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From platelet dust to gold dust: physiological importance and detection of platelet microvesicles

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This series of contemporary reviews focuses on the unique physiology and detection of platelet derived microvesicles. Platelet associated procoagulant activity, or “platelet dust”, has long been known to be important in haemostasis^{1,2}. Defects in vesicle associated procoagulant activity are indeed implicated with various bleeding disorders^{3,4}. Conversely, high levels of circulating platelet microvesicles are not only platelet specific activation markers, but are potential biomarkers of a variety of thrombotic⁵⁻⁷, inflammatory diseases⁸⁻¹⁰, and cancer^{11,12}. Thus, profound understanding together with accurate and reproducible detection of platelet microvesicles may turn platelet dust into gold dust.

Fig. 1 shows a transmission electron micrograph of platelet microvesicles labelled with gold nanoparticles targeting integrin beta-3 (CD61). This single image reveals the presence, functionality, heterogeneity, as well as the small size of platelet microvesicles. Because platelet microvesicles are more than 10-fold smaller in diameter than platelets themselves, platelet microvesicles are difficult to study.

For many years, haemostasis and thrombosis researchers have measured platelet procoagulant vesicle activity using relatively simple clotting assays^{2,13,14}. Although these assays are useful for measuring bulk procoagulant activity of vesicles, they provide little information on the individual properties of vesicles. Because vesicles are heterogeneous, access to the individual properties of vesicles is likely to provide more accurate clinical information.

Flow cytometry was the instrumentation of choice to characterize platelet microvesicles one by one^{3,4,9-14}. The development of flow cytometry not only revolutionized the phenotypic and functional measurement of platelets, but also facilitated deeper insight into the size range, phenotypes and functional characteristics of platelet vesicles in whole blood, platelet-rich plasma (PRP) or platelet-poor plasma (PPP). However, flow cytometers were originally designed to measure cells and platelets, thus soon it became clear that there are fundamental physical limitations to the application of flow cytometers to characterize microvesicles^{15,16}. The arrival of new biophysical approaches taught us that conventional flow cytometry is unable to characterise the full size range and potential enormous quantities of vesicles¹⁷. Despite this, an evolving series of studies led by the Scientific and Standardization Committee (SSC) on vascular biology of the International Society of Thrombosis and Haemostasis (ISTH), the International Society on Extracellular Vesicles (ISEV), and the International Society for Advancement of Cytometry (ISAC) have led to an optimal understanding of the size limitation of individual instruments¹⁸⁻²¹. Since one year, the joint flow cytometry working group of the ISTH, ISEV and ISAC provides important recommendations on pre-analytical and analytical variables to facilitate standardization of vesicle measurements (www.evflowcytometry.org)²².

Meanwhile, pioneering engineers pushed the lower limit of size detection downwards^{21,23}. Using these next generation cytometers, researchers showed that there was previously unknown biological information within the smaller vesicle populations. This tendency is now culminating in the development of dedicated nanocytometers²⁴, designed from first principles to detect all vesicle populations. Coupled with the recent advancements in the field of extracellular vesicle research, it has become apparent that vesicles are not just potential biomarkers and important in many areas of cell biology, but provide a novel intercellular transport mechanism between different cell types.

This commissioned series of up to date reviews begins with two articles on the role of platelet microvesicles in health and disease and their novel roles in intercellular communication by Boilard and Edelstein, respectively^{25,26}. Nieuwland and Gasecka then discuss whether platelet vesicles have utility as useful biomarkers of arterial thrombosis²⁷. The remaining reviews focus on vesicle detection, including classical functional and genetic assays by Amiral et al.²⁸; the detection of vesicles by immuno-based bulk techniques by Coumans et al.²⁹, the detection of single vesicles in suspension using a variety of novel approaches by Buzas, Gardiner, Lee and Smith³⁰; and the recent exciting developments in flow cytometry and nanocytometry by Nolan and Jones³¹. This series culminates with a new research paper by Brisson et al.³², demonstrating the beautiful structure of vesicles from activated platelets by cryo-electron microscopy.

In sum, the contributions to this review series justify that the field has progressed enormously in the last 20 years. It is evident that we are at the threshold of capitalizing on the enormous potential utility of vesicle measurements as biomarkers and as novel therapeutic agents for many of the major diseases that afflict mankind.

Figure legend

Figure 1: Transmission electron microscopy (TEM) image of platelet microvesicles labelled with gold nanoparticles targeting integrin beta-3 (CD61). CD61 is a cluster of differentiation found on platelets. Microvesicles imaged by TEM often have a cup-shaped morphology, which is attributed to the thin (~5 nm) and thus fragile phospholipid membrane, which often collapses due to adhesion and dehydration. In suspension, however, most (>95%) microvesicles are spherical particles^{32,33}. Other particles in this image may be proteins, lipoprotein particles or vesicles from other cell types than platelets.

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References

- 1 Wolf, P. The nature and significance of platelet products in human plasma. *Br J Haematol* **13**, 269-288 (1967).
- 2 Chargaff, E. & West, R. The biological significance of the thromboplastic protein of blood. *J Biol Chem* **166**, 189-197 (1946).
- 3 Dachary-Prigent, J. *et al.* Aminophospholipid exposure, microvesiculation and abnormal protein tyrosine phosphorylation in the platelets of a patient with Scott syndrome: a study using physiologic agonists and local anaesthetics. *Br J Haematol* **99**, 959-967 (1997).

- 4 Castaman, G., Yu-Feng, L., Battistin, E. & Rodeghiero, F. Characterization of a novel bleeding disorder with isolated prolonged bleeding time and deficiency of platelet microvesicle generation. *Br J Haematol* **96**, 458-463 (1997).
- 5 Giannopoulos, G. *et al.* Red blood cell and platelet microparticles in myocardial infarction patients treated with primary angioplasty. *Int J Cardiol* **176**, 145-150, doi:10.1016/j.ijcard.2014.07.022 (2014).
- 6 Montoro-Garcia, S. *et al.* Small-size circulating microparticles in acute coronary syndromes: relevance to fibrinolytic status, reparative markers and outcomes. *Atherosclerosis* **227**, 313-322, doi:10.1016/j.atherosclerosis.2013.01.028 (2013).
- 7 Chen, Y. *et al.* The Role of Circulating Platelets Microparticles and Platelet Parameters in Acute Ischemic Stroke Patients. *J Stroke Cerebrovasc Dis* **24**, 2313-2320, doi:10.1016/j.jstrokecerebrovasdis.2015.06.018 (2015).
- 8 Pelletier, F. *et al.* Increased levels of circulating endothelial-derived microparticles and small-size platelet-derived microparticles in psoriasis. *J Invest Dermatol* **131**, 1573-1576, doi:10.1038/jid.2011.57 (2011).
- 9 Pereira, J. *et al.* Circulating platelet-derived microparticles in systemic lupus erythematosus. Association with increased thrombin generation and procoagulant state. *Thromb Haemost* **95**, 94-99 (2006).
- 10 Knijff-Dutmer, E. A., Koerts, J., Nieuwland, R., Kalsbeek-Batenburg, E. M. & van de Laar, M. A. Elevated levels of platelet microparticles are associated with disease activity in rheumatoid arthritis. *Arthritis Rheum* **46**, 1498-1503, doi:10.1002/art.10312 (2002).
- 11 Toth, B. *et al.* Platelet-derived microparticles and coagulation activation in breast cancer patients. *Thromb Haemost* **100**, 663-669 (2008).
- 12 Mege, D. *et al.* The origin and concentration of circulating microparticles differ according to cancer type and evolution: A prospective single-center study. *Int J Cancer* **138**, 939-948, doi:10.1002/ijc.29837 (2016).
- 13 Nieuwland, R. *et al.* Cellular origin and procoagulant properties of microparticles in meningococcal sepsis. *Blood* **95**, 930-935 (2000).
- 14 Nieuwland, R. *et al.* Cell-derived microparticles generated in patients during cardiopulmonary bypass are highly procoagulant. *Circulation* **96**, 3534-3541 (1997).
- 15 van der Pol, E., van Gemert, M. J., Sturk, A., Nieuwland, R. & van Leeuwen, T. G. Single vs. swarm detection of microparticles and exosomes by flow cytometry. *J Thromb Haemost* **10**, 919-930, doi:10.1111/j.1538-7836.2012.04683.x (2012).
- 16 Harrison, P. & Gardiner, C. Invisible vesicles swarm within the iceberg. *J Thromb Haemost* **10**, 916-918, doi:10.1111/j.1538-7836.2012.04711.x (2012).
- 17 van der Pol, E. *et al.* Particle size distribution of exosomes and microvesicles determined by transmission electron microscopy, flow cytometry, nanoparticle tracking analysis, and resistive pulse sensing. *J Thromb Haemost* **12**, 1182-1192, doi:10.1111/jth.12602 (2014).
- 18 Lacroix, R. *et al.* 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* **8**, 2571-2574, doi:10.1111/j.1538-7836.2010.04047.x (2010).
- 19 Cointe, S. *et al.* Standardization of microparticle enumeration across different flow cytometry platforms: results of a multicenter collaborative workshop. *J Thromb Haemost*, doi:10.1111/jth.13514 (2016).
- 20 Arraud, N., Gounou, C., Turpin, D. & Brisson, A. R. Fluorescence Triggering: A General Strategy for Enumerating and Phenotyping Extracellular Vesicles by Flow Cytometry. *Cytom Part A* **89a**, 184-195, doi:10.1002/cyto.a.22669 (2016).
- 21 Stoner, S. A. *et al.* High sensitivity flow cytometry of membrane vesicles. *Cytometry A* **89**, 196-206, doi:10.1002/cyto.a.22787 (2016).

- 22 van der Pol, E., Boing, A. N., Gool, E. L. & Nieuwland, R. Recent developments in the nomenclature, presence, isolation, detection and clinical impact of extracellular vesicles. *J Thromb Haemost* **14**, 48-56, doi:10.1111/jth.13190 (2016).
- 23 van der Vlist, E. J., Nolte-'t Hoen, E. N., Stoorvogel, W., Arkesteijn, G. J. & Wauben, M. H. Fluorescent labeling of nano-sized vesicles released by cells and subsequent quantitative and qualitative analysis by high-resolution flow cytometry. *Nat Protoc* **7**, 1311-1326, doi:10.1038/nprot.2012.065 (2012).
- 24 Zhu, S. *et al.* Light-scattering detection below the level of single fluorescent molecules for high-resolution characterization of functional nanoparticles. *ACS Nano* **8**, 10998-11006, doi:10.1021/nn505162u (2014).
- 25 Melki, I., Tessandier, N., Zufferey, A. & Boilard, E. Platelet microvesicles in health and disease. *Platelets Submitted* (2017).
- 26 Edelstein, L. C. The role of platelet microvesicles in intercellular communication. *Platelets Submitted* (2017).
- 27 Gasecka, A., Boing, A., Filipiak, K. & Nieuwland, R. Platelet extracellular vesicles as biomarkers for arterial thrombosis. *Platelets Submitted* (2017).
- 28 Laroche, M., Dunois, C., Vissac, A. M. & Amiral, J. Functional and genetic laboratory assays for detection of platelet microvesicles. *Platelets Submitted* (2017).
- 29 Coumans, F. A. W., Gool, E. L. & Nieuwland, R. Bulk immunoassays for analysis of extracellular vesicles. *Platelets Submitted* (2017).
- 30 Buzás, E., Gardiner, C., Lee, C. & Smith, Z. Single particle analysis: methods for detection of platelet extracellular vesicles in suspension. *Platelets Submitted* (2017).
- 31 Jones, J. & Nolan, J. P. Detection of platelet microvesicles by flow cytometry *Platelets Submitted* (2017).
- 32 Brisson, A. R., Tan, S., Linares, R., Gounou, C. & arraud, N. Extracellular vesicles from activated platelets: a quantitative cryo-electron microscopy and immuno-gold labeling study. *Platelets Submitted* (2017).
- 33 Arraud, N. *et al.* Extracellular vesicles from blood plasma: determination of their morphology, size, phenotype and concentration. *J Thromb Haemost* **12**, 614-627, doi:10.1111/jth.12554 (2014).