Grönholm M, Siljander PR-M. Isolation and characterization of platelet-derived extracellular vesicles. *J Extracell Vesicles* 2014; 3: 24692.
- [16] Gremmel T, Frelinger AL, Michelson AD. Platelet physiology. *Semin Thromb Hemost* 2016; 42: 191-204.
- [17] Lacroix R, Robert S, Poncelet P, Kasthuri RS, Key NS, Dignat-George F. Standardization of platelet-derived microparticle enumeration by flow cytometry with calibrated beads: results of the International Society on Thrombosis and Haemostasis SSC Collaborative workshop. *J Thromb Haemost* 2010; 8: 2571-4.
- [18] Cocucci E, Meldolesi J. Ectosomes and exosomes: shedding the confusion between extracellular vesicles. *Trends Cell Biol* 2015; 25: 364-72.
- [19] van der Pol E, Hoekstra AG, Sturk A, Otto C, Van Leeuwen TG, Nieuwland R. Optical and non-optical methods for detection and characterization of microparticles and exosomes. *J Thromb Haemost* 2012; 8: 2596-607.
- [20] Heijnen HFG., Schiel AE, Fijnheer R, Geuze HJ, Sixma JJ. Exocytosis of multivesicular bodies and  $\alpha$ -granules microvesicles by surface shedding and exosomes derived from activated platelets release two types of membrane vesicles. *Blood* 1999; 94: 3791-9.
- [21] Leytin V, Allen DJ, Mykhaylov S, Lyubimov E, Freedman J. Thrombin-triggered platelet apoptosis. *J Thromb Haemost* 2006; 4: 2656-63.
- [22] Arraud N, Linares R, Tan S, Gounou C, Pasquet J-M, Mornet S, Brisson AR. Extracellular vesicles from blood plasma: determination of their morphology, size, phenotype and concentration. *J Thromb Haemost* 2014; 12: 614-27.
- [23] Thushara RM, Hemshekhar M, Kemparaju K, Rangappa KS, Devaraja S, Girish KS. Therapeutic drug-induced platelet apoptosis: an overlooked issue in pharmacotoxicology. *Arch Toxicol* 2014; 88: 185-98.
- [24] Zubairova LD, Nabiullina RM, Nagaswami C, Zuev YF, Mustafin IG, Litvinov RI, Weisel JW. Circulating microparticles alter formation, structure, and properties of fibrin clots. *Sci Rep* 2015; 5: 176110.
- [25] James G, White MD. Exocytosis of secretory organelles from blood platelets incubated with cationic polypeptides. *Am J Pathol* 1972; 69: 41-54.
- [26] Zucker WH, Shermer RW, Mason RG. Ultrastructural comparison of human platelets separated from blood by various means. *Am J Pathol* 1974; 77: 255-68.
- [27] Neumüller J, Meisslitzer-Ruppitscha C, Ellinger A, Pavelkaa M, Jungbauer C, Renzb R, Leitner G, Wagner T. Monitoring of platelet activation in platelet concentrates using transmission electron microscopy. *Transfus Med Hemother* 2013; 40: 101-7.

- [28] Boral BM, Williams DJ, Boral LI. Disseminated intravascular coagulation. *Am J Clin Pathol* 2016; 146: 670–80.
- [29] Cines DB, McMillan R. Pathogenesis of chronic immune thrombocytopenic purpura. *Curr Opin Hematol* 2007; 14: 511–4.
- [30] Shatzel JJ, Taylor JA. Syndromes of thrombotic microangiopathy. *Med Clin North Am* 2017; 101: 395–415.
- [31] Stein JM, Luzio JP. Ectocytosis caused by sublytic autologous complement attack on human neutrophils. The sorting of endogenous plasma-membrane proteins and lipids into shed vesicles. *Biochem J* 1991; 274: 381–6.
- [32] Johnstone RM, Adam M, Hammond JR, Orr L, Turbide C. Vesicle formation during reticulocyte maturation. Association of plasma membrane activities with released vesicles (exosomes). *J Biol Chem* 1987; 262: 9412–20.
- [33] Akers JC, Gonda D, Kim R, Carter BS, Chen CC. Biogenesis of extracellular vesicles (EV): exosomes, microvesicles, retrovirus-like vesicles, and apoptotic bodies. *J Neurooncol* 2013; 113: 1–11.
- [34] Spees JL, Olson SD, Whitney MJ, Prockop DJ. Mitochondrial transfer between cells can rescue aerobic respiration. *Proc Natl Acad Sci USA* 2006; 103: 1283–8.
- [35] Polasek J. The appearance of multivesicular structures during platelet activation as observed by scanning electron microscopy. *Thromb Res* 1982; 28: 433–7.
- [36] George JN, Thoi LL, McManus LM, Reimann TA. Isolation of human platelet membrane microparticles from plasma and serum. *Blood* 1982; 60: 834–40.
- [37] Biró E, Akkerman JW, Hoek FJ, Gorter G, Pronk LM, Sturk A, Nieuwland R. The phospholipid composition and cholesterol content of platelet-derived microparticles: a comparison with platelet membrane fractions. *J Thromb Haemost* 2005; 3: 2754–63.
- [38] Shai E, Rosa I, Parguina AF, Motahedeh S, Varon D, García Á. Comparative analysis of platelet-derived microparticles reveals differences in their amount and proteome depending on the platelet stimulus. *J Proteomics* 2012; 5: 287–96.
- [39] Milioli M, Ibáñez-Vea M, Sidoli S, Palmisano G, Careri M, Larsen MR. Quantitative proteomics analysis of platelet-derived microparticles reveals distinct protein signatures when stimulated by different physiological agonists. *J Proteomics* 2015; 21: 56–66.
- [40] Hoyer DP, Korkmaz Y, Grönke S, Addicks K, Wettschureck N, Offermanns S, Reuter H. Differential expression of protein kinase C isoforms in coronary arteries of diabetic mice lacking the G-protein  $G\alpha_{11}$ . *Cardiovasc Diabetol* 2010; 9: 93.
- [41] Hughes M, Hayward CPM, Warkentin TE, Horsewood P, Chorneyko KA., Kelton JG. Morphological analysis of microparticle generation in heparin-induced thrombocytopenia. *Blood* 2000; 96: 188–94.