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Review Article

Microparticles: biomarkers and beyond

Dylan BURGER*, Sarah SCHOCK†, Charlie S. THOMPSON†, Augusto C. MONTEZANO‡, Antoine M. HAKIM† and Rhian M. TOUYZ*‡

*Kidney Research Centre, Ottawa Hospital Research Institute, University of Ottawa, Ottawa, Canada
†Neuroscience Program, Ottawa Hospital Research Institute, University of Ottawa, Ottawa, Canada
‡BHF Glasgow Cardiovascular Research Centre, University of Glasgow, Glasgow, U.K.

Abstract
Membrane microparticles are submicron fragments of membrane shed into extracellular space from cells under conditions of stress/injury. They may be distinguished from other classes of extracellular vesicles (i.e. exosomes) on the basis of size, content and mechanism of formation. Microparticles are found in plasma and other biological fluids from healthy individuals and their levels are altered in various diseases, including diabetes, chronic kidney disease, pre-eclampsia and hypertension among others. Accordingly, they have been considered biomarkers of vascular injury and pro-thrombotic or pro-inflammatory conditions. In addition to this, emerging evidence suggests that microparticles are not simply a consequence of disease, but that they themselves may contribute to pathological processes. Thus microparticles appear to serve as both markers and mediators of pathology. The present review examines the evidence for microparticles as both biomarkers of, and contributors to, the progression of disease. Approaches for the detection of microparticles are summarized and novel concepts relating to the formation of microparticles and their biological effects are examined.

Key words: biomarker, cardiovascular disease, endothelial dysfunction, microparticle, predictive power

INTRODUCTION

Membranous vesicles are important mediators of physiological and pathological cellular processes. Within the cell, specialized vesicles including endosomes, lysosomes and transport vesicles are critical to cellular homoeostasis and the shuttling of proteins/molecules. Cells also release vesicles into the extracellular space under both normal and stress conditions. Previously overlooked as an artefact, it is increasingly clear that these extracellular vesicles are more than just inert cell debris and that they play an important role in intercellular communication. Among the various types of extracellular vesicles formed, membrane microparticles (also referred to as ectosomes or, more broadly, microvesicles) are emerging as both indices of vascular injury and as circulating biologically active entities. MP research is growing rapidly and, although there is increasing literature in the field, the present comprehensive review focuses on evidence pertaining to microparticles as biomarkers of pathology and as vectors of cellular communication.

Microparticles and extracellular vesicles

The term microparticle may be used to describe a number of similarly sized particles that comprise membrane, lipoprotein, protein aggregates and other debris. Membrane microparticles, henceforth referred to as 'microparticles' (MPs), consist of a cell-derived vesicle that is formed from the outward blebbing of the plasma membrane and subsequent shedding into extracellular space [1–3]. MPs are typically defined as 0.1–1.0 μm in size and consisting of membrane proteins and cytosolic material derived from the cell from which they originate [1–3]. Believed to be formed by all cell types, MP formation has been observed in cells of the vasculature (endothelial cells, platelets, leucocytes and vascular smooth muscle cells [4]), erythrocytes [5], cardiomyocytes [6] and podocytes (D. Burger, unpublished work), as well as cancer [7] and progenitor cell populations [8,9].

MPs are released from cells under conditions of stress; however, apoptosis/cell death is not necessarily a requisite for MP formation as activated cells (i.e. endothelial cells and platelets) also release MPs [2]. Once released, MPs retain cell-surface

Abbreviations: ABC transporter, ATP-binding cassette transporter; ADAM17, a disintegrin and metalloproteinase 17; ANCA, anti-neutrophil cytoplasmic antibody; AngII, angiotensin II; COX, cyclo-oxygenase; CRP, C-reactive protein; Del-1, developmental endothelial locus-1; EGF, epidermal growth factor; EGFR, EGF receptor; fMLP, *N*-formylmethionyl-leucyl-phenylalanine; HUVEC, human umbilical vein endothelial cell; ICAM, intercellular adhesion molecule; IL, interleukin; LPS, lipopolysaccharide; MCP, monocyte chemotactic protein; miRNA, microRNA; MMP, matrix metalloproteinase; MP, microparticle; NF-κB, nuclear factor κB; NOS, NO synthase; eNOS, endothelial NOS; O_2^{-}, superoxide anion; PAI-1, plasminogen activator inhibitor-1; ROS, reactive oxygen species; Shh, sonic Hedgehog; TLR, Toll-like receptor; TMEM16F, transmembrane protein 16F; TNF, tumour necrosis factor; TRAP, thrombin-receptor-activating peptide.

Correspondence: Dr Dylan Burger (email dburger@uottawa.ca).

proteins from the cell of origin, along with cytosolic contents including enzymes, RNA, miRNA (microRNA) and, possibly, DNA [2,3]. MPs may be distinguished from other common extracellular vesicles (such as exosomes or apoptotic bodies) on the basis of size, mechanism of formation and content. Exosomes are smaller (approximately 40–100 nm) than MPs and are formed through a multi-step process [10]. The first step involves inward membrane budding and the formation of intracellular vesicles of 40–100 nm in size [10]. These vesicles then accumulate within multivesicular bodies of anywhere from 0.5 μm to several micrometres in size. Mutivesicular bodies may then fuse with either lysosomes, where they are subjected to proteolytic degredation, or with the cell membrane, where the intracellular vesicles are released into the extracellular milieu as exosomes [11]. Exosomes are detectable in plasma, urine, bronchoalveolar lavage fluid and breast milk [12]. Their formation appears to occur in most, if not all, cell types and occurs both constitutively and in response to induction [10,13]. Exosomes may be identified by the presence of exosome-specific markers including Lamp1 (lysosomal-associated membrane protein 1), TSG101 (tumour susceptibility gene 101) and the membrane protein CD63 [10]. There is some debate as to whether exosomes externalize phosphatidylserine; however, it is likely that phosphatidylserine exposure, if present, is minimal. However, exosomes are enriched in lipid rafts have been shown to contain both membrane and cytosolic components, including protein, RNA, and miRNA, but lack nuclear material. For a comprehensive examination of exosomes and their biological relevance, readers are directed to more focused reviews [10,11].

Apoptotic bodies, in contrast, are much larger than exosomes or MPs with a size of 1–5 μm [13]. They are formed exclusively during the late stages of apoptosis during cell shrinkage/collapse and after externalization of phosphatidylserine, increases in membrane permeability and karyorrhexis (nuclear fragmentation) [14]. Apoptotic bodies contain significant nuclear material, tightly packed cellular organelles and membrane/cytosolic contents [14]. Similar to MPs, apoptotic bodies externalize phosphatidylserine on their membrane, but may be distinguished by size, the presence of significant nuclear material and/or cell organelles and a permeable membrane [2]. Apoptotic bodies are formed by all cell types.

Although the size definitions draw clear distinctions between exosomes, MPs and apoptotic bodies, it is likely that even the most carefully prepared vesicle preparations remain heterogeneous and there is at least some overlap in size among vesicular populations. The term 'microvesicle' is commonly used in the literature and can be a source of confusion [13]. Microvesicle refers to any intact vesicular membrane fragment which, on one hand, is a more specific term than MPs in that it excludes non-membranous particles. However, as the size of the particles is often not defined in microvesicle populations, the term often refers to heterogeneous extracellular populations of exosomes, MPs and apoptotic bodies obtained via ultracentrifugation. Figure 1 depicts the formation and physical properties of MPs, exosomes and apoptotic bodies. Less common terms for extracellular vesicles, such as exosome-like vesicles (implying 20–50 nm particles similar to exosomes but lacking lipid-rich domains) and membrane particles (which refer to 50–100 nm membrane fragments enriched in CD133/prominin-1), are more poorly defined and infrequently used [15]. Given the clear differences between classes of extracellular vesicles, there is a critical need for strict nomenclature and definitions in the reporting of all studies investigating extracellular vesicles. Indeed the International Society of Extracellular Vesicles (www.isev.org) has initiated efforts to establish a standardized nomenclature for various extracellular vesicles. Table 1 outlines the characteristics of commonly used terms for extracellular vesicles as typically defined.

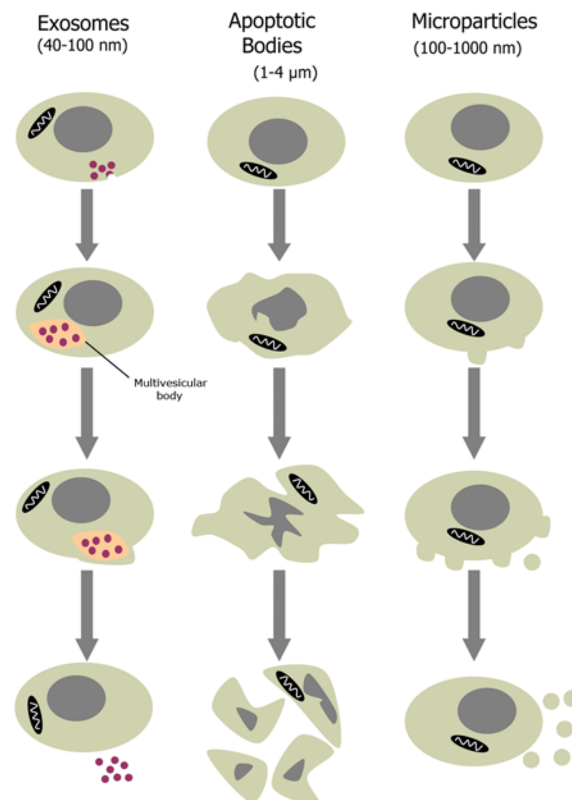

Figure 1 Origins of various classes of extracellular vesicles
Exosomes are formed through inward membrane budding, the formation of 40 – 100 nm intracellular vesicles which accumulate within multivesicular bodies and their subsequent release into the extracellular milieu. Apoptotic bodies are formed during the late stages of apoptosis, after cell shrinkage and may contain DNA and/or organelles. MPs are formed from the outward blebbing of membrane and release into the extracellular space.

How are MPs formed?

In contrast with exosome formation, which involves inward membrane invagination, MP formation involves the outward blebbing of the plasma membrane [3]. Although the precise molecular determinants of MP formation are not known, cytoskeletal re-organization and alterations in phospholipid symmetry are essential to formation.

As MP formation begins with the outward blebbing of the plasma membrane it is perhaps not surprising that cytoskeletal re-organization is a critical component of MP formation. In this regard, actin filament dynamics appear crucial for MP formation

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Table 1 Characteristics of various types of extracellular vesicles

Characteristic	Microparticles	Exosomes	Apoptotic bodies
Size	100–1000 nm	40–100 nm	>1 μm
Mechanism of formation	Outward blebbing of plasma membrane	Fusion of multivesicular bodies with plasma membrane	Cell shrinkage and death
How detected	Flow cytometry, capture-based assays and electron microscopy	Electron microscopy and Western blotting (characterization with exosome-enriched markers)	Flow cytometry and electron microscopy
Characteristic features	Annexin V-positivity and presence of cell-specific surface markers	LAMP1, CD63 and TSG101	Annexin V-positivity, DNA and permeable membrane
Composition	Protein, RNA and miRNA	Protein, RNA and miRNA	Protein, DNA, cell organelles, RNA and miRNA
Membrane properties	Externalized phosphatidylserine, rich in lipid rafts and impermeable	Rich in lipid rafts and impermeable	Externalized phosphatidylserine and permeable

from multiple cell types. For example, the inhibition of actin polymerization with cytochalasin D, latrunculin B or jasplakinolide increases MP formation from platelets, megakaryocytes and T-cells [16–18]. Inhibition of calpain, a Ca^{2+}-dependent protease, which is known to cleave cytoskeletal proteins including talin and α-actin, attenuates MP formation from platelets and neutrophils [19,20]. Interestingly, calpain-dependence does not appear to be ubiquitous as we have failed to observe alterations in calpain activity during AngII (angiotensin II)-induced MP formation from endothelial cells [21]. Conversely, our laboratory and others have implicated Rho kinase, an upstream regulator of MLCK (myosin light-chain kinase) and cytoskeletal dynamics, in the formation of MPs from endothelial cells, a process which may involve caspase 2 [21–23]. Finally, transglutaminase 2, an enzyme which catalyses protein cross-linking and governs cytoskeletal re-organization, has recently been implicated in MP release from vascular smooth muscle cells [24]. Thus, although the precise machinery necessary for MP formation is not fully understood and indeed may differ among various cell populations, cytoskeletal re-organization appears to represent a critical step in MP formation.

A second event implicated in MP formation is the externalization of phosphatidylserine. Phosphatidylserine is an aminophospholipid which is found preferentially (if not exclusively) on the inner leaflet of the plasma membrane of healthy cells [25]. The assymetric distribution of phosphatidylserine is regulated by three distinct enzymes: flippases, floppases and scramblases. Flippases promote the translocation of phosphatidylserine and phosphatidylethanolamine against their electrochemical gradient towards the inner membrane in an ATP-dependent manner and are constitutively expressed. Floppases, which include members of the ABC transporter (ATP-binding cassette transporter) family, catalyse the transport of phosphatidylserine to the outer membrane in an ATP-dependent fashion. Finally, scramblases are ATP-independent and facilitate movement of phosphatidylserine between both membrane leaflets and include TMEM16F (transmembrane protein 16F) [25,26]. The majority of studies examining MPs report some degree of outer-membrane phosphatidylserine exposure, and emerging evidence suggests that this exposure is a key mediator of the formation of MPs. The strongest evidence supporting this derives from individuals with Scott syndrome, a condition characterized by an impaired ability to externalize phosphatidylserine and impaired coagulation that may result from defects in TMEM16F or the floppase ABCA1 [26–29]. Individuals with Scott syndrome exhibit reductions in MP shedding from platelets [30]. Similarly, mice deficient in ABCA1 display impaired phosphatidylserine exposure and significant reductions in circulating MP levels [31,32]. Although some reports suggest that phosphatidylserine is not externalized in certain MP populations (an observation based on a lack of detectable Annexin V binding), it is unclear whether these populations truly lack externalized phosphatidylserine or whether the level of externalization is simply below limits of detection [33].

Recently, lipid-rich microdomains, including lipid rafts and caveolae, have also been implicated in the formation of endothelial, monocyte and platelet MPs. Biro et al. [34] first observed a high cholesterol content in platelet MPs relative to the plasma membrane, suggesting an enrichment in lipid rafts. Additionally, perturbation of lipid-rich domains is associated with alterations in MP formation. In this regard, Liu et al. [35] have shown that cholesterol enrichment in monocytes/macrophages is associated with enhanced MP formation [35]. Similarly, we have observed that disruption of lipid-rich domains, with methyl-β-cyclodextrin or nystatin impairs MP formation in endothelial cells [21]. Moreover, several proteins which localize to lipid rafts/caveolae have been identified in MPs, including CD39, flotillin-2, eNOS [endothelial NOS (NO synthase)] and caveolin-1 [21,36]. Figure 2 summarizes the proposed pathways governing MP formation.

What stimuli are responsible for MP formation?

In addition to the broad structural changes governing MP formation, several stimuli have been identified which may stimulate or inhibit MP formation. Knowledge of stimuli for MP formation largely stems from *in vitro* studies on cultured cells where effects on MP release may be examined directly.

In platelets, pro-inflammatory stimuli, including LPS (lipopolysaccharide) [37], Shiga toxin [37], soluble CD40 ligand [38] and cytokines, such as IL (interleukin)-6 and erythropoietin [39], are potent stimuli for membrane blebbing and MP release. Additionally, mediators of coagulation, including thrombin [40], collagen [41], proteinase-activated receptor agonists [42] and TRAP

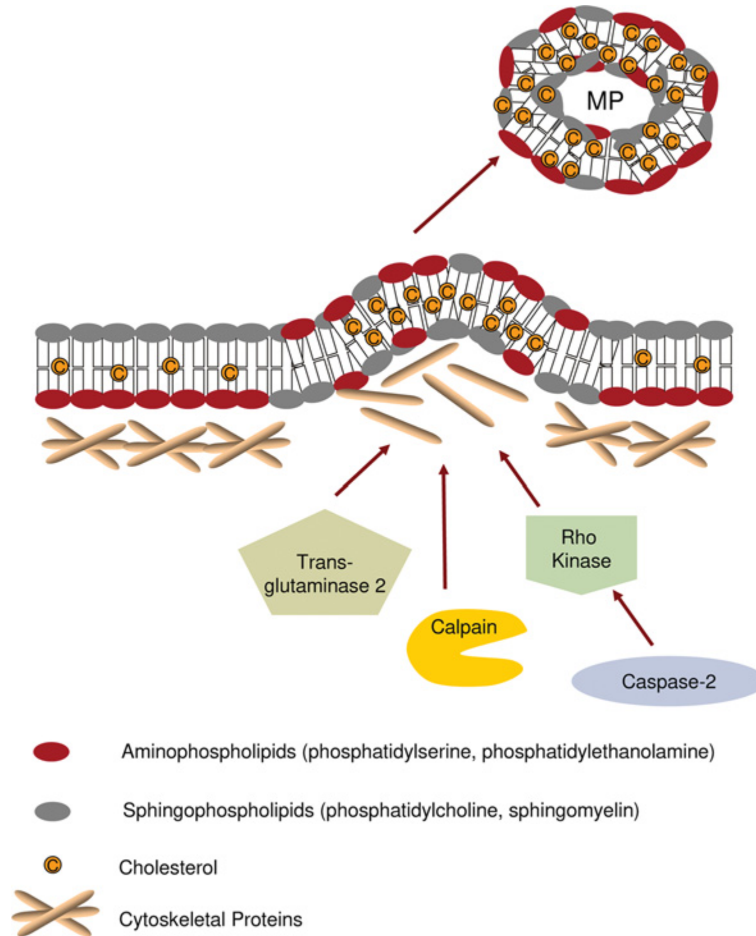

Figure 2 Proposed mechanisms responsible for remodelling of cytoskeleton leading to MP formation
Under normal conditions, aminophospholipids (phosphatidylserine and phosphatidylethanolamine) are found exclusively on the inner leaflet of the plasma membrane. During MP formation, membrane asymmetry is lost as aminophospholipids redistribute to the outer leaflet of the plasma membrane. Cytoskeletal re-organization results in the outward blebbing of the plasma membrane and may be dependent upon actin polymerization, caspase 2/Rho kinase, calpain and/or transglutaminase 2. Such processes may vary between different cell types. MP formation appears to occur selectively in lipid-rich microdomains (lipid rafts/caveolae) within the plasma membrane.

(thrombin-receptor-activating peptide) [43], promote platelet MP production. Other stimuli for platelet MP formation include noradrenaline (norepinephrine) [43] and the calcium ionophore A23187 [39]. Conversely, Syk inhibition [44] and epoprostenol (a synthetic salt of prostacyclin, [45]) are associated with reductions in platelet MP formation.

As was the case with platelets, pro-inflammatory stimuli are also potent stimuli of microparticle formation from endothelial cells. In this regard, TNF (tumour necrosis factor)-α [46,47], LPS (in the presence of fatty acids) [48], IL-1α [49] and CRP (C-reactive protein) [50,51] all promote MP release from endothelial cells. Similarly, the pro-coagulant factors thrombin [52] and PAI-1 (plasminogen activator inhibitor-1) [47,53] also increase endothelial MP formation. Uraemic toxins such as p-cresol [54], p-cresyl sulfate [55], indoxyl sulfate [54] and homocyteine [56] are also associated with increased MP formation from endothelial cells. Other stimuli implicated in endothelial MP formation include high glucose [40], AngII [21], camptothecin [57], growth factor deprivation [58] and ROS (reactive oxygen species) [21,59]. Shear stress has also been associated with endothelial MP formation *in vivo*, although direct effects on isolated endothelial cells have not been reported [60]. Conversely, statin treatment [61] and NO [50] suppress MP production from endothelial cells.

Leucocytes are also a significant source of microparticles in plasma. Several stimuli have also been identified which regulate MP formation from leucocytes. In T-cells, TNF-α, etoposide, actinomycin D, staurosporin, phytohemagglutinin and PMA induce MP formation [18,62–65]. Monocyte MP formation may be induced by TNF-α [66], etoposide [67], Fas ligand [40], LPS [68] and the calcium ionophore A23187 [69]. Macrophage MP production may be induced through exposure to TLR (Toll-like receptor) 3 and TLR4 ligands, an effect which may be inhibited by progesterone [70,71]. Formation of MPs from neutrophils may be induced by ANCAs (anti-neutrophil cytoplasmic antibodies) [72], fMLP (*N*-formylmethionyl-leucyl-phenylalanine) [73], PMA [74] and bacterial infection [74].

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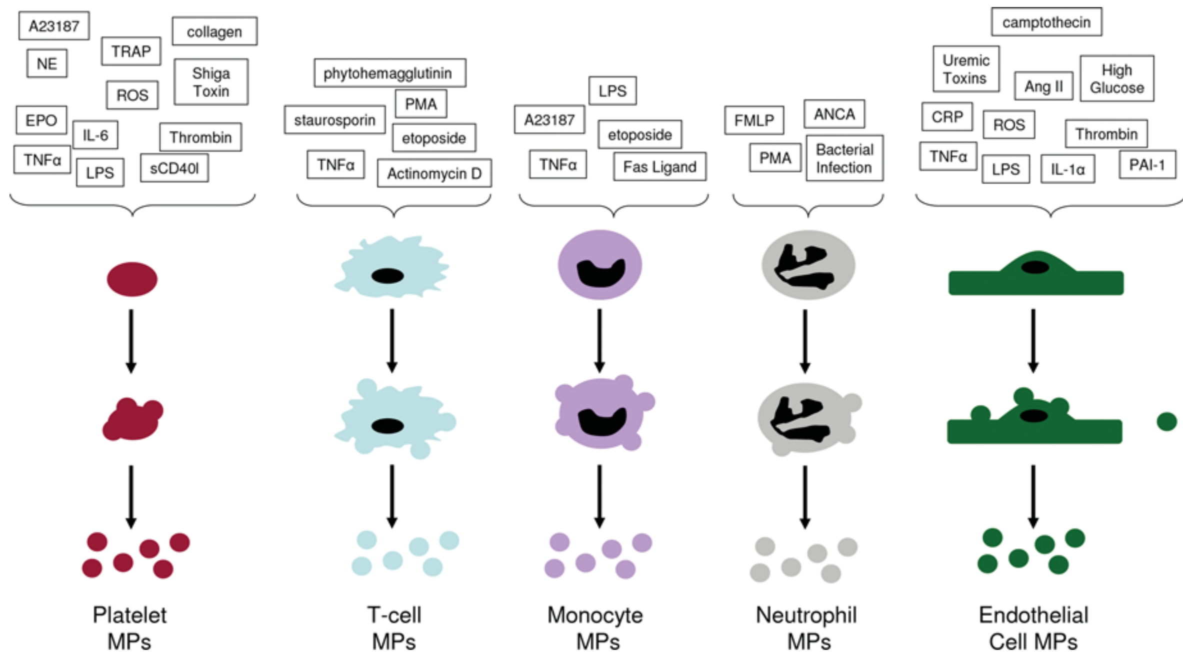

Figure 3 Stimuli for MP formation from platelets, endothelial cells and leucocytes
NE, noradrenaline; EPO, erythropoietin, sCD40l: soluble CD40 ligand.

The formation of MPs is less well understood in other cell populations. Nevertheless, emerging evidence suggests that cardiomyocytes produce MPs in response to TNF-α [6], mesangial cells produce MPs in response to high glucose and AGEs (advanced glycation end products) [75] and vascular smooth muscle cells produce MPs in response to Fas ligand [76]. A summary of the stimuli which promote MP formation from platelets, endothelial cells and leucocytes may be found in Figure 3.

MP clearance

In contrast with their formation, much less is known about the mechanisms of MP elimination. On the basis of the observation that MPs are engulfed within phagocytic cells, phagocytosis is widely believed to be the primary mechanism by which MPs are eliminated *in vivo*. In this regard, Distler et al. [18] have reported that co-culture of T-cell-derived MPs with macrophages *in vitro* results in phagocytosis of the MPs. Similarly, Willekens et al. [77] have shown that erythrocyte-derived vesicles are rapidly taken up by cells of the reticulo-endothelial system *in vivo*. The phagocytosis and internalization of MPs appear to result from the externalized phosphatidylserine, which may signal scavenger receptors, to promote endocytosis/efferocytosis of MPs [40,77]. Opsonization by IgM may also facilitate the binding and uptake of MPs by macrophages [78]. Interestingly, emerging evidence suggests that macrophages may discriminate and prioritize the clearance of MPs and other extracellular vesicles on the basis of glycosylation patterns [79].

Platelet MPs have been shown to be endocytosed by brain endothelial cells and HUVECs (human umbilical vein endothelial cells), suggesting a role for endothelial cells in the phagocytosis of MPs. [40,80]. In support of this, Dasgupta et al. [81] implicated Del-1 (developmental endothelial locus-1), an extracellular matrix protein expressed by endothelial cells. Inhibition of Del-1 blocked MP uptake in cultured endothelial cells and in mice deficient in Del-1. Moreover, mice deficient in Del-1 displayed a more pronounced increase in plasma MPs following endotoxin administration.

MPs AS BIOMARKERS

Much research has focused on the potential of MPs as biomarkers of endothelial dysfunction, coagulation, inflammation and other pathological processes. Biomarkers have been previously defined as characteristics that are objectively measured and evaluated as indicators of normal biological processes, pathogenic processes or pharmacological responses to therapeutic intervention [82,83]. As direct assessment of biological states is often too invasive, or too costly, biomarkers are of significant clinical utility in identifying pathology and evaluating risk of disease. Moreover, because hard end points often take years to emerge, biomarkers allow for early detection of pathology and may facilitate earlier therapeutic intervention.

How are MPs detected/measured?

Although MPs are believed to be formed by all cell populations in the body, their release into the extracellular space following formation results in access being limited, for the most part, to biological fluids. The majority of studies examine MPs isolated from plasma samples, which typically contain endothelial, leucocyte-, platelet- and erythrocyte-derived MPs [3]. Under pathological conditions, MPs derived from non-circulating cells, for example

vascular smooth muscle cells or fibroblasts, might also be detectable, although there is a dearth of knowledge in this area. Additionally, MPs of varying origins may be found in urine ([84], and D. Burger, unpublished work), bronchoalveolar lavage fluid [85], sputum [86], synovial fluid [87], ascites [88] and saliva [89]. Leroyer et al. [31,90] have also successfully isolated MPs of leucocyte, endothelial, erythrocyte and smooth muscle origin from atherosclerotic plaques. Unfortunately, although it is believed that MP formation is actively occurring within tissues, there is presently no reliable approach for obtaining and/or characterizing MPs from tissue samples and knowledge of MP biology in tissue is lacking.

Once obtained, several approaches may be used for the detection and characterization of MPs. The most widespread approach, and the one that best allows for enumeration, is flow cytometry. Flow cytometry examines the fluorescent and light-scattering properties of cells and particles in suspension as they are passed through a stream of fluid [91]. Through the use of fluorescent-conjugated antibodies to cell-surface antigens, or annexin V which labels externalized phosphatidylserine, flow cytometry allows for the determination of cellular origins and the enumeration of thousands of individual MPs in a single suspension. Flow cytometry offers the advantage of being commonly available at most research facilities and can rapidly analyse large numbers of samples. Within institutions, flow cytometry appears reliable for the detection of MPs, with reports of inter-assay and intra-assay variability of approximately 7–12% and 2–6% respectively [46,92,93]. Thus flow cytometry offers reproducible enumeration and characterization of MPs. Nevertheless flow cytometry also has a number of limitations. First, MPs operate at the lower detection limits of flow cytometry. For many instruments, the detection of particles of 100–400 nm in size is imperfect, and this can vary even between identical systems [91]. The practical offshoot of this is that significant numbers of particles may potentially be missed due to being too small or because multiple vesicles are identified as a single event. Secondly, although the use of antibodies for cell-surface markers allows for the determination of cellular origin, antibodies are labelling particles that contain a very small fraction of the surface antigens typically found on a cell. Thus, under certain conditions, there may be limited opportunity for antibody binding and subsequently weak fluorescence which, in turn, remains undetected. Nevertheless, flow cytometry remains the ‘gold standard’ for MP enumeration at this time.

An alternative to flow-cytometry-based approaches is the use of ELISAs for the detection of MPs. In this approach, MPs are captured using either Annexin V or antibodies to surface antigens (capture antibody) and subsequently detected using a distinct antibody to surface antigens (detection antibody) [94,95]. Detection of MPs by ELISA has the advantage of not having any size restrictions, being available to most research facilities and allowing for the semi-quantitative assessment of MP levels in biological samples. This approach has been used extensively for the enumeration of platelet MPs and has been reported to be similar to flow cytometry in reliability [94,95]. Drawbacks to this approach include the fact that MPs are analysed in bulk, rather than at an individual level, and as such changes in relative expression of a ligand at the surface of MPs might have an impact on quantification.

Functional assays aimed at the measurement of procoagulant or prothrombinase activity have also been employed for the relative enumeration of MPs [96,97]. Once again these assays analyse MPs in bulk and provide a relative measure of MPs, in this case based on functional activity. However, a major disadvantage of this approach is that it measures a single biological activity and provides no information, for example, on other functional activities, such as the ability to promote oxidative stress or inflammation. Additionally these assays provide no insight into the relative size of the MPs being analysed. Table 2 summarizes the principal methods for quantification of MPs.

Finally, a number of approaches may be used to provide phenotypic information for MPs, but do not presently allow for bulk quantification. These approaches include electron microscopy, atomic force microscopy and dynamic light scattering [91]. Emerging technologies such as nanoparticle tracking analysis allow for the visualization of particles of 25–1500 nm in size, the sizing of particles and may provide an alternative to flow cytometry for the enumeration of MP preparations [98]. However, as yet, the utility of nanoparticle tracking for absolute quantification of particle numbers is unclear. All of these approaches either lack or are limited in their ability to identify the cell source of MPs and the reliability and reproducibility of novel approaches to MP assessment are unclear. Regardless of the approach used to assess MPs, the pre-analytical handling of MPs represents an important source of variability and standardized methods need to be established (for which efforts are ongoing) and strictly adhered to [99,100].

Are MPs reflective of disease states?

As MPs are known to be formed under conditions of stress/injury, they have been investigated as putative biomarkers of disease. As discussed above, MP levels are determined by both their formation and elimination, and accordingly plasma levels may not be viewed as simply an index of MP formation, but rather a dynamic balance between formation and elimination. Nevertheless, increases in MP levels have been reported in a variety of disease states. In particular, diseases associated with vascular injury, inflammation and a pro-thrombotic state exhibit elevated plasma MP levels. On the basis of their respective origins, one might envisage that increases in endothelial MPs reflect vascular injury, that increases in leucocyte MPs signifies a pro-inflammatory state and increases in platelet MPs indicate aberrant coagulation. However, as there is significant overlap between these processes, MPs of each type may be elevated in multiple pathologies.

Plasma levels of MPs are increased in diseases that involve a degree of vascular injury. In this regard, several groups have reported elevations in plasma levels of endothelial, platelet and leucocyte MPs in diabetes [101–105]. In particular, elevations in platelet MPs appear to be exacerbated in diabetic patients with end-organ damage compared with those without organ damage [104]. In pulmonary hypertension, Amabile et al. [106,107] have reported increases in endothelial MPs, whereas Diehl et al. [108] have reported increases in endothelial-, platelet- and leucocyte-derived MPs. Chronic kidney disease is also frequently

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Table 2 Summary of methods of MP quantification

Protocol	Method of quantification	Advantages	Disadvantages	References
Flow cytometry	Fluorescence and light-scattering properties MPs in suspension	Available to most research facilities	Quantification of 100–400 nm may be imperfect	[21,22,91–93]
		Rapid	Cell origin identification is antibody-dependent	
		Multiple antigens may be analysed in a single sample		
		MPs analysed on an individual basis		
Immunoassays	Immunocapture of MPs and quantification based on the presence of surface antigen	Available to most research facilities	Quantification is done in bulk	[94,95]
		No size restrictions	Quantifies based on a single antigen	
			Does not allow for size determination	
Functional assays	Procoagulant or prothrombinase activity of MPs	Available to most research facilities	Quantification is done in bulk	[96,97]
		Provides an indication of biological activity	Measures only a single biological activity	
			Does not allow for size determination	
Atomic force microscopy	Cantilever is used to scan the surface of MPs and tip displacement is related to surface properties	Allows for very accurate sizing of MPs	Non-universal technology	[198]
		Allows for three-dimensional view of MP structure	Determination of cell origin requires development of specialized antibody-coated surfaces	
		May be used for quantification	Not conducive to large sample numbers	
Nanoparticle tracking analysis	MPs are visualized by light microscopy and light scattering is observed; Brownian motion of individual particles is tracked by video	Clear idea of MP size	Utility of assay for quantification is unclear	[98,199]
		Allows for quantification	Non-universal technology	
			May be time-consuming	

associated with elevations in plasma MPs (particularly endothelial MPs), where increases in laminar shear stress have been implicated in their formation [54,60,109–111]. Increases in MPs of various origins have also been reported in pre-eclampsia [112], atherosclerosis [113], sleep apnoea [114] and hypertension [115]. Importantly, elevations in MPs correlate with functional measures of endothelial function, other biomarkers of vascular injury and are associated with poor clinical outcomes. For example, Werner et al. [116] have reported that levels of $CD31^+$/annexin V^+ MPs are inversely correlated with endothelium-dependent vasorelaxation. Similarly, elevations in endothelial MPs are negatively correlated with flow-mediated vasodilation and positively correlated with pulse wave velocity and carotid intima-media thickness [114,117]. Additionally, elevations in plasma levels of platelet and/or endothelial MPs predict cardiovascular morbidity and mortality in atherosclerosis, pulmonary hypertension, end-stage kidney disease and heart failure [106,110,118,119]. Finally, interventions that improve vascular health (i.e. statins and angiotensin receptor blockers) have been shown to reduce plasma levels, suggesting that plasma MPs are a sensitive index of changing vascular health [120,121].

Increased plasma MP levels have also been reported in inflammatory diseases. Two independent groups reported increases in circulating MP levels (endothelial and platelet) in

individuals with psoriasis, an autoimmune disorder affecting the skin [122,123]. Additionally, Minagar et al. [124] have reported that endothelial MPs are increased in patients with multiple sclerosis. The same laboratory subsequently confirmed this observation using both *in vitro* and *in vivo* approaches and documented increases in platelet MPs in patients with multiple sclerosis [125–127]. MPs expressing Annexin V, as well as leucocyte- and platelet-derived MPs, are increased in systemic lupus erythematosus, where they may promote a pro-thrombotic state [128–131]. Increases in neutrophil-derived MPs have been reported in anti-neutrophil cytoplasmic autoantibody-associated vasculitis [72], whereas increases in endothelial-, erythrocyte-, platelet- and leucocyte-derived MPs have been reported in graft versus host disease [132], rheumatoid arthritis [133] and inflammatory bowel disease [134]. Importantly, treatment that improves disease conditions also reduces circulating MPs, at least in some inflammatory/immune diseases. In this regard, treatment of multiple sclerosis with IFN (interferon)-β1a reduces endothelial MPs [125]. Similarly, treatment of rheumatoid arthritis with leucocytapheresis, an extracorporeal circulation therapy where pro-inflammatory leucocytes are removed, is associated with reductions in platelet MPs [135]. Interestingly, levels of granulocyte-derived MPs, which have been suggested to have anti-inflammatory properties, were increased in this study. However, anti-inflammatory treatment does not appear to universally influence plasma MP levels, as treatment of rheumatoid arthritis with a combination of anti-inflammatory agents improved inflammatory status, but had no effect on levels of MPs which expressed complement components (CRP and serum amyloid protein) [136].

MPs may also provide information regarding the thrombotic state in individuals as MPs are increased in hypercoagulative states [137]. Conversely, individuals with Scott syndrome, who have a defect in procoagulant activity, exhibit decreased plasma MP levels [138]. Importantly, increases in circulating MPs of various origins correlate with indices of coagulation. van Beers et al. [139] reported that erythrocyte-derived MPs are increased in conjunction with thrombin generation and levels of von Willebrand factor [139]. Elevations in MPs of various origins have been proposed as a predictor of deep vein thrombosis, although this has not been conclusively established experimentally [140,141]. To date, the question of whether antithrombotic therapy has any impact on plasma MP levels in hypercoagulative conditions has not been answered. One study, examining platelet MPs in atrial fibrillation, reported that treatment with aspirin and/or warfarin had no effect [142]; however whether antithrombotic therapy has an effect on MP levels in more severe hypercoagulative states is unclear.

Finally, elevations in specific MP populations may be indicative of hormone imbalances. For example, elevations in platelet MPs have been reported in women with polycystic ovary syndrome where they correlate with serum testosterone [143]. In menopausal women, the numbers of platelet, endothelial, monocyte, granulocyte and total MPs are increased with declining oestrogen levels and may be influenced by triacylglycerols (triglycerides) and blood pressure [144,145]. Paradoxically, hormone-replacement therapy in postmenopausal women may also increase platelet MP formation [146]. MPs derived from ovarian carcinomas may be found in ascites, although this may only be present in advanced stages of the disease [88]. Finally, MPs found in urine may reflect the presence of bladder cancer [84]. The observation that MPs may be found in the urine suggests a potential role as biomarkers of renal injury; however, there is a paucity of information in this area.

In summary, extensive data suggest that MPs are elevated under pathological conditions and that this elevation may be associated with disease severity. However, the specificity of individual MP populations for specific disease states is unclear. Therefore elevations in circulating MPs appear to identify generalized stress/injury, rather than a specific pathological state. Nevertheless, MPs may be of use in determining the status of certain tissues, particularly the vasculature where injury may result in inflammation, coagulation and endothelial injury. Moreover, elevations in MP levels appear to have utility in predicting future risk in certain disease states, which may be of significant clinical utility. Therefore, although our understanding of the role of MPs in disease is far from comprehensive, MPs display promise as biomarkers of disease state.

Potential role of MPs as biomarkers in stratified medicine

If MPs truly reflect vascular injury and disease states, it may be possible to use them as biomarkers in stratified medicine and in healthcare administration, The ability to detect alterations in MPs during the pre-disease stage, when individuals are exposed to risk factors, or after the first organ is affected by vascular compromise, raises the possibility of being able to stratify individuals according to the risk that they are exposed to for further vascular compromise [147]. Thus resources can be focused on those individuals whose MP load indicates that they are at imminent risk for recurrence of organ damage. Such an approach requires confirmation and validation, but would be an attractive model in a 'vascular clinic' setting, where MPs may provide the opportunity to target care to those individuals most at risk of vascular compromise.

BEYOND BIOMARKERS: EVIDENCE FOR MP-MEDIATED BIOLOGICAL EFFECTS

In addition to their putative role as biomarkers of disease, growing evidence suggests that MPs may themselves exert biological effects. MPs represent a novel and potentially important method of cell–cell communication, regulating a number of physiological/pathophysiological processes. Here we examine the best-characterized effects of MPs and discuss the potential mechanisms by which MPs may achieve their effects. As we have described above, the definition of various classes of extracellular vesicles can be inconsistent in studies involving biological effects. As such, the primary focus of this section will be on studies which have used a definition of MPs consistent with that outlined in Table 1.

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Coagulation

Perhaps the best established property of MPs is their ability to promote coagulation. MPs are elevated in hypercoagulative disorders and this relationship is probably a result of their active participation in the coagulation process. The procoagulative properties of MPs are largely linked to their physical characteristics with two specific surface features thought to be responsible for this procoagulant activity. First, the externalization of anionic phospholipids (predominantly phosphatidylserine) results in a negatively charged surface; this negatively charged surface allows for interaction with cationic domains in clotting proteins, the subsequent assembly of coagulation factors and ultimately thrombin formation [148]. The externalization of phosphatidylserine is believed to be a property of all types of MPs and is a strong promoter of coagulation. Secondly, certain populations of MPs have been shown to display tissue factor on their surface [148]. Tissue factor is a critical component of the early stages of coagulation where it forms a complex with Factor VII/VIIa, ultimately leading to the initiation of coagulation [148]. The surface expression of tissue factor has been reported in monocyte and endothelial cell-derived MPs [92,149], whereas platelet MPs are not believed to express tissue factor [148]. At present, it is unclear to what degree MPs contribute to coagulation *in vivo*; however, an *in vitro* examination of platelet MPs recently observed that their surfaces were 50–100 times more procoagulant than those of activated platelets [150].

Oxidative stress

MPs have been shown to regulate the production of ROS. Brodsky et al. [151] first reported that endothelial MPs decreased NO and increased O_2^- (superoxide anion) production in rat aortic rings, which was associated with impaired endothelial function. Similarly, we have observed that endothelial MPs increase production of O_2^- and H_2O_2 in cultured endothelial cells through NADPH oxidase and mitochondria, but not xanthine oxidase [21,22]. Conversely, Terrisse et al. [40] have reported that the endothelial MP-mediated stimulation of ROS production in endothelial cells involves xanthine oxidase and NADPH oxidase. Lymphocytic MPs have been reported to promote endothelial cell O_2^- production through an NADPH oxidase- [152] or xanthine oxidase- [153] dependent process, whereas monocyte-derived MPs have been reported to facilitate endothelial cell ROS production through a combination of NADPH oxidase, mitochondria, xanthine oxidase, COX (cyclo-oxygenase) and uncoupled NOS [154]. The reasons for discrepancies with regards to the ROS-generating systems affected are unclear, but may result from subtle differences in the MP populations studied.

Interestingly, both the source of MPs and the manner in which they are formed appear to be a critical determinant of MP-mediated pro-oxidative effects. For example, MPs derived from the plasma of on-pump coronary artery bypass graft patients promoted O_2^- production in rat aortic rings, whereas MPs derived from off-pump patients had no effect, suggesting the potential for differing biological activity among various MP populations [155]. Agouni et al. [156] reported that MPs derived from activated T-cells actually decreased ROS production, an effect which was coupled with an increase in NO production and eNOS expression. However, subsequent studies from the same laboratory have observed pro-oxidative effects of monocyte-derived MPs [67]. Thus the majority of studies suggest that MPs (endothelial-, monocyte- and lymphocyte-derived) are capable of promoting oxidative stress in the endothelium through processes which may involve several enzymatic systems. However, the source of MPs appears to be critical to the determination of whether MPs are capable of promoting oxidative stress and the specific enzymatic systems that may be affected.

Inflammation

MPs appear to represent both a consequence of, and contributor to, inflammation. Various pro-inflammatory stimuli provoke the release of MPs. However, in addition to this, emerging *in vitro* and *in vivo* evidence suggests that MPs may directly contribute to an inflammatory response. As MPs appear capable of promoting an inflammatory response in the absence of any micro-organisms, this process may be considered a form of ‘sterile inflammation’, which involves the production of pro-inflammatory mediators (cytokines and chemokines) and the recruitment of inflammatory cells [157].

Mesri and Altieri [158] reported that MPs derived from polymorphonuclear leucocytes promote the release of inflammatory cytokines IL-6 and MCP (monocyte chemotactic protein) in cultured endothelial cells [158]. Additionally, T-cell-derived MPs have been reported to promote production of TNF-α, and IL-1β by monocytes [63]. Monocyte-derived MPs have been shown to stimulate production of IL-8 and MCP in human airway epithelial cells [69,159], and both monocyte and endothelial MPs stimulate IL-6 and MCP production in podocytes [66]. The extent to which MPs may access the latter two cell populations *in vivo* is unclear. Conversely, a recent study suggested that endothelial MPs had no effect on release of cytokines/chemokines from endothelial cells, but that these effects were mediated by apoptotic bodies [160].

MPs may also promote the interaction and adhesion of leucocytes to endothelial cells. In this regard, treatment with platelet MPs is associated with increased expression of ICAM (intercellular adhesion molecule) and monocyte–endothelial cell interaction in cultured endothelial cells [161]. Similarly, we have observed that endothelial MPs increase expression of cell adhesion molecules in endothelial cells and facilitate monocyte–endothelial cell interactions [21]. Monocyte-derived MPs have been reported to activate NF-κB (nuclear factor κB) and increase the expression of cell adhesion molecules [162]. On the other hand, endothelial MPs have been shown to bind to monocytes and promote their transendothelial migration [127].

The most conclusive evidence of a pro-inflammatory role for MPs comes from the observation that exogenous administration of endothelial MPs to rats is associated with acute lung injury, as shown by increased systemic and alveolar levels of pro-inflammatory cytokines (IL-1β and TNF-α), neutrophil infiltration into the perivascular space and histological injury [163,164]. Similarly, injection of MPs from the blood of patients with sepsis into mice leads to increased expression of iNOS (inducible NOS), COX-2 and NF-κB in the heart and lung [165].

Angiogenesis

MPs have been implicated in the regulation of angiogenesis, with several proposed mechanisms of action. Platelet-derived MPs were the first population reported to influence angiogenesis. This is perhaps not surprising as platelets are known to influence angiogenesis and contain at least 20 angiogenesis-regulating factors [166]. Kim et al. [167] first reported that platelet MPs stimulate the proliferation, survival, migration and tube formation of HUVECs *in vitro* via G-protein-coupled receptor and kinase signalling pathways. Similarly, injection of platelet MPs increases post-ischaemic capillary density following myocardial ischemia in rats [168]. Subsequent studies have revealed that MPs from other cell populations also influence angiogenesis. In this regard, MPs isolated from atherosclerotic plaques are involved in neovessel formation and the progression of plaques to a vulnerable state prone to rupture [169]. In that study, the authors observed that exposure of HUVECs, to MPs isolated from atherosclerotic plaques *in vitro* led to an increase in cell proliferation, and injection of these MPs into Balb/CNude mice stimulated angiogenesis *in vivo*. Importantly, the authors observed that angiogenic activity was absent from the supernatant following a 20 500 ***g*** spin, suggesting that exosomes are not involved in such processes. The same group have subsequently reported that MPs released during limb ischaemia in mice induce the differentiation of bone marrow mononuclear cells to an endothelial phenotype and promote neovascularization *in vivo* [31]. MPs isolated from atherosclerotic plaques did not promote endothelial differentiation.

The morphogen Shh (sonic Hedgehog) has also been implicated in some of the pro-angiogenic effects of MPs. Shh-positive MPs from human T-cells have been shown to induce the formation of capillary structures *in vitro* and increase the expression of adhesion molecules and other pro-angiogenic factors by Eahy 926 endothelial cells [170]. When these MPs were injected into mice subjected to hindlimb ischaemia, there was enhanced recovery of blood flow at 21 days and this was associated with activation of the Shh pathway in ischaemic muscle [171].

In contrast with these studies, MPs have also been reported to inhibit the process of angiogenesis under certain conditions. For example, Yang et al. [152] found that MPs generated from CEM T-cells inhibit angiogenesis *in vitro* in the aortic ring assay and *in vivo* in a corneal neovascularization model, probably through the production of ROS. These authors went on to show that intratumoral injection of lymphocytic MPs from apoptotic lymphocytes reduced microvascular density, VEGF (vascular endothelial growth factor)-A levels and tumour size in a mouse model of Lewis lung carcinoma and that lymphocyte MPs suppress angiogenesis *in vivo* in the retina [172,173].

Apoptosis

In addition to being a potent stimulus for MP formation, apoptosis also appears to be a consequence of MP signalling. For example Huang et al. [174] found that MPs isolated from hypertensive patients induce H_2O_2 production, cellular senescence and apoptosis [174]. In patients with SSc (systemic sclerosis), the number of MPs derived from endothelial cells was greatly increased and induced apoptosis in circulating angiogenic cells [175]. *In vitro* studies from the same laboratory report that MPs derived from monocytes and T-cells induce apoptosis in a dose-dependent manner [175]. This process appears to be dependent upon phagocytosis of MPs and, in particular, high amounts of arachidonic acid within MPs. Conversely, inhibition of phagocytosis prevented the induction of apoptosis [175]. Huber et al. [176] have reported that T-cell-derived MPs induce apoptosis in macrophages via ERK (extracellular-signal-regulated kinase) 1/2 activation, an upstream regulator of phospholipase A_2 and arachidonic acid release. Moreover, treating macrophages with an inhibitor of ceramide production blocked MP-induced caspase 8 activity and apoptosis, suggesting that membrane lipids may also play a critical role in the pro-apoptotic effects of MPs [176].

Interestingly, MPs derived from various origins, including monocytes, erythrocytes, endothelial cells and platelets, have been reported to contain caspase 3 [177,178]. This process is believed to be a protective mechanism directed at removing pro-apoptotic machinery, since inhibition of MP-mediated caspase 3 release in endothelial cells is associated with increased apoptosis [49]. It has been suggested that MPs may deliver caspase 3 to target cells, leading to the induction of apoptosis [178]. However, as caspase 3 is involved in numerous cellular processes beyond cell death, the delivery of caspases may have an impact on several cellular processes.

In contrast with the observations described above, microvesicles derived from mesenchymal stem cells may actually prevent apoptosis in ischaemic tissue injury [179]. However, microvesicles are a heterogeneous population including exosomes, MPs and apoptotic bodies. Thus it is unclear whether the anti-apoptotic effects of these populations are directly attributable to MPs.

Mechanisms of MP-mediated effects

One critical question regarding the biological effects of MPs is how these cell fragments influence cellular processes. To date, this question has not been definitively answered; however, putative mechanisms of action include the *de novo* production of ROS by MPs, physical interaction with target cells, effects on the extracellular matrix, and the fusion and transfer of MP contents to target cells. Additionally, the surface properties of MPs play a role in their effects on coagulation.

One possible mechanism of action that has been proposed is the potential for MPs to directly produce ROS. In this regard, MPs from rat microvascular endothelial cells contain the NADPH oxidase subunit p22^{phox} and produce $O_2^{\,-}$ *in vitro* [151]. The presence of other NADPH oxidase subunits was not examined in that study; however, NOX1 and NOX4 subunits have been identified in lymphocyte MPs, suggesting that MPs may contain enzymes with ROS-generating capabilities [153]. Although the *de novo* production of ROS by MPs has not, as yet, been conclusively proven, ROS have been implicated in cell growth, apoptosis, inflammation and endothelial dysfunction and could ultimately represent an important mechanism of MP-mediated action [180]. Interestingly, erythrocyte-derived MPs have been shown to scavenge NO in solution, which could further contribute to oxidative

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stress in biological systems [181]. This ability is probably exclusive to erythrocyte-derived MPs, however, as it required the presence of haemoglobin.

As MPs contain proteolytic enzymes, it is possible that certain effects of MPs may be attributed to alterations in the extracellular matrix or the proteolytic cleavage of signalling molecules. For example, MPs derived from microvascular endothelial cells have been reported to contain MMP (matrix metalloproteinase) 1, MMP2, MMP7 and MMP13 and to bind and degrade fibronectin *in vitro* [182]. Additionally, MPs isolated from human atherosclerotic plaques contain an active form of human ADAM17 (a disintegrin and metalloproteinase 17) which can stimulate the shedding of ADAM17 substrates, including the pro-inflammatory cytokine TNF-α [183]. Not surprisingly, this requires the presence of ADAM17 in the cell of origin as MPs derived from endothelial cells which do not detectably express ADAM17 fail to demonstrate ADAM17 enzymatic activity [183]. Although not directly examined, one can speculate that the presence of ADAM17 on the surface of MPs may exert effects on other ADAM17 substrates, such as L-selectin, EGF (epidermal growth factor) ligands, L1 adhesion molecule and CD30 [184]. Thus the presence of proteolytic enzymes on the surface of MPs could contribute to MP-mediated alterations in the extracellular environment and to MP-mediated release of multiple biologically active cytokines.

Our laboratory and others have suggested that MPs may mediate their effects through direct physical interaction with the surface of target cells [21,40,80]. Close physical interaction between MP and target cell results in a juxtacrine signal transmission. For example, platelet MPs interact with neutrophils through glycoprotein Ib and interaction with neutrophil-expressed integrin $\alpha M\beta 2$ (also known as Mac-1) [185]. Integrin-mediated communication between MPs and target cells have also been reported for endothelial [40], smooth muscle [186] and neutrophil-derived [187] MPs. We suggested that endothelial MPs induce their pro-oxidative and pro-inflammatory effects on endothelial cells through the activation of EGFR (EGF receptor) [21]. We observed that fluorescently labelled MPs physically interact at the surface of endothelial cells. Furthermore, we have shown that endothelial MPs carry the EGFR ligand HB-EGF (heparin-binding EGF) and that EGFR antagonism blocked MP-mediated effects on ROS production and inflammation, suggesting a physical interaction and activation of EGFR by MPs. Nevertheless the EGFR is widely known to be transactivated by other signalling pathways (i.e. AngII), so it is possible that the receptor is activated independent of juxtracrine activation by EGFR ligands [188].

Another potential mechanism for MP signalling which has been widely speculated on is the fusion of MPs with a target cell and transfer of its contents. This process of membrane fragment transfer has previously been termed ‘trogocytosis’ in antigen-presenting cells [189]. The mechanisms by which this fusion may occur are unknown at this time; however, ATP hydrolysis and Ca^{2+} elevation are believed to be required for vesicle-membrane fusion in exocytic processes [190,191]. Consistent with the transfer of MP contents, platelet- and megakaryocyte-derived MP populations have been shown to transfer CXCR4 (CXC chemokine receptor 4) to target cells [192]. This transfer appears to functional as cells which are otherwise deficient in the receptor and resistant to viral entry become susceptible to infection [192]. Similarly, Tang et al. [193] have reported that platelet MPs may functionally transfer lipoxygenase to mast cells. MP fusion has also been implicated in multidrug resistance through the transfer of P-glycoprotein from drug-resistant to drug-sensitive cancer cells [194].

miRNA transcripts have been found in MPs, with the MP membrane probably functioning to protect circulating miRNAs from degradation by RNAses in blood. It has been speculated that MP fusion with target cells may facilitate transfer of miRNA transcripts and therein alter cell activity [195]. In contrast with these observations, Sabatier et al. [196] have reported that endothelial MP-mediated modulation of monocyte pro-coagulant activity is independent of fusion, based on the observation that low temperature did not affect MP–monocyte interactions. Thus it is not clear to what degree this process mediates the biological effects of MPs. Additionally, attempts to visualize MP–target cell interaction by our laboratory and others have most commonly observed a punctate staining at the cell surface and/or internally, which would seemingly contradict the notion of any fusion of MPs and transfer of their contents [18,21,40,80,195,196]. In contrast with this, Rautou et al. [197] recently provided scanning electron microscopic evidence of a potential transfer of ICAM-1 from MPs derived from apoptotic plaques to endothelial cells. These images appeared to show transfer of labelled ICAM-1 to smooth areas of endothelial cells, suggesting functional transfer. Nevertheless, given that the largest of MPs may constitute up to 10% of the size of certain cells, it seems unlikely that MP fusion and transfer of contents represents a ubiquitous mechanism of action. More likely, fusion and transfer of contents occurs between exosomes and smaller MP populations which would represent only a small fraction of the size of a target cell.

An alternative mechanism of ‘transfer’ of MP contents is internalization through phagocytosis or macropinocytosis by target cells. In such a process, the contents of the MPs would be internalized within a membrane pocket rather than transferred directly following fusion with the membrane, which would be more consistent with a punctate staining. Faille et al. [80] have reported such a process where platelet MPs are internalized by brain endothelial cells and targeted to lysosomes. Under these conditions, the phagocytosis of MPs and targeting to lysosomes would result in a transfer of content; however, the material transferred would probably never serve a functional purpose.

The mechanisms implicated in MP-mediated biological effects are as diverse as the biological effects themselves. MP-mediated alterations of the extracellular environment, their own *de novo* production of ROS and physical interactions which may result in juxtacrine signalling, fusion and transfer of contents or internalization through phagocytosis/macropinocytosis have been identified and implicated in their biological effects. A summary of the putative mechanisms of action for MPs is shown in Figure 4. The relative contributions of these pathways is presently unknown; however, the biological effects of MPs probably occur as a result of a combination of these and other, yet undiscovered, pathways working in concert.

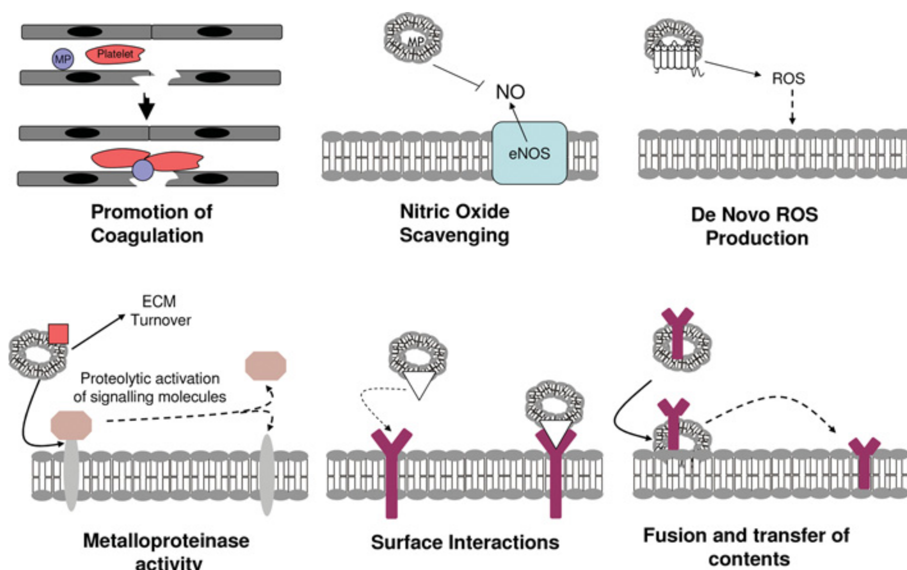

Figure 4 Putative mechanisms by which MPs achieve their biological effects
MPs may promote coagulation, scavenge NO, directly produce ROS, regulate extracellular matrix (ECM) turnover and activate signalling pathways through proteolytic pathways, physically interact with the surface of a target cell or fuse with a target cell and transfer content.

CONCLUSIONS: THE FUTURE OF MP RESEARCH

Originally believed to behave simply as inert cellular debris, MPs are now emerging as biomarkers of underlying endothelial injury and inflammatory or thrombotic states. Beyond their putative role as biomarkers of pathology, mounting evidence suggests that MPs actively modulate a variety of cellular events thereby conveying biological information and having an impact on numerous (patho)physiological processes. The field of MP research faces a number of challenges with regards to standardization of methodology and the need to better understand how MPs achieve their biological effects. Future research relating to MPs as biomarkers should focus on the optimization and implementation of standardized pre-analytical handling of samples and the development and validation of novel approaches to MP enumeration. Only then will MPs have true clinical utility as biomarkers of pathology. Additionally, a critical step in our understanding of the biological effects of MPs will be the development of selective pharmacological agents which modulate MP formation and/or signalling. Such inhibitors would better allow for the examination of the cellular processes which MPs may regulate and the relative contribution of MPs to *in vivo* pathophysiological processes. Accordingly, there remains much work to be done in this exciting field of research. Nevertheless, our understanding of MPs has developed enormously in a short period of time and seems poised to expand significantly in the future.

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