

Microparticles: Reliable Biomarkers of Thrombosis?

Shelly Chopra

Bachelor of Health Sciences (Honours) Program, Class of 2014

Microparticles are vesicles that bud off cells in response to extracellular stimulation or apoptosis. They play an important role in the regulation of physiological and pathological pathways such as clot formation and the progression of thrombotic disease by influencing the expression of critical proteins and cofactors. The following critical review evaluates research findings on the potential significance of microparticles as indicators of thrombotic risk.

From “platelet dust” to “trigger[s] of vascular repair,” the description of microparticles (MPs) has evolved over the past four decades.^{1,2} These submicron (0.1-1 μm diameter) vesicles shed off cellular plasma membrane in response to cell activation, stress, or apoptosis (Figure 1). MPs have been observed to bind and fuse with endothelial and blood cells, enabling the delivery of protein and RNA contained within them.³ In platelets and monocytes, this fusion promotes haemostasis, suggesting the procoagulant nature of certain MPs. This homeostatic role of MPs seems to depend on the quantity and type of MPs. As demonstrated in many studies, MPs that bear elevated levels of tissue factor (TF), a potent initiator of platelet activation, play a role in the pathogenesis of thrombotic and hypertensive conditions.⁴ This review will discuss the potential of MPs as biomarkers of thrombosis and the clinical implications of current MP research.

MICROPARTICLE PHYSIOLOGY

A. Procoagulant Properties of Microparticles

MPs are a key component of haemostasis because they directly activate protein factors and enzymes responsible for initiating the coagulation cascade. The membranes of platelet-derived MPs

are known to contain receptor sites for procoagulant factors IIa, Va, VII, and IXa. In addition, certain MPs also express the phospholipid phosphatidylserine (PS) on their cell membrane, which catalyzes clotting by providing a surface for the reaction to occur.⁵ Interestingly, the surface provided by PS on MPs was found to have up to 100 times greater procoagulant activity than an equal area on an activated platelet.⁶

The significance of microparticle formation in directing haemostasis is highlighted in Scott syndrome, a condition characterized by a deficiency in platelet coagulant activity. This deficiency is directly linked to a defective scramblase, an enzyme in the plasma membrane responsible for the externalization of PS. As a result, PS expression on MP membranes is reduced, inhibiting the binding and enzymatic activity of procoagulant factors.⁷

B. Microparticles are Mediators of Apoptosis

Both inhibition of MP clearance and excessive MP in circulation have been linked to a number of immunosuppressive and thrombotic diseases. Normally, the presence of PS on MPs is an immune-system recognition marker as it is externalized on apoptotic cells to trigger macrophage-mediated removal of cellular debris. However, elevated levels of MPs can stimulate unnecessary

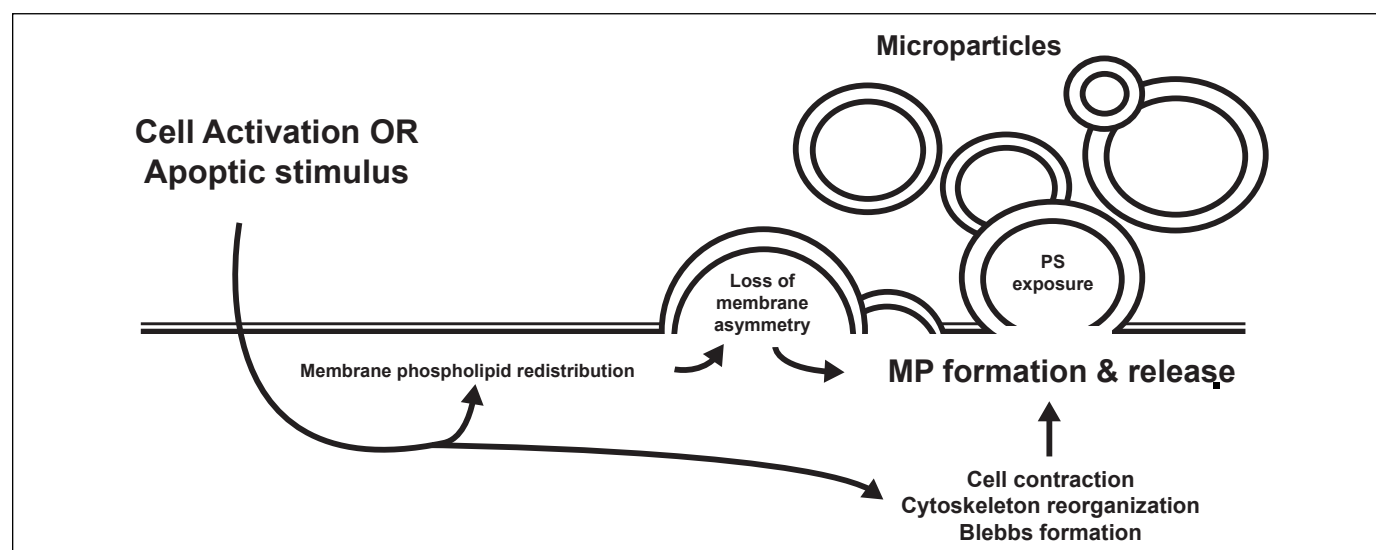


FIGURE 1: Basic mechanism of MP formation and release.⁴ Microparticle formation is initiated in response to extracellular activation of apoptotic stimulus. A series of membrane phospholipid reorganization events follow, destabilizing the physiological asymmetry of the membrane. This step in the budding of microparticles with external PS exposure.

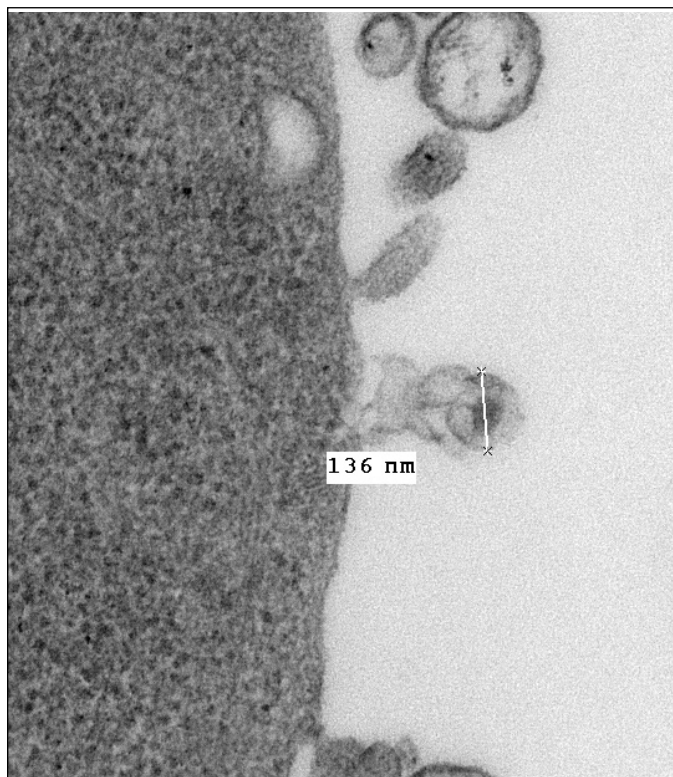


FIGURE 2: Electron micrograph of MPs shedding from parent monocyte. Taken by Hilary Christensen, Department of Pathology, University of Toronto.

apoptosis, an outcome that demonstrates the pathological role of MPs in immunosuppressive and thrombotic diseases.⁸

MICROPARTICLES AND THROMBOTIC DISEASE

A. Thrombosis

Many disorders associated with elevated levels of MPs involve abnormal thrombosis.⁴ Thrombosis refers to the process of forming a clot inside a blood vessel, thereby restricting blood flow (occlusion). In addition to PS expression, the presence of tissue factor (TF) on MPs promotes thrombosis. TF is a protein cofactor required to initiate coagulation. Under normal conditions, circulating blood contains very low levels (10^{-9} M) of active TF as it is expressed mainly in tissues outside the vascular system. During inflammation and elevated immune response, however, monocytes and endothelial cells express TF and release it into circulation via microparticles.⁹ Some TF-bearing MPs also contain adhesion molecules, such as PSGL-1, that allow them to bind to platelets and further stimulate the clotting cascade.³

TF-bearing MPs bud from lipid raft domains on parent monocytic cell membranes. Lipid rafts are regions of the plasma membrane with a proportionally higher concentration of glycolipids (such as cholesterol) and glycoproteins that associate with PSGL-1 and TF. The role of lipid raft proteins in generating prothrombotic MPs is best characterized by the effect of statins, a class of drugs commonly used to lower blood cholesterol and inhibit atherosclerosis

development. Statins function by inhibiting the enzyme responsible for cholesterol synthesis. This decreases the supply of cholesterol available for lipid-raft formation, and as a result, disrupts lipid-raft structure and stability.¹⁰ In turn, the destabilization of lipid rafts inhibits appropriate externalization of PS and TF, both of which are necessary to generate viable prothrombotic MPs.¹⁰ The essential role of lipid rafts in formation of TF-bearing MPs may explain the correlation between high cholesterol levels and incidence of thrombotic diseases.¹²

B. Microparticle Tissue Factor Activity

Although elevated levels of TF-bearing MPs in cardiovascular disease are a consistent finding in many research studies, the specific characteristics of TF are debated. TF-bearing MPs are found in healthy individuals, but their measurable activity is generally very low.⁹ This may be attributed to a latent, inactive form of TF present on the MPs. TF-bearing MPs may only be activated upon assembly at the site of vascular damage—a theory that explains why MPs do not initiate coagulation on their own.⁹ However, MPs detected in cardiovascular disease may bear an active, pathologic form of TF that contributes directly to thrombus formation. Adding to the complexity of TF-bearing MPs, recent research suggests certain MPs carrying TF pathway inhibitor are also present in blood circulation.¹³ As the name suggests, these MPs function to reduce thrombus formation. Although this finding may explain the specific physiological and pathological functions of MPs, the interaction between MPs bearing TF pathway inhibitors and active/inactive TF-carrying MPs remains to be clarified.

C. Cancer-associated Thrombosis

The role of MPs in cancer-associated thrombosis has been clearly established. As the second leading cause of death in cancer patients, thrombosis has been researched for the past 150 years.¹⁴ In 1865, Armand Trousseau discovered abnormal, migratory thrombi in patients to be associated with visceral malignancy—a condition now fittingly referred to as Trousseau syndrome.¹⁵ Tumour microparticle formation has been observed in many animal studies and more recently in the blood of patients with leukemia.¹⁶ Tumour-induced MPs are characterized typically by PS and mucins, proteins that promote a prothrombotic state. The presence of TF on these MPs further the progression of systemic thrombosis and enhances tumour angiogenesis (blood vessel growth within the tumour). Increased levels of TF- or mucin-bearing MPs have been associated with tumour size and survival.¹⁷ A recent clinical study found that patients with metastatic pancreatic cancer have high levels of MP-linked TF activity, confirming the link between tumours and thrombosis.¹⁸

FUTURE DIRECTIONS

The measurable impact of MPs has contributed to the development of a clearer understanding of the pathogenesis of cardiovascular and haematological disorders. The normal physiologic role of MPs to promote haemostasis and mediate clearance of apoptotic bodies has been shown to transform into pathologic outcomes when MP levels rise in circulation. This duality in MP function

remains unresolved but MP structure specificity and origin may be responsible. For example, a high concentration of active TF-bearing MPs are a risk factor for thrombotic disease.³

Despite their association with various thrombotic diseases, the measurement of MP levels has not yet been used as a diagnostic tool. In addition to the need for better characterization of MPs, standardization of detection and quantification methods is needed to further the clinical potential of MPs. Currently, researchers tend to use different parameters of MP detection, which can be problematic when comparing data across studies.^{3,12}

The therapeutic role of MPs in diseases characterized by excessive bleeding or low MP concentration has been recently investigated.

Prothrombotic MPs promote haemostasis in models of thrombocytopenia, a condition characterized by low platelet count. In a clinical trial, administration of old, frozen platelets displayed similar haemostatic effects.¹⁹ Platelet MP shedding is known to increase during storage, a finding that may explain the results of the trial and lead to more MP-based therapies.¹²

Circulating MPs are important for maintaining haemostatic balance and are biomarkers of cardiovascular disease. This positions them as promising candidates for use in a diagnostic tool or treatment of blood disorders. However, further research on MP physiology, pathology, and quantification techniques is necessary before the full potential of MPs can be realized. ■

Reviewed by Peter Gross, MD, MSc, FRCPC and Nima Vaezzadeh, BSc

Dr. Peter Gross is an Associate Professor in the Division of Hematology and Thromboembolism of the Department of Medicine at McMaster University. His research interests include thrombosis prevention, thrombus stability, and the role of statins on haemostasis. His lab employs a variety of methodological techniques ranging from intravital microscopy to flow cytometry.

Nima Vaezzadeh is a PhD Candidate under the supervision of Dr. Peter Gross. He is currently studying the therapeutic potential of monocyte-derived microparticles as inhibitors of bleeding.

REFERENCES

- ¹Wolf P. The nature and significance of platelet products in human plasma. *Br J Haematol* 1967 May;13(3):269-288.
- ²Boulanger CM. Microparticles, vascular function and hypertension. *Curr Opin Nephrol Hypertens* 2010 Mar;19(2):177-180.
- ³Davizon P, Lopez JA. Microparticles and thrombotic disease. *Curr Opin Hematol* 2009 Sep;16(5):334-341.
- ⁴Boulanger CM, Amabile N, Tedgui A. Circulating microparticles: a potential prognostic marker for atherosclerotic vascular disease. *Hypertension* 2006 Aug;48(2):180-186.
- ⁵Bernimoulin M, Waters EK, Foy M, Steele BM, Sullivan M, Falet H, et al. Differential stimulation of monocytic cells results in distinct populations of microparticles. *J Thromb Haemost* 2009 Jun;7(6):1019-1028.
- ⁶Sinauridze EI, Kireev DA, Popenko NY, Pichugin AV, Pantelev MA, Krymskaya OV, et al. Platelet microparticle membranes have 50- to 100-fold higher specific procoagulant activity than activated platelets. *Thromb Haemost* 2007 Mar;97(3):425-434.
- ⁷Toti F, Satta N, Fressinaud E, Meyer D, Freyssinet JM. Scott syndrome, characterized by impaired transmembrane migration of procoagulant phosphatidylserine and hemorrhagic complications, is an inherited disorder. *Blood* 1996 Feb 15;87(4):1409-1415.
- ⁸Rak J. Microparticles in cancer. *Semin Thromb Hemost* 2010 Nov;36(8):888-906.
- ⁹Furie B, Furie BC. Mechanisms of thrombus formation. *N Engl J Med* 2008; 359:938-949.
- ¹⁰Ponce J, de la Ossa NP, Hurtado O, Millan M, Arenillas JF, Davalos A, et al. Simvastatin reduces the association of NMDA receptors to lipid rafts: a cholesterol-mediated effect in neuroprotection. *Stroke* 2008 Apr;39(4):1269-1275.
- ¹¹Del Conde I, Shrimpton CN, Thiagarajan P, Lopez JA. Tissue-factor-bearing microvesicles arise from lipid rafts and fuse with activated platelets to initiate coagulation. *Blood* 2005 Sep 1;106(5):1604-1611.
- ¹²Piccin A, Murphy WG, Smith OP. Circulating microparticles: pathophysiology and clinical implications. *Blood Rev* 2007 May;21(3):157-171.
- ¹³Steppich B, Mattisek C, Sobczyk D, Kastrati A, Schomig A, Ott I. Tissue factor pathway inhibitor on circulating microparticles in acute myocardial infarction. *Thromb Haemost* 2005 Jan;93(1):35-39.
- ¹⁴Pruemer J. Prevalence, causes, and impact of cancer-associated thrombosis. *Am J Health Syst Pharm* 2005 Nov 15;62(22 Suppl 5):S4-6.
- ¹⁵Varki A. Trousseau's syndrome: multiple definitions and multiple mechanisms. *Blood* 2007 Sep 15;110(6):1723-1729.
- ¹⁶Carr JM, Dvorak AM, Dvorak HF. Circulating membrane vesicles in leukemic blood. *Cancer Res* 1985 Nov;45(11 Pt 2):5944-5951.
- ¹⁷Toth B, Nieuwland R, Liebhardt S, Ditsch N, Steinig K, Stieber P, et al. Circulating microparticles in breast cancer patients: a comparative analysis with established biomarkers. *Anticancer Res* 2008 Mar-Apr;28(2A):1107-1112.
- ¹⁸Khorana AA, Francis CW, Menzies KE, Wang JG, Hyrien O, Hathcock J, et al. Plasma tissue factor may be predictive of venous thromboembolism in pancreatic cancer. *J Thromb Haemost* 2008 Nov;6(11):1983-1985.
- ¹⁹George JN, Pickett EB, Heinz R. Platelet membrane microparticles in blood bank fresh frozen plasma and cryoprecipitate. *Blood* 1986 Jul;68(1):307-309.