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Review Article

Dynamic microvesicle release and clearance within the cardiovascular system: triggers and mechanisms

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Abstract

Interest in cell-derived microvesicles (or microparticles) within cardiovascular diagnostics and therapeutics is rapidly growing. Microvesicles are often measured in the circulation at a single time point. However, it is becoming clear that microvesicle levels both increase and decrease rapidly in response to certain stimuli such as hypoxia, acute cardiac stress, shear stress, hypertriglyceridaemia and inflammation. Consequently, the levels of circulating microvesicles will reflect the balance between dynamic mechanisms for release and clearance. The present review describes the range of triggers currently known to lead to microvesicle release from different cellular origins into the circulation. Specifically, the published data are used to summarize the dynamic impact of these triggers on the degree and rate of microvesicle release. Secondly, a summary of the current understanding of microvesicle clearance via different cellular systems, including the endothelial cell and macrophage, is presented, based on reported studies of clearance in experimental models and clinical scenarios, such as transfusion or cardiac stress. Together, this information can be used to provide insights into potential underlying biological mechanisms that might explain the increases or decreases in circulating microvesicle levels that have been reported and help to design future clinical studies.

Key words: cardiovascular disease, clinical studies, microparticles, microvesicles.

INTRODUCTION

Interest in extracellular vesicles within cardiovascular diagnostics and therapeutics is growing rapidly. These vesicles encompass both those released from the surface of cells, typically referred to as MVs (microvesicles) or microparticles, and smaller particles released from multivesicular bodies that are described as exosomes. MVs released from the surface of cells have been of particular interest because they express markers that indicate changes in the apoptosis or activation state of their cell of origin, thereby providing a means to deduct the status of a tissue or organ from the nature and quantity of the vesicles present in the circulation [1]. The present review focuses on these larger cell-surface-derived MVs as there is now consistent evidence that the absolute level of these circulating MVs in the circulation varies in different cardiovascular conditions [2–6]. It is important to note emerging evidence which suggests that exosomes cannot be easily divided from MVs by size, and that many techniques are unable to distinguish between them. Therefore, although the present review focuses on MVs, some of the cited studies may well have included exosomes in their analysis.

MVs have been identified in other body fluids including saliva [7], synovial fluid [8], seminal fluid [9] and urine [10], but it is differences in circulating levels that may have the greatest clinical significance as they have been shown to predict the future risk of cardiovascular events in some patients [11–17]. Therefore quantitative circulating MV measurement in cardiovascular patients has been proposed as a way to generate diagnostic and prognostic information [18–22].

To fully evaluate the likely clinical significance of a change in circulating MV levels, a deeper understanding of the technical and biological factors that determine these levels is required. There

Abbreviations: CPAP, continuous positive airway pressure; CRP, C-reactive protein; IL, interleukin; ISEV, International Society for Extracellular Vesicles; MV, microvesicle; OSA, obstructive sleep apnoea; PS, phosphatidylserine; TF, tissue factor; TNF, tumour necrosis factor.

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are a number of technical limitations, as well as inconsistencies in terminology and preparation techniques, which have previously made it difficult to compare studies [23]. In fact, because of differences in the detection limit of various methodologies, such as flow cytometry, nanoparticle tracking analysis and resistive pulse sensing, it is likely that these technologies are measuring very different vesicle populations. Even within a single technology, such as flow cytometry, there are great discrepancies between detected levels of vesicles, partly due to the sensitivity of the machine [23].

An area that still requires consideration, and forms the basis of the present review, is the traditional measurement of MVs in the circulation of patients or healthy controls at a single time point. MV levels both increase and decrease very rapidly in response to certain physiological stimuli such as hypoxia, vessel shear stress and inflammation. Furthermore, how effectively MVs are cleared from the circulation depends on the efficiency of clearance mechanisms known to exist within different cellular compartments. Therefore levels of circulating MVs at any one time point are likely to reflect the balance between these dynamic mechanisms rather than a static marker of cellular state. For example, if rates of MV production and removal are balanced, no changes in levels will be detected, even if there is a markedly increased rate of MV production related to a disease state.

The present review draws together evidence from experimental studies to identify key triggers for MV release based on pathophysiological mechanisms as well as environmental exposures (Figure 1). These are each interpreted within the context of *in vivo* studies to understand their potential clinical relevance. Key clearance mechanisms are then defined and discussed on the basis of their potential clinical relevance. In particular, the rates at which these two processes, release and clearance, occur *in vivo* are considered and the likely resultant impact on degree of change in circulating MV levels over time is discussed. An understanding of these mechanisms is likely to provide deeper insights into the clinical relevance, and underlying biological background, of changes in MV levels increasingly being reported in cardiovascular conditions. Furthermore, knowledge of these processes will ensure more informed design of future clinical studies that investigate changes in MV levels.

TRIGGERS FOR RELEASE

Pathophysiological triggers

Hypoxia

Hypoxia has long been considered a mechanism which can trigger the release of MVs. In cell culture, hypoxic conditions have been shown to trigger the release of vesicles from several cell types, particularly from tumour cell lines [24–26]. It would therefore be reasonable to expect hypoxia to trigger MV release *in vivo* and this has been confirmed by experiments that artificially induce hypoxia in a laboratory setting. When eight healthy males were exposed to a level of hypoxia for 80 min, equivalent to that experienced at an altitude of 3000 m, they had an elevation in circulating $CD106^+$ endothelial-cell-derived MVs [27]. Similarly, Lichtenauer et al. [28] found an increase in $CD31^+$ MVs in 14 healthy controls following a slightly different hypoxic exposure regime based on normobaric hypoxia in an air-conditioned chamber equivalent to hypoxia at 5500 m altitude. Use of CD31 positivity, in conjunction with negativity for CD41 or CD42, excludes platelet MVs, and is often used to define endothelial-cell-derived MVs, but may also comprise MVs derived from leucocytes and activated platelets. Therefore the precise nature of the MV rise in this experiment is not clear, but does confirm MV release in response to acute hypoxia *in vivo*.

However, this response appears to change after more prolonged exposure to hypoxia. Two studies using high altitude as a model for inducing hypoxia have reported a reduction in circulating platelet-derived MVs under hypoxic conditions [29,30]. In a study of 51 healthy male volunteers, a two-day stay at moderate altitude (2590 m) also led to a significant reduction in MVs derived from platelets and erythrocytes with no change in endothelial or leucocyte-derived MVs [29]. The changes in this study were associated with a reduction in inflammatory markers and an improvement in lipid profiles [31]. Therefore general systemic effects on vascular health may be of relevance to this change in MV levels. However, similar differences related to chronic exposure to hypoxia have been reported in exercise training studies. Training under hypoxic conditions appears to attenuate hypoxia-driven elevations in MVs. Hypoxic-relative (50% maximal heart rate) exercise training for 4 weeks depressed the high-shear-stress-mediated platelet MV increase in the circulation [32]. Hypoxic exercise also triggers the release of TF (tissue factor)$^+$ monocyte-derived MVs [33] and TF^+ neutrophil-derived MVs [34], and this release can be suppressed by exercise training over 5 weeks in sedentary healthy males [33,34]. Therefore sustained hypoxia exposure may provide tolerance to hypoxia.

OSA (obstructive sleep apnoea) is an *in vivo* disease model of recurrent intermittent hypoxia with normoxia in between episodes. Several investigations have used flow cytometry and MV ELISA to demonstrate that patients with OSA of different severity have elevated circulating platelet, leucocyte and endothelial-cell-derived MVs compared with controls [35–39] consistent with the laboratory human studies of acute hypoxia. Furthermore, effective treatment of this condition with CPAP (continuous positive airway pressure) to reduce the frequency of hypoxic episodes leads to a reduction in platelet and endothelial MVs [37,39]. Withdrawal from CPAP treatment can then lead to subsequent $CD62E^+$ endothelial MV increases [40]. However, it is possible that hypoxia alone may not be responsible for these changes. Oxidative stress has been identified as an important trigger for altered levels of circulating MVs in chronic obstructive pulmonary disease patients [41]. Oxidative stress can also trigger the release of MVs from human neutrophils [42], human endothelial cells [43] and mouse endothelial cells [44] *in vitro*. Furthermore, there appears to be a close positive-feedback relationship between MVs and oxidative stress, as MVs from mouse endothelial cells can trigger endothelial injury and reactive oxygen species production [44].

Despite the differences in these study designs and potential for the role of linked biological pathways, such as inflammation and

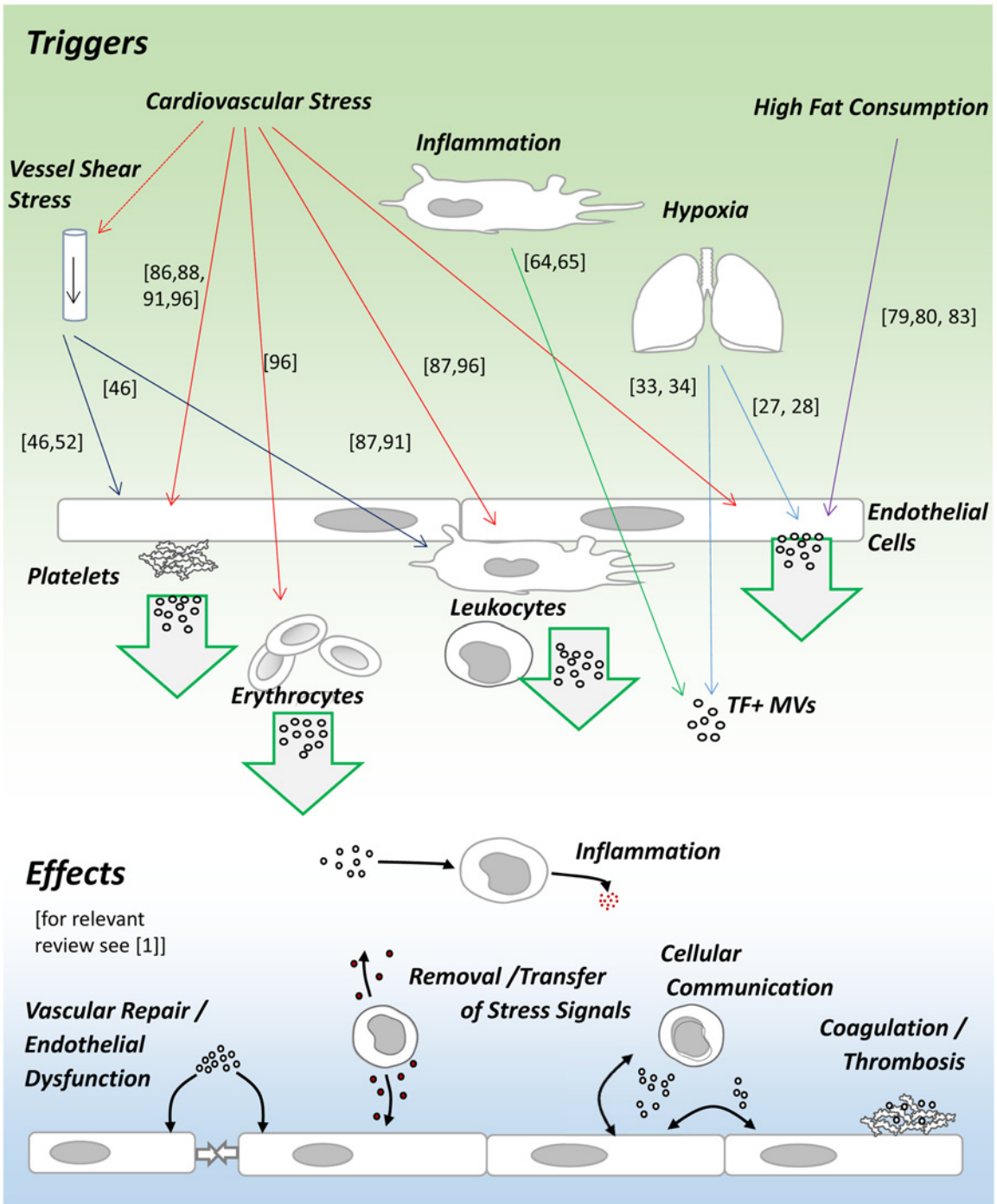

Figure 1 **Triggers, release and effects of circulating-cell-derived MVs**
Summary of the cellular triggers, release and effects of MVs in the circulation. The cellular triggers are detailed at the top, including vessel shear stress, cardiovascular stress, inflammation, hypoxia and high-fat consumption. Arrows then indicate where there is evidence in the literature of a relationship between this trigger and release of a specific cellular MV, with relevant references cited. The cellular effects of these circulating MVs, both physiological and pathological, although not specifically reviewed in the present paper, are then provided for information to help interpret the potential clinical impact of release of these MVs.

oxidative stress, there are a growing number of publications that have tried to focus on the *in vivo* impact of hypoxia. These appear to indicate that circulating MVs from different cell types show various responses to hypoxia. Furthermore, the MV release in response to chronic hypoxia exposure differs from that to acute hypoxia. Therefore timing and duration of exposure as well as measurement of cell-specific MVs is of importance in conditions in which hypoxia forms a component of the stimulus for MV release.

Vessel shear stress

A common feature of many cardiac conditions, such as atherosclerosis or hypertension, is an association with changes in vessel shear stress. *In vitro* studies suggest that shear stress may be an important regulator of MV release. Chen et al. [32] used a rotational viscometer to generate high shear stress and found release of platelet-derived MVs in platelet rich plasma. The use of viscometers and perfusion systems have also revealed the release of both megakaryocyte MVs [45] and platelet MVs [46–50] during periods of high shear stress. In contrast, low shear stress appears to trigger endothelial MV release, probably through a Rho kinase and ERK (extracellular-signal-regulated kinase) 1/2 pathway [51]. Therefore, *in vitro*, the degree of shear stress appears to determine the type of MV release.

In vivo changes in shear stress are more difficult to divide into high and low shear exposures. Furthermore, any disease process that alters shear stress has other associated biological conditions, which themselves may lead to MV release. For instance, patients with severe aortic valve stenosis have high shear stress around the valve and aorta, and this condition has been associated with increased platelet MVs, but also elevated leucocytes and endothelial MVs [46]. However, aortic stenosis can be linked with atherosclerotic coronary disease and changes in cardiac function. Evidence for a variable impact of shear stress on circulating MV type and levels may be stronger from studies of artificial devices. External counter-pulsation is a therapy that reduces ischaemia by increasing preload and cardiac output, but also leads to significant increases in vessel shear stress. When given for sustained periods amounting to 30 h over 4 weeks, an increase in platelet-derived MVs was observed, but no change in those derived from monocytes or endothelial cells [52]. Again this could relate in part to disease modification. Nevertheless, a study of patients with continuous-flow left ventricular assist devices, which directly expose blood cells to high shear stress, also found increased levels of PS (phosphatidylserine)-exposing MVs associated with the activity of the device [53].

The most convincing evidence has come from studies that have tried to directly relate the degree of vessel wall shear stress to MV release in humans. Patients who receive transcatheter aortic valve implantation to correct aortic stenosis have been found to have an increase in brachial artery wall shear stress related to the increase in cardiac output. This has been linked to improved endothelial function and also a decrease in endothelial MVs, but no change in platelet-derived MVs [54]. This would be consistent with lower shear stress being more commonly associated with endothelial MV release. This is supported by the findings in patients with end-stage renal disease, who have reduced shear stress. Their aortic and brachial artery shear stress were both found to be inversely correlated with endothelial derived MVs, but not platelet-derived MVs. Treatment of these patients with erythropoietin and intravenous iron for an average of 15 weeks led to increased brachial artery shear stress and an associated decrease in endothelial MVs [55]. This differential response to the level of shear stress highlights the need to interpret changes in MV levels on the basis of knowledge of the haemodynamic impact of different diseases and a careful consideration of the type of MV being studied.

Inflammation

Inflammation and MV release are often considered to be closely linked and function in a positive-feedback loop. Several studies, which have utilized both flow cytometry and microscopy, show release of endothelial and monocyte MVs from cell lines after exposure to pro-inflammatory cytokines [56–59]. This release has then been linked to the up-regulation of adhesion molecules and release of inflammatory markers in response to the MVs themselves when they are incubated with cells [2,57,58,60–62]. It might therefore be expected that pro-inflammatory conditions also trigger MV release *in vivo* and that treatments which reduce inflammation in turn reduce circulating MVs.

Initial evidence to support this hypothesis can be drawn from studies that show correlations of MVs with levels of inflammatory markers in the circulation. A study of 154 chronic heart failure patients found correlations between endothelial MVs and several markers of inflammation including TNF (tumour necrosis factor)-α, soluble Fas and Fas-ligand [22]. In a study of patients with myocardial infarction or angina, significant correlations were noted between levels of platelet and endothelial-cell-derived MVs and the inflammatory markers IL (interleukin)-6 and CRP (C-reactive protein) [63]. More direct experimental evidence for a link between MV release and inflammation comes from work in healthy individuals. In these studies, acute inflammation has been modelled by injection of endotoxin. This leads to an increase in TF-expressing MVs, determined by microscopy and by MV-capture assays, which is associated with a rise in both pro-inflammatory and anti-inflammatory cytokines [64,65]. Further support for a potential direct role of inflammatory mediators has been drawn from work with anti-TNF-α therapies. TNF-α has been shown to be a potent stimulator of MVs *in vitro*, including from cardiomyocytes [58,59,66–68] and it is correlated with MV levels in the circulation [22]. In patients with psoriasis, treatment with anti-TNF-α, a commonly used therapy in autoimmune conditions, led to a decrease of platelet and endothelial-cell-derived MVs following 3 months of treatment [69]. This finding supports findings from Sari et al. [70] who showed that platelet and endothelial MVs were significantly reduced in an ankylosing spondylitis patient receiving anti-TNF-α therapy, compared with conventional therapy.

In the more extreme clinical inflammatory condition of sepsis, systemic inflammation predominates and these patients also have been found to exhibit elevated levels of MVs [71–74]. Interestingly, the positive-feedback loop between MVs and inflammation may not be so clear cut, as MVs from patients with sepsis have been shown to enhance aortic contraction in mice and it has been

proposed they may play a protective role by reversing the fall in peripheral resistance and hypotension seen in severe sepsis [71] as well as promoting the release of IL-10 [75]. MVs, particularly those derived from granulocytes, have also been noted to have anti-inflammatory properties [75–78]. Together, these studies suggest that pro-inflammatory cytokines are potent triggers for MV release and the response can be modified with targeted cytokine therapies. In turn, MVs are capable of triggering both pro-inflammatory and anti-inflammatory responses dependent on the clinical scenario, reinforcing the importance of understanding the type of trigger and MV being studied in clinical studies.

Environmental and lifestyle triggers

High fat consumption

As well as being a risk factor for cardiovascular disease, the consumption of high-fat meals has previously been shown to impact on various biomarkers. This also seems to be the case for MVs. Ferreira et al. [79] demonstrated an elevation of $CD31^+CD42^-$ endothelial MVs 1 and 3 h following a high-fat meal in 18 healthy subjects. This appeared to correlate with a postprandial elevation in serum triacylglycerols and a direct association with exposure to triacylglycerols has been proposed [79]. Harrison et al. [80] also noted elevated $CD31^+CD42b^-$ endothelial MVs following a high-fat meal. This effect appears to last for only the first few hours after ingestion of fatty foods, as no change in either $CD31^+CD42^-$ or $CD62E^+$ endothelial-cell-derived MVs was evident 4 hours following ingestion of a high-fat meal in ten healthy men [81]. Tushuizen et al. [82] found that two high-fat meals in one day led to increased levels of total MVs, but not endothelial-cell-specific MVs, in the circulation, along with impaired flow-mediated dilatation in healthy individuals. Intriguingly, the drink consumed alongside the high-fat meal may also have an impact on the circulating MV levels, with cola giving the greatest increase in MVs at 1 and 2 h following consumption and red wine ameliorating the impact on MV release [83]. Exercise before consumption of the high-fat meal also altered the release of MVs although with different effects between studies. Harrison et al. [80] noted that the rise in endothelial MVs was not attenuated by exercise before the meal, despite a considerable reduction in postprandial lipaemia [80], whereas another study found that prior moderate intensity cycling can blunt the postprandial increases in endothelial MVs [84].

The range of biological changes that result from a high-fat meal as well as the ability of various dietary and lifestyle factors to modify release make identification of the precise mechanism for release difficult to identify. A high-fat meal is known to lead to an increase in markers of inflammation, such as CRP [85]. As inflammation causes elevated MVs, it is possible that this drives the changes in MV levels. Alternatively, several studies have correlated changes in MVs with levels of lipaemia or triglyceridaemia. There are also haemodynamic effects of a high-fat meal with redistribution of blood flow to the mesenteric circulation that could alter vascular shear stress. Whatever the precise mechanism, it would appear that timing of MV measurement in clinical studies and interpretation of changes in levels should take into account the recent dietary history of research study participants. This raises the question of whether all blood samples for MV analysis should be taken from fasting participants or patients? At the very least, the consumption of high-fat foods should be avoided for several hours before sampling to avoid elevations in levels.

Exercise and cardiovascular stress

Several studies have investigated the impact of acute cardiovascular stress on circulating MV levels, using both exercise and pharmacological models to generate changes in heart rate and cardiac contractility. Exercise or acute cardiac stress is likely to lead to changes in circulating MVs due to a combination of the pathophysiological mechanisms described above, including hypoxia and shear stress. It is also possible that cardiac stress itself serves as a trigger for MV release. The time course for release of MVs into the circulation is similar to those observed with other triggers. For example, moderate exercise for 90 min has been shown to lead to an immediate increase in platelet-derived MVs [86] in healthy males. In addition, moderate exercise causes an increase in endothelial-cell- and monocyte-derived MVs in highly trained healthy individuals [87].

Elevated platelet MVs measured by ELISA have also been shown in healthy males and females after completing treadmill exercise [88]. Even exercise carried out while scuba diving has been shown to increase PS-expressing MV levels, when compared with remaining stationary at depth [89]. Strenuous exercise has also been shown to induce a release of MVs from platelets in sedentary healthy men performing a graded exercise test [90]. Furthermore, maximal exercise, achieved by a graded exercise test, was associated with an elevation of MVs from platelets and neutrophils, but not erythrocytes in healthy males [91].

The length of time MVs remain elevated has been studied to a limited extent. Durrer et al. [92] took blood 18 h after high-intensity exercise and found an increase in endothelial MVs. However, intriguingly, this was only evident in females, with a decrease in endothelial MVs in males [92]. Interestingly, the interpretation of levels may be further complicated by the pre-existing fitness of individuals taking part in studies as a 5-day reduction in daily exercise in normally active young men leads to increased $CD31^+CD42b^-$ (apoptotic), but not $CD62E^+$ (activated), endothelial-cell-derived MVs [93]. This suggests that regular moderate cardiac stress may be necessary to keep apoptotic endothelial MVs at baseline levels. Furthermore, in the study of scuba divers, exercise before diving actually reduced levels of PS expressing MVs while diving [94].

The majority of these studies of acute cardiac stress and exercise were conducted in healthy volunteers and consistently showed significant elevations in circulating MVs. In contrast with the above studies, a study of high-intensity interval training in patients with coronary heart disease revealed no significant change in platelet or endothelial derived MVs [95]. This lack of MV response in those with cardiovascular disease is further supported by the findings of Augustine et al. [96] who used dobutamine during stress echocardiogram to trigger cardiac stress.

Table 1 ***In vivo*** **triggers of microvesicle release into the circulation**

Trigger	Generated by	Findings	Ref
Hypoxia	Withdrawal of CPAP in OSA patients (2 weeks)	↑ CD62E+ endothelial MVs in 18 OSA patients	[40]
	Laboratory Induced hypoxia (15% O2, 80 min)	↑ CD106+ endothelial MVs in 8 healthy males	[27]
	Hypoxic chamber (equivalent to 5500m)	↑ CD31+ MVs in 14 healthy controls	[28]
	Hypoxic Exercise (12% O2, 30 min)	↑ TF+ neutrophil MVs in 60 sedentary males	[34]
	Hypoxic Exercise (12% O2, 30 min)	↑ TF+ monocyte MVs in 40 sedentary healthy males	[33]
	Altitude (2590m, 2 days)	↓ Platelet and erythrocyte MVs and no change in CD31+CD41-, CD144+ or CD62E+ endothelial or leucocyte MVs in 51 healthy males	[29]
Vessel shear stress	External counter-pulsation (30 hours in 4 weeks)	↑ Platelet MVs, no change in CD62E+ and CD146+ endothelial or monocyte MVs in 7 CAD patients	[52]
	Continuous-flow left ventricular assist devices (average 28 days)	↑ PS exposing MVs in 20 patients	[53]
	Transcatheter aortic valve implantation (3 months)	↓ CD31+CD41-, CD144+ and CD62E+ endothelial MVs, no change in platelet MVs in 56 aortic valve stenosis patients	[54]
	Erythropoietin and intravenous Iron treatment (15 weeks)	↓ CD31+CD41- endothelial MVs, no change in platelet MVs in 25 end-stage renal disease patients	[55]
Inflammation	Injection of LPS (3 hours post)	↑ Tissue Factor MV activity in 8 healthy males	[64]
	Injection of LPS (3 hours post)	↑ Tissue Factor MVs in 18 healthy individuals	[65]
	Anti-TNF (3 months treatment)	↓ Platelet and endothelial MVs after 3 months treatment in 20 psoriasis patients	[69]
Exercise and cardiovascular stress	Moderate exercise (Immediately and 45 min and 2 h post)	↑ Platelet-MVs, no change in CD62E+ endothelial or monocyte MVs in 16 healthy males	[86]
	Moderate exercise (45 min post)	↑ Platelet MVs, which fall to baseline within 2 hours but only in trained healthy males	[87]
	Strenuous exercise (Immediately and 2 h post)	↑ Platelet and neutrophil MVs, no change in erythrocyte MVs in 7 healthy males.	[91]
	High intensity training (20 min post)	No change in platelet or CD31+CD42b- or CD62E+ endothelial MVs in 19 CHD patients	[95]
	Stress echocardiogram (Immediately)	↑ Platelet, CD31+CD41- endothelial and erythrocyte MVs in individuals without vascular disease, no change in those with vascular disease	[96]
High fat consumption	High fat meal (1 and 3 hours post)	↑ CD31+CD42- endothelial MVs in 18 healthy individuals	[79]
	High fat meal (2, 4 and 6 hours post)	↑ CD31+CD42b- endothelial MVs in 8 healthy males	[80]
	High fat meal (4 hours post)	No change in CD31+CD42- or CD62E+ endothelial MVs in 10 healthy males	[81]
	2 x High fat meal (2 hours post)	↑ Total MVs in 17 healthy males	[82]
	High fat meal (1 and 2 hours post)	↑ Total and endothelial MVs in 10 healthy males	[83]

They found that platelet, endothelial cell and erythrocyte MVs measured by flow cytometry and a coagulation assay are increased during cardiac stress in those with normal stress responses, but not in those with vascular disease [96]. Exercise- and dobutamine-induced stress both cause increased heart rate and can induce hypoxia as well as increased vascular shear stress. The physiological effects of exercise and dobutamine are not identical and therefore these studies cannot be necessarily directly compared. However, these findings raise the possibility that individuals with cardiovascular disease lose the ability to release MVs during cardiac stress. The findings from stress studies in healthy volunteers, and those with disease, do demonstrate that the background level of fitness, degree of stress exposure and underlying disease process needs to be considered in interpretation of results from studies that use cardiac stress as a stimulus for MV release.

RATE OF RELEASE

Acute stimuli

The studies described above and listed in Table 1 demonstrate that the time course of MV release differs with different

stimuli. However, all of these studies are limited in their fidelity for characterization of MV release by the relatively broad selection of sampling period they use. What is clear from these studies is that most acute stimuli such as shear stress, inflammation and hypoxia will have led to an increase in MV levels that can be detected within minutes and it remains possible that changes occur earlier. Exercise and stress echocardiogram studies indicate presence of platelet-derived MVs after 90 min of moderate exercise [87] or 5–10 min of cardiac stress induced by dobutamine [96]. Similar timeframes are reported in studies of laboratory-induced hypoxia, which have identified increases in MV levels immediately after hypoxic states that are then still detectable 80 min later [27]. High-fat meals also lead to evidence of MVs in the circulation within 1 h of consumption [79,80] and TF-exposing MVs, which are normally rare in the circulation, are elevated within hours of administration of endotoxin in healthy individuals [64,65].

These timeframes are consistent with the known time rate of the signalling cascades and cellular mechanisms that lead to MV release. MV release from the cell membrane is generated by two mechanisms: cell activation and cell apoptosis. In cell activation, agonists are detected by a cell receptor causing cytosolic calcium to increase, leading to activation of kinases and calpain, inhibition of phosphatases, talin breakdown and a loss of membrane asymmetry [97]. These mechanisms cause disruption of the membrane cytoskeleton and consequent budding of the membrane, with formation of MVs [98]. In apoptosis, Rho-associated kinase 1 is activated by caspase 3 [99], causing cell contraction, redistribution of fragmented DNA and membrane blebbing [100]. MVs formed by apoptosis differ from those formed by cell activation in size, in lipid and protein composition, and in their surface markers [98]. The MVs described in the studies of acute response to a stimulus are likely to be a combination of MVs produced by both cell activation and apoptosis. Both of the mechanisms described above are likely to be able to result in MV release within minutes of the stimulus.

Chronic stimuli

The impact of sustained stimuli on MV levels suggest prolonged exposure to different factors may be able to induce chronic changes in the rate of release. This is suggested by studies which have looked at release following prolonged therapies that study MV levels after several weeks or even months from the initial baseline assessment [52,53] and show significant sustained changes in circulating MV levels. However, such studies are limited by the potential modifying effect of therapies on the underlying triggers for MV release. More convincingly, as can be seen from Figure 2, studies of sustained hypoxia appear to demonstrate a differential effect on MV levels depending on whether measurements are performed in response to the acute stimulus or prolonged exposure. In these more prolonged studies, however, consideration needs to be given to the clearance mechanisms for MVs and prolonged effects of exposure on these processes and how this alters the MV balance within the circulation. We therefore now consider potential clearance mechanisms.

MECHANISMS OF CLEARANCE

Overview

Once an appropriate trigger has resulted in MV release, then, for those MVs to be detectable in the blood, they need to persist in the circulation until the time of sampling. It was thought that, because of their smaller size and greater ability to diffuse and escape phagocytosis, MVs may be able to survive in the circulation longer than the cells from which they originate [101]. However, this is by no means clear, as several mechanisms have now been identified that actively clear MVs from the circulation through cellular uptake. Figure 3 summarizes the cells and organs identified to be involved in MV clearance. The predominant route of uptake appears to be by endocytosis (clathrin- and caveolin-dependent and lipid-raft-mediated), micropinocytosis, phagocytosis and membrane fusion [102]. This is largely driven by exposure of PS by MVs that are recognized by phagocytes, as well as endothelial cells. These then bind PS directly or by receptors for the proteins, such as the complement component C3b, that leads to opsonization of MVs [103] and activation of the complement cascade. PS is known to be involved in the clearance of red cells and platelets from the circulation [104,105], through a lactadherin-mediated process. This process has also been shown to be relevant in the removal of PS-exposing platelet MVs from the circulation [106].

The extent of exposure of PS, or other cellular proteins, in different situations or on different MVs would therefore be expected to alter the ease and rate of clearance. Consistent with this, exploratory studies using modified liposomes have shown that retention and clearance behaviour is affected by the molecular composition of the vesicle membrane and size [107]. However, the extent of PS exposure on MVs *in vivo* remains unclear [108]. It may be that much of the exposure of PS on MVs detected in the circulation is due to post-venepuncture manipulation of the vesicles, such as freeze/thawing, centrifugation and filtration. It is becoming increasingly clear that many circulating MVs do not bind to annexin V and therefore may not expose PS on their surface [109]. This puts the role of PS in the clearance of MVs under scrutiny (Figure 4). Furthermore, rate of clearance may vary depending on the way that cells interact with their environment as it is known that live cells actively react to changing environmental cues, and will therefore alter their membrane composition leading to different retention times for their MVs within the circulation. Below we summarize the known *in vivo* mechanisms for specific clearance pathways and the rates at which these are thought to operate.

Cellular uptake

Endothelium

Several studies have now demonstrated that MVs can be cleared from the circulation through endothelial uptake by endocytosis. It has been proposed that this is through a lipid-raft-mediated process [102,110]. PS exposure on MVs appears to be particularly important for effective clearance [111] and Del-1 (developmental endothelial locus 1), a glycoprotein expressed on the endothelium, has been shown to mediate uptake of MVs through

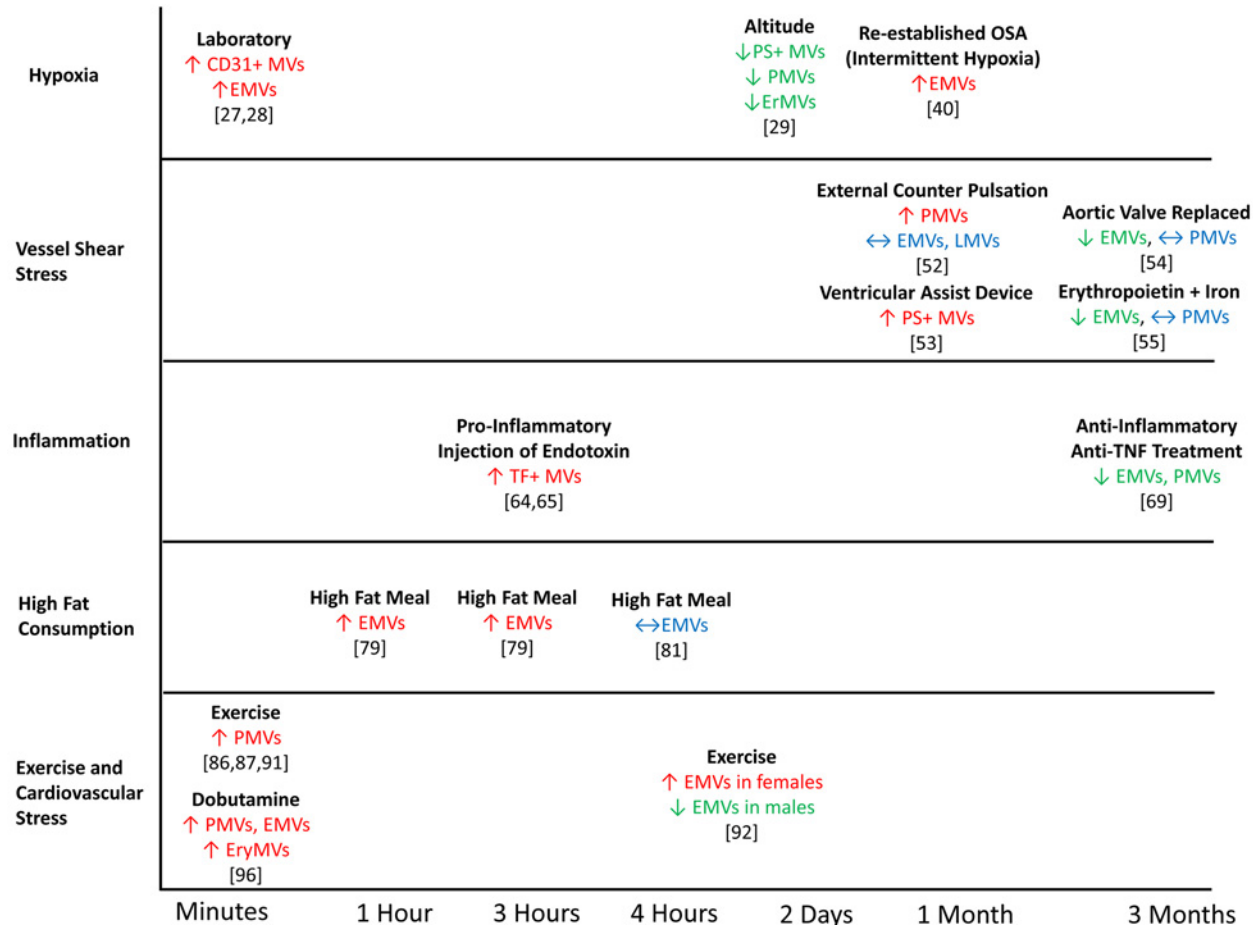

Figure 2 **Time course of MV release**

Different stimuli are listed on the left and then the impact of these stimuli on MV levels over time are detailed across the Figure. The timescale along the bottom describes the time between the stimulus and when the venepuncture for the study reported took place. Red indicates MV levels that had increased, blue represents MVs that had remained the same and green shows MVs that had decreased from baseline levels. References are provided for each study presented. The cellular origin of MVs have been defined, where known. PMVs, platelet MVs; EMVs, endothelial cell MVs; EryMVs, erythrocyte MVs; LMVs, leucocyte MVs.

endothelial endocytosis by acting as a bridge between integrins on endothelial cells and PS on MVs [112]. Endothelial clearance has also been demonstrated in *in vivo* studies. Wahl et al. [113] studied levels of $CD31^{+}CD42b^{-}$ endothelial MVs, which they generated with high-volume and high-intensity training exercise, and linked reducing levels with increased uptake by endothelial cells in a PS-dependent manner.

One reason endothelial cells may take up MVs is that it provides a mechanism for transmission of signals to the endothelium from remote cells. This could be of importance particularly in view of the range of specific functions performed by the endothelium, such as pro-coagulant responses [114], vascular repair [115,116] and vascular control. In particular, the carriage of coding (mRNA) and non-coding (miRNA) RNAs within membrane vesicles, which confers protection on the RNAs against degradation, can ensure endothelial target specificity for these factors. Endothelial uptake of MVs may constitute a pathway of MV clearance that leads to the deliverance of intact miRNAs [117], and therefore changes in gene expression in the endothelial cell. In support of this targeted approach, the uptake of MVs by endothelial cells has been shown to be dependent on how the MV was produced. Alexy et al. [67] found that two distinct populations of MVs were produced following stimulation of human aortic endothelial cells with TNF-α: one Rho-associated-kinase-dependent, rich in miRNAs, and one caspase-dependent, poor in miRNAs [67]. Of these, the MVs rich in miRNAs were much more readily taken up, which suggests that endothelial cells are able to recognize MVs generated upon different molecular cues, thereby removing MVs from the circulation that contain high levels of miRNAs. Uptake by endothelial cells is therefore less likely to constitute a waste removal system and is much more likely to be a targeted mechanism of cellular communication.

Macrophages

Macrophages are the prototypical phagocytes and, as MVs often represent by-products of apoptotic cells, it is not surprising

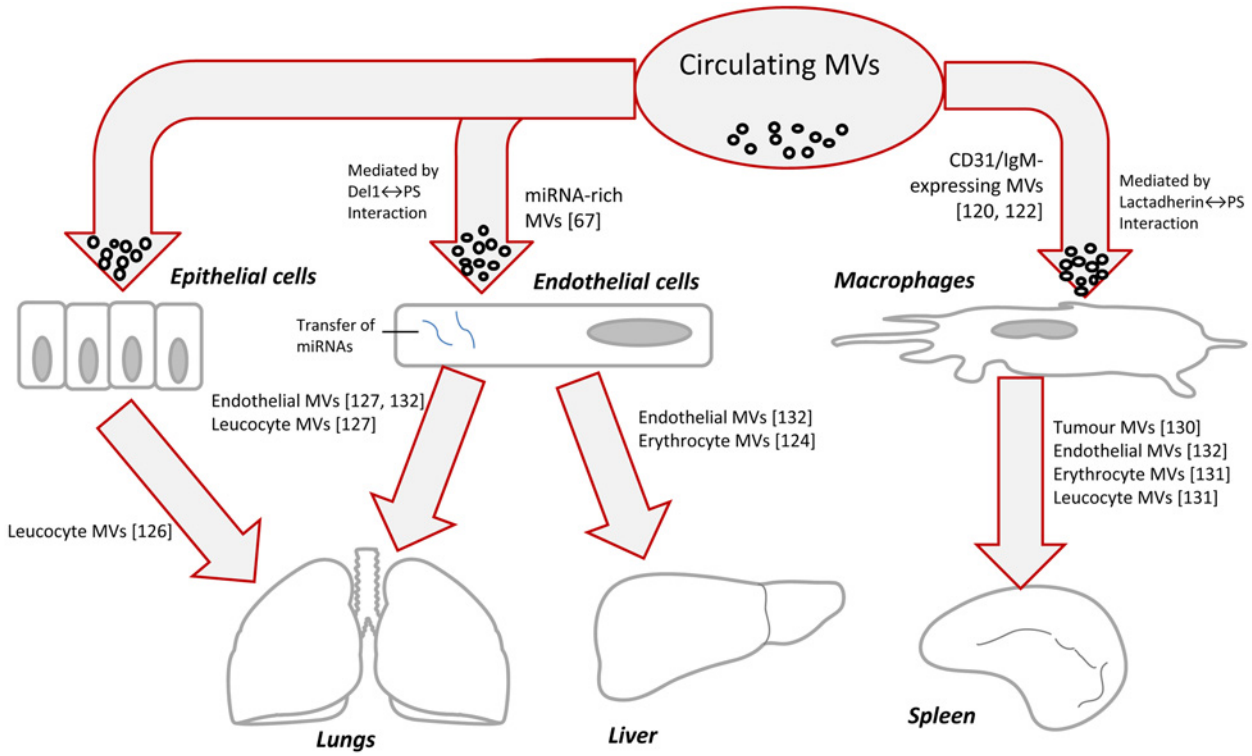

Figure 3 **Mechanisms of MV clearance**
The cells and organs involved in MV clearance are described in the centre and bottom with the passage of MVs from the circulation to cell and organ then illustrated by arrows. References are stated for the studies that have provided evidence for this passage of MVs and their targeted clearance in specific cells and organs.

that many pathways of MV uptake by macrophages overlap with the mechanisms governing the removal of apoptotic cells. The ubiquitous presence of macrophages, in their different forms, across a broad range of tissues also means macrophages could be a major site for MV clearance. PS are richly expressed on MVs and are central to the 'eat me' signals used in phagocytic processes [118]. Lactadherin, which binds to PS, appears to play an important role in the uptake of MVs by splenic macrophages, as lactadherin deficiency leads to increased levels of circulating MVs [106,119]. Another key factor that governs attachment to, as well as detachment from, macrophages is CD31 [120]. CD31 is abundant on many vascular cells such as platelets, leucocytes and endothelial cells, and is therefore also readily expressed on their MVs. Additionally, size and density of Fc or IgM, found to be present on MVs, appears to influence their uptake by macrophages [121,122]. Therefore uptake of MVs from different cell types by phagocytes is likely to vary depending on the degree of expression of PS, CD31, Fc or IgM on the MV surface. Technical factors or disease processes that influence the degree of expression of these factors will therefore influence how long MVs remain in the circulation. Interestingly, MVs themselves appear to trigger phagocytic activity by macrophages, which is not limited to the MVs. Therefore increased presence and clearing of MVs may induce clean-up processes of surrounding damaged tissue [123].

Clearance in organs

Liver

To understand whether there is variation in the site of MV clearance within the body, Willekens et al. [124] injected rats with sodium [^{51}Cr]chromate-labelled MVs and tracked their uptake. Within 5 min, 80% of the labelled MVs were cleared from the circulation and, of these, 55% had been taken up by the liver. It was noted that the Kupffer cells contained the majority of activity and also haemoglobin, suggesting their importance in clearance of erythrocyte MVs [124]. This clearance mechanism appears to be modifiable, depending on different environmental clues as, when injected with the artificial oestrogen diethylstilboestrol, mice show an enhanced clearance of liposomes associated with an increase in liver weight. Both the resident Kupffer cells and recruited macrophages may be responsible for the increased clearance of liposomes by the liver in this context, as Mac-1 has been implicated, but is also not essential, in this process of liver uptake [125].

Lungs

The lungs are rich in a variety of cell types, including endothelial cells, leucocytes and epithelial cells [126], known to facilitate MV clearance and it is therefore proposed that the lungs may act as an important site for MV clearance. This may explain why patients with pulmonary hypertension show elevated levels of

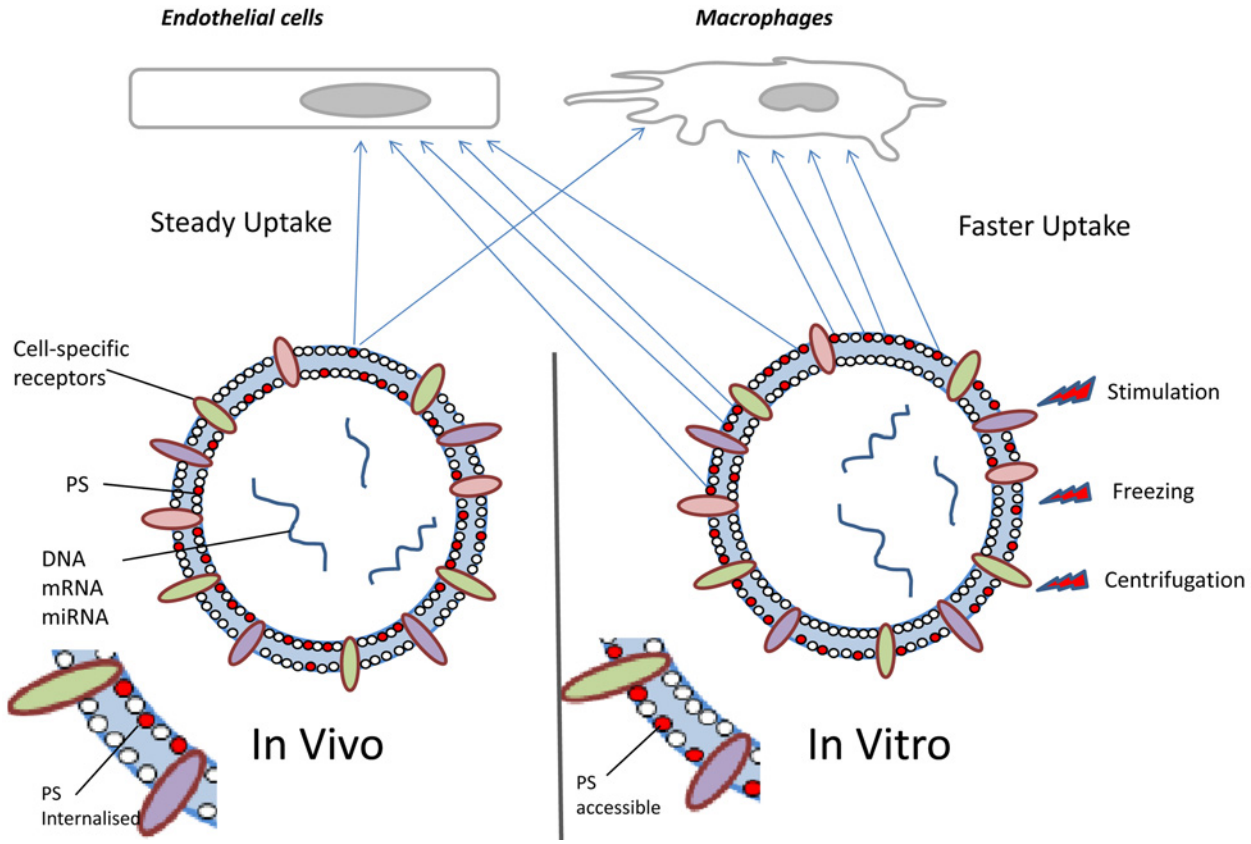

Figure 4 Rate of MV clearance

Clearance of MVs is known to be dependent on expression of PS on the surface. This Figure describes how differences in *in vivo* and *in vitro* conditions could lead to different external expression of PS and therefore alter clearance rates. *In vitro* processing of MVs (on the right), including stimulation, freezing and centrifugation, leads to increased external PS expression on MVs and therefore faster uptake by endothelial cells and macrophages. Therefore how samples have been processed *in vitro* should be considered when interpreting relevance of findings to *in vivo* clearance rates.

circulating leucocyte- and endothelial-cell-derived MVs as the available vascular beds for MV clearance are reduced [127]. Efferocytosis, a process of 'silent' removal of apoptotic cells, but also of MVs, has also been reported to be disturbed in various diseases of the lung [128], and patients suffering from various diseases of the lung show increased levels of MVs within samples of lavage fluid or alveolar oedema [129]. Nevertheless, this elevation in MV levels could be in part driven by greater release of MVs in these pulmonary inflammatory states and further work is required to define the relative balance of production and lung clearance in these conditions.

Spleen

Splenectomized mice infused with tumour cell MVs have increased mortality compared with non-splenectomized mice, an observation that has highlighted the potential importance of the spleen in clearance of MVs [130]. The importance of the spleen has also been highlighted in human studies in which splenectomized patients with ITP (idiopathic thrombocytopenic purpura) have been demonstrated to have increased MVs, in the form of red blood cell and leucocyte-derived MVs, compared with non-splenectomized patients [131]. However, some of the most convincing evidence has come from the use of endothelial-cell-derived MVs labelled with fluorescence or iron oxide nanoparticles. When injected into mice they have been found to localize mainly in the spleen (within monocytes and macrophages) and to a lesser extent in the liver and lungs [132]. This result appears discordant to studies that have identified significant liver uptake. However, as noted above, MV clearance is dependent on the surface receptors, protein and lipid composition of the MVs, which will vary with disease state and type of MV. Therefore there may be preferential uptake of certain MVs in different organs [133].

Rate of clearance from the circulation

Knowledge of clearance rates of microparticles is largely derived from experimental models. However, in each experiment, a key finding has been the extremely rapid clearance of MVs from the circulation. When platelet-derived MVs from platelet preparations were injected into rabbits, they were no longer identifiable in the circulation within 10 min of injection and remained absent from the circulation for up to 50 min, suggesting that, once cleared, they were not being re-released [134]. Flaumenhaft [103]

also reported clearance of platelet-derived MVs within 30 min of infusion in mice. Up to 80% of erythrocyte vesicles have also been shown to be cleared from the circulation within 5 min of injection into rats [124], and endothelial MVs can be detected in the spleen within 5 min of injection into mice [132]. However, in some more unusual settings, MV clearance may be delayed, for example, elevated circulating platelet-derived MVs in canines caused by insertion of material test segments to a shunt, were cleared from the circulation over a 3–24 h period following removal of the test segment from the shunt [135].

It should be noted that some of these studies of MV clearance use vesicles that have been through a freeze–thaw cycle or were artificially stimulated using calcium ionophores. The processing of samples, particularly freeze–thawing or artificial stimulation, can induce 'flip-flop' of the plasma membrane and a higher exposure of PS on the surface of vesicles than that found in naturally circulating MVs [136–138]. As discussed above, rate of MV clearance is likely to be dependent on the degree of exposure of PS on the MV surface and therefore uptake of manipulated MVs may not be representative of *in vivo* uptake [109] and care should be taken when using such data to define clearance times *in vivo* (Figure 4).

Injecting labelled MVs into humans is ethically complex, but some studies have found alternative ways in which to monitor human MV clearance. As with animal studies, certain types of MV must clear very rapidly from the circulation. For example, re-transfusion of pericardial blood, which contains high levels of TF-exposing MVs, during cardiopulmonary bypass, does not cause systemic coagulation suggesting the elevated MVs must be cleared within ∼15 min [139]. Consistent with this rapid clearance, Augustine et al. [96] demonstrated that elevated platelet MV levels generated by dobutamine-induced cardiac stress, had returned to baseline within 1 h. However, interestingly, this study supported the concept that variation in clearance rate depends on the cellular origin of the MV [96]. Erythrocyte MVs appeared to have slower clearance as they were still elevated at 1 h, whereas endothelial MVs had fallen below baseline levels, suggesting an ability to up-regulate clearance in the presence of a stress stimulus [96]. This differential rate of clearance according to cellular origin or stimulus was also found by Rank et al. [140] who identified that the half-life of PS-expressing MVs, derived from transfusion of platelet concentrates, in humans was 5.8 h, and that the removal of platelet MVs was quicker than the removal of platelets. Vince et al. [27] also noted that elevated $CD106^+$ endothelial MVs, induced by breathing hypoxic air, took 10 h to fall to baseline levels after hypoxia exposure.

Importantly, the rate of clearance for a single cellular type of MV may also vary with the physical state of the individual. Sossdorf et al. [86,87] carried out two studies using moderate endurance exercise as a model for excess MV generation *in vivo*. They found that, in physically untrained individuals, platelet MVs remained elevated in the circulation (even continuing to rise) up to 2 h following exercise [86,87]. Supporting this, Chaar et al. [91] also noted that elevated platelet- and neutrophil-derived MVs remained elevated 2 h following maximal exercise in healthy males, whereas in trained individuals, exercise-elevated platelet MVs returned to baseline within 2 h [87] and high-intensity training in triathletes/cyclists led to a fall in $CD31^+CD42^-$ endothelial MVs below baseline within 1 h [113].

CONCLUSIONS

It is increasingly becoming clear that an understanding of the triggers and regulators of the dynamic change in MV levels observed in different clinical scenarios is required to interpret findings from clinical investigations. The present review has drawn together the evidence from experimental and clinical studies that demonstrate that changes in circulating MV levels are due to both cellular release and clearance. Furthermore, it has described how these factors differ in their impact on both the degree and time course of change in circulating MV levels. There remain discrepancies in the change of MV levels reported in these studies, even when considering the same triggers, and some of this variation may relate to differences in techniques used to identify and quantify the cell-specific vesicles. The majority of studies use flow cytometry, but even among these there is great variation in the levels reported, partly explainable by pre-analytical (sampling, centrifugation, storage) and analytical (antibody choice, flow cytometer resolution, flow cytometer set-up) variables [138,141–145]. Standardization of MV methodologies will be important to improve consistency and analysis across multiple centres [141,143,146,147]. A collaborative working group between ISEV (International Society for Extracellular Vesicles), ISTH (International Society on Thrombosis and Haemostasis) and ISAC (International Society for Advancement of Cytometry) has recently been formed (at ISEV Meeting 2015) to focus on the standardization of EV measurement by flow cytometry, which should address some of these issues. However, in addition, it is now clear that careful consideration needs to be given to the different stimuli and cellular processes that may underlie changes in circulating MV levels. By taking these factors into account, it should be possible to start to gain deeper insights into the likely biological background for changes in MV levels and also lead to technically superior design of future clinical studies that assess MV levels in cardiovascular disease states.

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REFERENCES

1 Montoro-Garcia, S., Shantsila, E., Marin, F., Blann, A. and Lip, G.Y. (2011) Circulating microparticles: new insights into the biochemical basis of microparticle release and activity. Basic Res. Cardiol. **106**, 911–923 CrossRef PubMed

2 Lu, Y., Li, L., Yan, H., Su, Q., Huang, J. and Fu, C. (2013) Endothelial microparticles exert differential effects on functions of Th1 in patients with acute coronary syndrome. Int. J. Cardiol. **168**, 5396–5404 CrossRef PubMed

3 Bernal-Mizrachi, L., Jy, W., Jimenez, J.J., Pastor, J., Mauro, L.M., Horstman, L.L., de Marchena, E. and Ahn, Y.S. (2003) High levels of circulating endothelial microparticles in patients with acute coronary syndromes. Am. Heart J. **145**, 962–970 CrossRef PubMed

4 Koga, H., Sugiyama, S., Kugiyama, K., Watanabe, K., Fukushima, H., Tanaka, T., Sakamoto, T., Yoshimura, M., Jinnouchi, H. and Ogawa, H. (2005) Elevated levels of VE-cadherin-positive endothelial microparticles in patients with type 2 diabetes mellitus and coronary artery disease. J. Am. Coll. Cardiol. **45**, 1622–1630 CrossRef PubMed

5 Skeppholm, M., Mobarrez, F., Malmqvist, K. and Wallen, H. (2012) Platelet-derived microparticles during and after acute coronary syndrome. Thromb. Haemost. **107**, 1122–1129 CrossRef PubMed

6 Michelsen, A.E., Brodin, E., Brosstad, F. and Hansen, J.B. (2008) Increased level of platelet microparticles in survivors of myocardial infarction. Scand. J. Clin. Lab. Invest. **68**, 386–392 CrossRef PubMed

7 Berckmans, R.J., Sturk, A., van Tienen, L.M., Schaap, M.C. and Nieuwland, R. (2011) Cell-derived vesicles exposing coagulant tissue factor in saliva. Blood **117**, 3172–3180 CrossRef PubMed

8 Berckmans, R.J., Nieuwland, R., Tak, P.P., Boing, A.N., Romijn, F.P., Kraan, M.C., Breedveld, F.C., Hack, C.E. and Sturk, A. (2002) Cell-derived microparticles in synovial fluid from inflamed arthritic joints support coagulation exclusively via a factor VII-dependent mechanism. Arthritis Rheum. **46**, 2857–2866 CrossRef PubMed

9 Franz, C., Boing, A.N., Hau, C.M., Montag, M., Strowitzki, T., Nieuwland, R. and Toth, B. (2013) Procoagulant tissue factor-exposing vesicles in human seminal fluid. J. Reprod. Immunol. **98**, 45–51 CrossRef PubMed

10 Murakami, T., Oakes, M., Ogura, M., Tovar, V., Yamamoto, C. and Mitsuhashi, M. (2014) Development of glomerulus-, tubule-, and collecting duct-specific mRNA assay in human urinary exosomes and microvesicles. PLoS One **9**, e109074 CrossRef PubMed

11 Bulut, D., Tuns, H. and Mugge, A. (2009) $CD31^+$/Annexin V^+ microparticles in healthy offsprings of patients with coronary artery disease. Eur. J. Clin. Invest. **39**, 17–22 CrossRef PubMed

12 Nozaki, T., Sugiyama, S., Koga, H., Sugamura, K., Ohba, K., Matsuzawa, Y., Sumida, H., Matsui, K., Jinnouchi, H. and Ogawa, H. (2009) Significance of a multiple biomarkers strategy including endothelial dysfunction to improve risk stratification for cardiovascular events in patients at high risk for coronary heart disease. J. Am. Coll. Cardiol. **54**, 601–608 CrossRef PubMed

13 Ueba, T., Nomura, S., Inami, N., Nishikawa, T., Kajiwara, M., Iwata, R. and Yamashita, K. (2010) Plasma level of platelet-derived microparticles is associated with coronary heart disease risk score in healthy men. J. Atheroscler. Thromb. **17**, 342–349 CrossRef PubMed

14 Berezin, A.E., Kremzer, A.A., Samura, T.A., Martovitskaya, Y.V., Malinovskiy, Y.V., Oleshko, S.V. and Berezina, T.A. (2015) Predictive value of apoptotic microparticles to mononuclear progenitor cells ratio in advanced chronic heart failure patients. J. Cardiol. **65**, 403–411 CrossRef PubMed

15 Fan, Y., Wang, L., Li, Y., Yin, Z., Xu, Z. and Wang, C. (2014) Quantification of endothelial microparticles on modified cytometric bead assay and prognosis in chest pain patients. Circ. J. **78**, 206–214 CrossRef PubMed

16 Lee, S.T., Chu, K., Jung, K.H., Kim, J.M., Moon, H.J., Bahn, J.J., Im, W.S., Sunwoo, J., Moon, J., Kim, M. et al. (2012) Circulating $CD62E^+$ microparticles and cardiovascular outcomes. PLoS One **7**, e35713 CrossRef PubMed

17 Sinning, J.M., Losch, J., Walenta, K., Bohm, M., Nickenig, G. and Werner, N. (2011) Circulating $CD31^+$/Annexin V^+ microparticles correlate with cardiovascular outcomes. Eur. Heart J. **32**, 2034–2041 CrossRef PubMed

18 Jung, C., Sorensson, P., Saleh, N., Arheden, H., Ryden, L. and Pernow, J. (2012) Circulating endothelial and platelet derived microparticles reflect the size of myocardium at risk in patients with ST-elevation myocardial infarction. Atherosclerosis **221**, 226–231 CrossRef PubMed

19 Sarlon-Bartoli, G., Bennis, Y., Lacroix, R., Piercecchi-Marti, M.D., Bartoli, M.A., Arnaud, L., Mancini, J., Boudes, A., Sarlon, E., Thevenin, B. et al. (2013) Plasmatic level of leukocyte-derived microparticles is associated with unstable plaque in asymptomatic patients with high-grade carotid stenosis. J. Am. Coll. Cardiol. **62**, 1436–1441 CrossRef PubMed

20 Montoro-García, S., Shantsila, E., Tapp, L.D., López-Cuenca, A., Romero, A.I., Hernández-Romero, D., Orenes-Piñero, E., Manzano-Fernández, S., Valdés, M., Marín, F. and Lip, G.Y. (2013) Small-size circulating microparticles in acute coronary syndromes: Relevance to fibrinolytic status, reparative markers and outcomes. Atherosclerosis **227**, 313–322 CrossRef PubMed

21 Steppich, B.A., Braun, S.L., Stein, A., Demetz, G., Groha, P., Schomig, A., von Beckerath, N., Kastrati, A. and Ott, I. (2009) Plasma TF activity predicts cardiovascular mortality in patients with acute myocardial infarction. Thromb. J. **7**, 11 CrossRef PubMed

22 Berezin, A.E., Kremzer, A.A., Samura, T.A. and Martovitskaya, Y.V. (2014) Circulating endothelial-derived apoptotic microparticles in the patients with ischemic symptomatic chronic heart failure: relevance of pro-inflammatory activation and outcomes. Int. Cardiovasc. Res. J. **8**, 116–123 PubMed

23 Lacroix, R., Robert, S., Poncelet, P. and Dignat-George, F. (2010) Overcoming limitations of microparticle measurement by flow cytometry. Semin. Thromb. Hemost. **36**, 807–818 CrossRef PubMed

24 Wysoczynski, M. and Ratajczak, M.Z. (2009) Lung cancer secreted microvesicles: underappreciated modulators of microenvironment in expanding tumors. Int. J. Cancer **125**, 1595–1603 CrossRef PubMed

25 Zhang, H.C., Liu, X.B., Huang, S., Bi, X.Y., Wang, H.X., Xie, L.X., Wang, Y.Q., Cao, X.F., Lv, J., Xiao, F.J. et al. (2012) Microvesicles derived from human umbilical cord mesenchymal stem cells stimulated by hypoxia promote angiogenesis both *in vitro* and *in vivo*. Stem Cells Dev. **21**, 3289–3297 CrossRef PubMed

26 Wang, T., Gilkes, D.M., Takano, N., Xiang, L., Luo, W., Bishop, C.J., Chaturvedi, P., Green, J.J. and Semenza, G.L. (2014) Hypoxia-inducible factors and RAB22A mediate formation of microvesicles that stimulate breast cancer invasion and metastasis. Proc. Natl. Acad. Sci. U.S.A. **111**, E3234–E3242 CrossRef PubMed

27 Vince, R.V., Chrismas, B., Midgley, A.W., McNaughton, L.R. and Madden, L.A. (2009) Hypoxia mediated release of endothelial microparticles and increased association of S100A12 with circulating neutrophils. Oxid. Med. Cell. Longev. **2**, 2–6 CrossRef PubMed

28 Lichtenauer, M., Goebel, B., Fritzenwanger, M., Forster, M., Betge, S., Lauten, A., Figulla, H.R. and Jung, C. (2014) Simulated temporary hypoxia triggers the release of $CD31^+$/Annexin$^+$ endothelial microparticles: a prospective pilot study in humans. Clin. Hemorheol. Microcirc. CrossRef PubMed

29 Ayers, L., Stoewhas, A.C., Ferry, B., Latshang, T.D., Lo Cascio, C.M., Sadler, R., Stadelmann, K., Tesler, N., Huber, R., Achermann, P. et al. (2014) Circulating levels of cell-derived microparticles are reduced by mild hypobaric hypoxia: data from a randomised controlled trial. Eur. J. Appl. Physiol. **114**, 1067–1073 CrossRef PubMed

30 Pichler Hefti, J., Stutz, M., Huber, A.R. and Geiser, T. (2012) Procoagulatory state during high altitude expedition: thrombocytic and endothelial microparticles as source of coagulation activation? Respiration **83**, 435

31 Stoewhas, A., Latshang, T., LoCascio, C., Lautwein, S., Stadelmann, K., Tesler, N., Ayers, L., Berneis, K., Gerber, P., Huber, R. et al. (2013) Effects of acute exposure to moderate altitude on vascular function, metabolism and systemic inflammation. PLoS One **8**, e70081 CrossRef PubMed

32 Chen, Y.W., Chen, Y.C. and Wang, J.S. (2013) Absolute hypoxic exercise training enhances *in vitro* thrombin generation by increasing procoagulant platelet-derived microparticles under high shear stress in sedentary men. Clin. Sci. (Lond.) **124**, 639–649 CrossRef PubMed

33 Wang, J.S., Chang, Y.L., Chen, Y.C., Tsai, H.H. and Fu, T.C. (2015) Effects of normoxic and hypoxic exercise regimens on monocyte-mediated thrombin generation in sedentary men. Clin. Sci. (Lond.) **129**, 363–374 CrossRef PubMed

34 Chen, Y.C., Ho, C.W., Tsai, H.H. and Wang, J.S. (2015) Interval and continuous exercise regimens suppress neutrophil-derived microparticle formation and neutrophil-promoted thrombin generation under hypoxic stress. Clin. Sci. (Lond.) **128**, 425–436 PubMed

35 Ayers, L., Ferry, B., Craig, S., Nicoll, D., Stradling, J.R. and Kohler, M. (2009) Circulating cell-derived microparticles in patients with minimally symptomatic obstructive sleep apnoea. Eur. Respir. J. **33**, 574–580 CrossRef PubMed

36 Jelic, S., Lederer, D.J., Adams, T., Padeletti, M., Colombo, P.C., Factor, P. and Le Jemtel, T.H. (2009) Endothelial repair capacity and apoptosis are inversely related in obstructive sleep apnea. Vasc. Health Risk Manag. **5**, 909–920 CrossRef PubMed

37 Yun, C.H., Jung, K.H., Chu, K., Kim, S.H., Ji, K.H., Park, H.K., Kim, H.C., Lee, S.T., Lee, S.K. and Roh, J.K. (2010) Increased circulating endothelial microparticles and carotid atherosclerosis in obstructive sleep apnea. J. Clin. Neurol. **6**, 89–98 CrossRef PubMed

38 Kim, J., Bhattacharjee, R., Kheirandish-Gozal, L., Spruyt, K. and Gozal, D. (2011) Circulating microparticles in children with sleep disordered breathing. Chest **140**, 408–417 CrossRef PubMed

39 Maruyama, K., Morishita, E., Sekiya, A., Omote, M., Kadono, T., Asakura, H., Hashimoto, M., Kobayashi, M., Nakatsumi, Y., Takada, S. and Ohtake, S. (2012) Plasma levels of platelet-derived microparticles in patients with obstructive sleep apnea syndrome. J. Atheroscler. Thromb. **19**, 98–104 CrossRef PubMed

40 Ayers, L., Stoewhas, A.C., Ferry, B., Stradling, J. and Kohler, M. (2013) Elevated levels of endothelial cell-derived microparticles following short-term withdrawal of continuous positive airway pressure in patients with obstructive sleep apnea: data from a randomized controlled trial. Respiration **85**, 478–485 CrossRef PubMed

41 Takahashi, T. and Kubo, H. (2014) The role of microparticles in chronic obstructive pulmonary disease. Int. J. Chron. Obstruct. Pulmon. Dis. **9**, 303–314 PubMed

42 Thom, S.R., Bhopale, V.M. and Yang, M. (2014) Neutrophils generate microparticles during exposure to inert gases due to cytoskeletal oxidative stress. J. Biol. Chem. **289**, 18831–18845 CrossRef PubMed

43 Takahashi, T., Kobayashi, S., Fujino, N., Suzuki, T., Ota, C., Tando, Y., He, M., Yamada, M., Kurosawa, S., Yamaya, M. and Kubo, H. (2013) Differences in the released endothelial microparticle subtypes between human pulmonary microvascular endothelial cells and aortic endothelial cells *in vitro*. Exp. Lung Res. **39**, 155–161 CrossRef PubMed

44 Burger, D., Montezano, A.C., Nishigaki, N., He, Y., Carter, A. and Touyz, R.M. (2011) Endothelial microparticle formation by angiotensin II is mediated via Ang II receptor type I/NADPH oxidase/Rho kinase pathways targeted to lipid rafts. Arterioscler. Thromb. Vasc. Biol. **31**, 1898–1907 CrossRef PubMed

45 Jiang, J., Woulfe, D.S. and Papoutsakis, E.T. (2014) Shear enhances thrombopoiesis and formation of microparticles that induce megakaryocytic differentiation of stem cells. Blood **124**, 2094–2103 CrossRef PubMed

46 Diehl, P., Nagy, F., Sossong, V., Helbing, T., Beyersdorf, F., Olschewski, M., Bode, C. and Moser, M. (2008) Increased levels of circulating microparticles in patients with severe aortic valve stenosis. Thromb. Haemost. **99**, 711–719 PubMed

47 Reininger, A.J., Heijnen, H.F., Schumann, H., Specht, H.M., Schramm, W. and Ruggeri, Z.M. (2006) Mechanism of platelet adhesion to von Willebrand factor and microparticle formation under high shear stress. Blood **107**, 3537–3545 CrossRef PubMed

48 Ikeda, M., Iwamoto, S., Imamura, H., Furukawa, H. and Kawasaki, T. (2003) Increased platelet aggregation and production of platelet-derived microparticles after surgery for upper gastrointestinal malignancy. J. Surg. Res. **115**, 174–183 CrossRef PubMed

49 Nomura, S., Tandon, N.N., Nakamura, T., Cone, J., Fukuhara, S. and Kambayashi, J. (2001) High-shear-stress-induced activation of platelets and microparticles enhances expression of cell adhesion molecules in THP-1 and endothelial cells. Atherosclerosis **158**, 277–287 CrossRef PubMed

50 Chow, T.W., Hellums, J.D. and Thiagarajan, P. (2000) Thrombin receptor activating peptide (SFLLRN) potentiates shear-induced platelet microvesiculation. J. Lab. Clin. Med. **135**, 66–72 CrossRef PubMed

51 Vion, A.C., Ramkhelawon, B., Loyer, X., Chironi, G., Devue, C., Loirand, G., Tedgui, A., Lehoux, S. and Boulanger, C.M. (2013) Shear stress regulates endothelial microparticle release. Circ. Res. **112**, 1323–1333 CrossRef PubMed

52 Al Kaabi, A., Traupe, T., Stutz, M., Buchs, N. and Heller, M. (2012) Cause or effect of arteriogenesis: compositional alterations of microparticles from CAD patients undergoing external counterpulsation therapy. PLoS One **7**, e46822 CrossRef PubMed

53 Nascimbene, A., Hernandez, R., George, J.K., Parker, A., Bergeron, A.L., Pradhan, S., Vijayan, K.V., Civitello, A., Simpson, L., Nawrot, M. et al. (2014) Association between cell-derived microparticles and adverse events in patients with nonpulsatile left ventricular assist devices. J. Heart Lung Transplant. **33**, 470–477 CrossRef PubMed

54 Horn, P., Stern, D., Veulemans, V., Heiss, C., Zeus, T., Merx, M.W., Kelm, M. and Westenfeld, R. (2015) Improved endothelial function and decreased levels of endothelium-derived microparticles after transcatheter aortic valve implantation. EuroIntervention **10**, 1456–1463 CrossRef PubMed

55 Boulanger, C.M., Amabile, N., Guerin, A.P., Pannier, B., Leroyer, A.S., Mallat, C.N., Tedgui, A. and London, G.M. (2007) *In vivo* shear stress determines circulating levels of endothelial microparticles in end-stage renal disease. Hypertension **49**, 902–908 CrossRef PubMed

56 Buendia, P., Montes de Oca, A., Madueno, J.A., Merino, A., Martin-Malo, A., Aljama, P., Ramirez, R., Rodriguez, M. and Carracedo, J. (2015) Endothelial microparticles mediate inflammation-induced vascular calcification. FASEB J. **29**, 173–181 CrossRef PubMed

57 Lee, S.K., Yang, S.H., Kwon, I., Lee, O.H. and Heo, J.H. (2014) Role of tumour necrosis factor receptor-1 and nuclear factor-κB in production of TNF-α-induced pro-inflammatory microparticles in endothelial cells. Thromb. Haemost. **112**, 580–588 CrossRef PubMed

58 Eyre, J., Burton, J.O., Saleem, M.A., Mathieson, P.W., Topham, P.S. and Brunskill, N.J. (2011) Monocyte- and endothelial-derived microparticles induce an inflammatory phenotype in human podocytes. Nephron Exp. Nephrol. **119**, e58–e66 CrossRef PubMed

59 Combes, V., Simon, A.C., Grau, G.E., Arnoux, D., Camoin, L., Sabatier, F., Mutin, M., Sanmarco, M., Sampol, J. and Dignat-George, E. (1999) *In vitro* generation of endothelial microparticles and possible prothrombotic activity in patients with lupus anticoagulant. J. Clin. Invest. **104**, 93–102 CrossRef PubMed

60 Brown, G.T. and McIntyre, T.M. (2011) Lipopolysaccharide signaling without a nucleus: kinase cascades stimulate platelet shedding of proinflammatory IL-1β-rich microparticles. J. Immunol. **186**, 5489–5496 CrossRef PubMed

61 Scanu, A., Molnarfi, N., Brandt, K.J., Gruaz, L., Dayer, J.M. and Burger, D. (2008) Stimulated T cells generate microparticles, which mimic cellular contact activation of human monocytes: differential regulation of pro- and anti-inflammatory cytokine production by high-density lipoproteins. J. Leukoc. Biol. **83**, 921–927 CrossRef PubMed

62 Mesri, M. and Altieri, D.C. (1999) Leukocyte microparticles stimulate endothelial cell cytokine release and tissue factor induction in a JNK1 signaling pathway. J. Biol. Chem. **274**, 23111–23118 CrossRef PubMed

63 Cui, Y., Zheng, L., Jiang, M., Jia, R., Zhang, X., Quan, Q., Du, G., Shen, D., Zhao, X., Sun, W. et al. (2013) Circulating microparticles in patients with coronary heart disease and its correlation with interleukin-6 and C-reactive protein. Mol. Biol. Rep. **40**, 6437–6442 CrossRef PubMed

64 Woei, A.J.F.J., De Kruif, M.D., Garcia Rodriguez, P., Osanto, S. and Bertina, R.M. (2012) Microparticles expressing tissue factor are concurrently released with markers of inflammation and coagulation during human endotoxemia. J. Thromb. Haemost. **10**, 1185–1188 CrossRef PubMed

65 Aras, O., Shet, A., Bach, R.R., Hysjulien, J.L., Slungaard, A., Hebbel, R.P., Escolar, G., Jilma, B. and Key, N.S. (2004) Induction of microparticle- and cell-associated intravascular tissue factor in human endotoxemia. Blood **103**, 4545–4553 CrossRef PubMed

66 Pihusch, V., Rank, A., Steber, R., Pihusch, M., Pihusch, R., Toth, B., Hiller, E. and Kolb, H.J. (2006) Endothelial cell-derived microparticles in allogeneic hematopoietic stem cell recipients. Transplantation **81**, 1405–1409 CrossRef PubMed

67 Alexy, T., Rooney, K., Weber, M., Gray, W.D. and Searles, C.D. (2014) TNF-α alters the release and transfer of microparticle-encapsulated miRNAs from endothelial cells. Physiol. Genomics **46**, 833–840 CrossRef PubMed

68 Antoniak, S., Boltzen, U., Eisenreich, A., Stellbaum, C., Poller, W., Schultheiss, H.P. and Rauch, U. (2009) Regulation of cardiomyocyte full-length tissue factor expression and microparticle release under inflammatory conditions *in vitro*. J. Thromb. Haemost. **7**, 871–878 CrossRef PubMed

69 Pelletier, F., Garnache-Ottou, F., Biichle, S., Vivot, A., Humbert, P., Saas, P., Seilles, E. and Aubin, F. (2014) Effects of anti-TNF-α agents on circulating endothelial-derived and platelet-derived microparticles in psoriasis. Exp. Dermatol. **23**, 924–925 CrossRef PubMed

70 Sari, I., Bozkaya, G., Kirbiyik, H., Alacacioglu, A., Ates, H., Sop, G., Can, G., Taylan, A., Piskin, O., Yildiz, Y. and Akkoc, N. (2012) Evaluation of circulating endothelial and platelet microparticles in men with ankylosing spondylitis. J. Rheumatol. **39**, 594–599 CrossRef PubMed

71 Mostefai, H.A., Meziani, F., Mastronardi, M.L., Agouni, A., Heymes, C., Sargentini, C., Asfar, P., Martinez, M.C. and Andriantsitohaina, R. (2008) Circulating microparticles from patients with septic shock exert protective role in vascular function. Am. J. Respir. Crit. Care Med. **178**, 1148–1155 CrossRef PubMed

72 Nieuwland, R., Berckmans, R.J., McGregor, S., Boing, A.N., Romijn, F.P., Westendorp, R.G., Hack, C.E. and Sturk, A. (2000) Cellular origin and procoagulant properties of microparticles in meningococcal sepsis. Blood **95**, 930–935 PubMed

73 Ogura, H., Kawasaki, T., Tanaka, H., Koh, T., Tanaka, R., Ozeki, Y., Hosotsubo, H., Kuwagata, Y., Shimazu, T. and Sugimoto, H. (2001) Activated platelets enhance microparticle formation and platelet-leukocyte interaction in severe trauma and sepsis. J. Trauma **50**, 801–809 CrossRef PubMed

74 Fujimi, S., Ogura, H., Tanaka, H., Koh, T., Hosotsubo, H., Nakamori, Y., Kuwagata, Y., Shimazu, T. and Sugimoto, H. (2002) Activated polymorphonuclear leukocytes enhance production of leukocyte microparticles with increased adhesion molecules in patients with sepsis. J. Trauma **52**, 443–448 CrossRef PubMed

75 Mostefai, H.A., Bourget, J.M., Meziani, F., Martinez, M.C., Leonetti, D., Mercat, A., Asfar, P., Germain, L. and Andriantsitohaina, R. (2013) Interleukin-10 controls the protective effects of circulating microparticles from patients with septic shock on tissue-engineered vascular media. Clin. Sci. (Lond.) **125**, 77–85 CrossRef PubMed

76 Gasser, O. and Schifferli, J.A. (2004) Activated polymorphonuclear neutrophils disseminate anti-inflammatory microparticles by ectocytosis. Blood **104**, 2543–2548 CrossRef PubMed

77 Pliyev, B.K., Kalintseva, M.V., Abdulaeva, S.V., Yarygin, K.N. and Savchenko, V.G. (2014) Neutrophil microparticles modulate cytokine production by natural killer cells. Cytokine **65**, 126–129 CrossRef PubMed

78 Koppler, B., Cohen, C., Schlondorff, D. and Mack, M. (2006) Differential mechanisms of microparticle transfer to B cells and monocytes: anti-inflammatory propertiesof microparticles. Eur. J. Immunol. **36**, 648–660 CrossRef PubMed

79 Ferreira, A.C., Peter, A.A., Mendez, A.J., Jimenez, J.J., Mauro, L.M., Chirinos, J.A., Ghany, R., Virani, S., Garcia, S., Horstman, L.L. et al. (2004) Postprandial hypertriglyceridemia increases circulating levels of endothelial cell microparticles. Circulation **110**, 3599–3603 CrossRef PubMed

80 Harrison, M., Murphy, R.P., O'Connor, P.L., O'Gorman, D.J., McCaffrey, N., Cummins, P.M. and Moyna, N.M. (2009) The endothelial microparticle response to a high fat meal is not attenuated by prior exercise. Eur. J. Appl. Physiol. **106**, 555–562 CrossRef PubMed

81 Jenkins, N.T., Landers, R.Q., Thakkar, S.R., Fan, X., Brown, M.D., Prior, S.J., Spangenburg, E.E. and Hagberg, J.M. (2011) Prior endurance exercise prevents postprandial lipaemia-induced increases in reactive oxygen species in circulating $CD31^+$ cells. J. Physiol. **589**, 5539–5553 CrossRef PubMed

82 Tushuizen, M.E., Nieuwland, R., Scheffer, P.G., Sturk, A., Heine, R.J. and Diamant, M. (2006) Two consecutive high-fat meals affect endothelial-dependent vasodilation, oxidative stress and cellular microparticles in healthy men. J. Thromb. Haemost. **4**, 1003–1010 CrossRef PubMed

83 Bulut, D., Jelich, U., Dacanay-Schwarz, R. and Mugge, A. (2013) Red wine ingestion prevents microparticle formation after a single high-fat meal: a crossover study in healthy humans. J. Cardiovasc. Pharmacol. **61**, 489–494 CrossRef PubMed

84 Strohacker, K., Breslin, W.L., Carpenter, K.C., Davidson, T.R., Agha, N.H. and McFarlin, B.K. (2012) Moderate-intensity, premeal cycling blunts postprandial increases in monocyte cell surface CD18 and CD11a and endothelial microparticles following a high-fat meal in young adults. Appl. Physiol. Nutr. Metab. **37**, 530–539 CrossRef PubMed

85 Raz, O., Steinvil, A., Berliner, S., Rosenzweig, T., Justo, D. and Shapira, I. (2013) The effect of two iso-caloric meals containing equal amounts of fats with a different fat composition on the inflammatory and metabolic markers in apparently healthy volunteers. J. Inflamm. **10**, 3 CrossRef

86 Sossdorf, M., Otto, G.P., Claus, R.A., Gabriel, H.H. and Losche, W. (2010) Release of pro-coagulant microparticles after moderate endurance exercise. Platelets **21**, 389–391 CrossRef PubMed

87 Sossdorf, M., Otto, G.P., Claus, R.A., Gabriel, H.H. and Losche, W. (2011) Cell-derived microparticles promote coagulation after moderate exercise. Med. Sci. Sports Exerc. **43**, 1169–1176 CrossRef PubMed

88 Maruyama, K., Kadono, T. and Morishita, E. (2012) Plasma levels of platelet-derived microparticles are increased after anaerobic exercise in healthy subjects. J. Atheroscler. Thromb. **19**, 585–587 CrossRef PubMed

89 Thom, S.R., Milovanova, T.N., Bogush, M., Yang, M., Bhopale, V.M., Pollock, N.W., Ljubkovic, M., Denoble, P., Madden, D., Lozo, M. and Dujic, Z. (2013) Bubbles, microparticles, and neutrophil activation: changes with exercise level and breathing gas during open-water SCUBA diving. J. Appl. Physiol. **114**, 1396–1405 CrossRef PubMed

90 Chen, Y.W., Chen, J.K. and Wang, J.S. (2010) Strenuous exercise promotes shear-induced thrombin generation by increasing the shedding of procoagulant microparticles from platelets. Thromb. Haemost. **104**, 293–301 CrossRef PubMed

91 Chaar, V., Romana, M., Tripette, J., Broquere, C., Huisse, M.G., Hue, O., Hardy-Dessources, M.D. and Connes, P. (2011) Effect of strenuous physical exercise on circulating cell-derived microparticles. Clin. Hemorheol. Microcirc. **47**, 15–25 PubMed

92 Durrer, C., Robinson, E., Wan, Z., Martinez, N., Hummel, M.L., Jenkins, N.T., Kilpatrick, M.W. and Little, J.P. (2015) Differential impact of acute high-intensity exercise on circulating endothelial microparticles and insulin resistance between overweight/obese males and females. PLoS One **10**, e0115860 CrossRef PubMed

93 Boyle, L.J., Credeur, D.P., Jenkins, N.T., Padilla, J., Leidy, H.J., Thyfault, J.P. and Fadel, P.J. (2013) Impact of reduced daily physical activity on conduit artery flow-mediated dilation and circulating endothelial microparticles. J. Appl. Physiol. **115**, 1519–1525 CrossRef PubMed

94 Madden, D., Thom, S.R., Milovanova, T.N., Yang, M., Bhopale, V.M., Ljubkovic, M. and Dujic, Z. (2014) Exercise before scuba diving ameliorates decompression-induced neutrophil activation. Med. Sci. Sports Exerc. **46**, 1928–1935 CrossRef PubMed

95 Guiraud, T., Gayda, M., Juneau, M., Bosquet, L., Meyer, P., Théberge-Julien, G., Galinier, M., Nozza, A., Lambert, J., Rhéaume, E. et al. (2013) A single bout of high-intensity interval exercise does not increase endothelial or platelet microparticles in stable, physically fit men with coronary heart disease. Can. J. Cardiol. **29**, 1285–1291 CrossRef PubMed

96 Augustine, D., Ayers, L.V., Lima, E., Newton, L., Lewandowski, A.J., Davis, E.F., Ferry, B. and Leeson, P. (2014) Dynamic release and clearance of circulating microparticles during cardiac stress. Circ. Res. **114**, 109–113 CrossRef PubMed

97 Hugel, B., Martinez, M.C., Kunzelmann, C. and Freyssinet, J.M. (2005) Membrane microparticles: two sides of the coin. Physiology **20**, 22–27 CrossRef PubMed

98 VanWijk, M.J., VanBavel, E., Sturk, A. and Nieuwland, R. (2003) Microparticles in cardiovascular diseases. Cardiovasc. Res. **59**, 277–287 CrossRef PubMed

99 Coleman, M.L., Sahai, E.A., Yeo, M., Bosch, M., Dewar, A. and Olson, M.F. (2001) Membrane blebbing during apoptosis results from caspase-mediated activation of ROCK I. Nat. Cell Biol. **3**, 339–345 CrossRef PubMed

100 Ardoin, S.P., Shanahan, J.C. and Pisetsky, D.S. (2007) The role of microparticles in inflammation and thrombosis. Scand. J. Immunol. **66**, 159–165 CrossRef PubMed

101 Freyssinet, J.M. (2003) Cellular microparticles: what are they bad or good for? J. Thromb. Haemost. **1**, 1655–1662 CrossRef PubMed

102 Mulcahy, L.A., Pink, R.C. and Carter, D.R. (2014) Routes and mechanisms of extracellular vesicle uptake. J. Extracell. Vesicles **3**, 24641

103 Flaumenhaft, R. (2006) Formation and fate of platelet microparticles. Blood Cells Mol. Dis. **36**, 182–187 CrossRef PubMed

104 Schroit, A.J., Madsen, J.W. and Tanaka, Y. (1985) *In vivo* recognition and clearance of red blood cells containing phosphatidylserine in their plasma membranes. J. Biol. Chem. **260**, 5131–5138 PubMed

105 Manfredi, A.A., Rovere-Querini, P. and Maugeri, N. (2010) Dangerous connections: neutrophils and the phagocytic clearance of activated platelets. Curr. Opin. Hematol. **17**, 3–8 CrossRef PubMed

106 Dasgupta, S.K., Abdel-Monem, H., Niravath, P., Le, A., Bellera, R.V., Langlois, K., Nagata, S., Rumbaut, R.E. and Thiagarajan, P. (2009) Lactadherin and clearance of platelet-derived microvesicles. Blood **113**, 1332–1339 CrossRef PubMed

107 Kaur, R., Bramwell, V.W., Kirby, D.J. and Perrie, Y. (2012) Manipulation of the surface pegylation in combination with reduced vesicle size of cationic liposomal adjuvants modifies their clearance kinetics from the injection site, and the rate and type of T cell response. J. Control. Release **164**, 331–337 CrossRef PubMed

108 van der Pol, E., Boing, A.N., Harrison, P., Sturk, A. and Nieuwland, R. (2012) Classification, functions, and clinical relevance of extracellular vesicles. Pharmacol. Rev. **64**, 676–705 CrossRef PubMed

109 Connor, D.E., Exner, T., Ma, D.D. and Joseph, J.E. (2010) The majority of circulating platelet-derived microparticles fail to bind annexin V, lack phospholipid-dependent procoagulant activity and demonstrate greater expression of glycoprotein Ib. Thromb. Haemost. **103**, 1044–1052 CrossRef PubMed

110 Faille, D., El-Assaad, F., Mitchell, A.J., Alessi, M.C., Chimini, G., Fusai, T., Grau, G.E. and Combes, V. (2012) Endocytosis and intracellular processing of platelet microparticles by brain endothelial cells. J. Cell. Mol. Med. **16**, 1731–1738 CrossRef PubMed

111 Jansen, F., Yang, X., Hoyer, F.F., Paul, K., Heiermann, N., Becher, M.U., Abu Hussein, N., Kebschull, M., Bedorf, J., Franklin, B.S. et al. (2012) Endothelial microparticle uptake in target cells is annexin I/phosphatidylserine receptor dependent and prevents apoptosis. Arterioscler. Thromb. Vasc. Biol. **32**, 1925–1935 CrossRef PubMed

112 Dasgupta, S.K., Le, A., Chavakis, T., Rumbaut, R.E. and Thiagarajan, P. (2012) Developmental endothelial locus-1 (Del-1) mediates clearance of platelet microparticles by the endothelium. Circulation **125**, 1664–1672 CrossRef PubMed

113 Wahl, P., Jansen, F., Achtzehn, S., Schmitz, T., Bloch, W., Mester, J. and Werner, N. (2014) Effects of high intensity training and high volume training on endothelial microparticles and angiogenic growth factors. PLoS One **9**, e96024 CrossRef PubMed

114 Collier, M.E., Mah, P.M., Xiao, Y., Maraveyas, A. and Ettelaie, C. (2013) Microparticle-associated tissue factor is recycled by endothelial cells resulting in enhanced surface tissue factor activity. Thromb. Haemost. **110**, 966–976 CrossRef PubMed

115 Jansen, F., Yang, X., Hoelscher, M., Cattelan, A., Schmitz, T., Proebsting, S., Wenzel, D., Vosen, S., Franklin, B.S., Fleischmann, B.K. et al. (2013) Endothelial microparticle-mediated transfer of MicroRNA-126 promotes vascular endothelial cell repair via SPRED1 and is abrogated in glucose-damaged endothelial microparticles. Circulation **128**, 2026–2038 CrossRef PubMed

116 Wen, B., Combes, V., Bonhoure, A., Weksler, B.B., Couraud, P.O. and Grau, G.E. (2014) Endotoxin-induced monocytic microparticles have contrasting effects on endothelial inflammatory responses. PLoS One **9**, e91597 CrossRef PubMed

117 Diehl, P., Fricke, A., Sander, L., Stamm, J., Bassler, N., Htun, N., Ziemann, M., Helbing, T., El-Osta, A., Jowett, J.B. and Peter, K. (2012) Microparticles: major transport vehicles for distinct microRNAs in circulation. Cardiovasc. Res. **93**, 633–644 CrossRef PubMed

118 Henson, P.M., Bratton, D.L. and Fadok, V.A. (2001) Apoptotic cell removal. Curr. Biol. **11**, R795–R805 CrossRef PubMed

119 Ait-Oufella, H., Kinugawa, K., Zoll, J., Simon, T., Boddaert, J., Heeneman, S., Blanc-Brude, O., Barateau, V., Potteaux, S., Merval, R. et al. (2007) Lactadherin deficiency leads to apoptotic cell accumulation and accelerated atherosclerosis in mice. Circulation **115**, 2168–2177 CrossRef PubMed

120 Brown, S., Heinisch, I., Ross, E., Shaw, K., Buckley, C.D. and Savill, J. (2002) Apoptosis disables CD31-mediated cell detachment from phagocytes promoting binding and engulfment. Nature **418**, 200–203 CrossRef PubMed

121 Pacheco, P., White, D. and Sulchek, T. (2013) Effects of microparticle size and Fc density on macrophage phagocytosis. PLoS One **8**, e60989 CrossRef PubMed

122 Litvack, M.L., Post, M. and Palaniyar, N. (2011) IgM promotes the clearance of small particles and apoptotic microparticles by macrophages. PLoS One **6**, e17223 CrossRef PubMed

123 Dalli, J. and Serhan, C.N. (2012) Specific lipid mediator signatures of human phagocytes: microparticles stimulate macrophage efferocytosis and pro-resolving mediators. Blood **120**, e60–e72 CrossRef PubMed

124 Willekens, F.L., Werre, J.M., Kruijt, J.K., Roerdinkholder-Stoelwinder, B., Groenen-Dopp, Y.A., van den Bos, A.G., Bosman, G.J. and van Berkel, T.J. (2005) Liver Kupffer cells rapidly remove red blood cell-derived vesicles from the circulation by scavenger receptors. Blood **105**, 2141–2145 CrossRef PubMed

125 Moghimi, S.M. and Patel, H.M. (2002) Modulation of murine liver macrophage clearance of liposomes by diethylstilbestrol: the effect of vesicle surface charge and a role for the complement receptor Mac-1 (CD11b/CD18) of newly recruited macrophages in liposome recognition. J. Control. Release **78**, 55–65 CrossRef PubMed

126 Qiu, Q., Xiong, W., Yang, C., Gagnon, C. and Hardy, P. (2013) Lymphocyte-derived microparticles induce bronchial epithelial cells' pro-inflammatory cytokine production and apoptosis. Mol. Immunol. **55**, 220–230 CrossRef PubMed

127 Amabile, N., Heiss, C., Real, W.M., Minasi, P., McGlothlin, D., Rame, E.J., Grossman, W., De Marco, T. and Yeghiazarians, Y. (2008) Circulating endothelial microparticle levels predict hemodynamic severity of pulmonary hypertension. Am. J. Respir. Crit. Care Med. **177**, 1268–1275 CrossRef PubMed

128 Vandivier, R.W., Henson, P.M. and Douglas, I.S. (2006) Burying the dead: the impact of failed apoptotic cell removal (efferocytosis) on chronic inflammatory lung disease. Chest **129**, 1673–1682 CrossRef PubMed

129 Bastarache, J.A., Fremont, R.D., Kropski, J.A., Bossert, F.R. and Ware, L.B. (2009) Procoagulant alveolar microparticles in the lungs of patients with acute respiratory distress syndrome. Am. J. Physiol. Lung Cell. Mol. Physiol. **297**, L1035–L1041 CrossRef PubMed

130 Davila, M., Amirkhosravi, A., Coll, E., Desai, H., Robles, L., Colon, J., Baker, C.H. and Francis, J.L. (2008) Tissue factor-bearing microparticles derived from tumor cells: impact on coagulation activation. J. Thromb. Haemost. **6**, 1517–1524 CrossRef PubMed

131 Fontana, V., Jy, W., Ahn, E.R., Dudkiewicz, P., Horstman, L.L., Duncan, R. and Ahn, Y.S. (2008) Increased procoagulant cell-derived microparticles (C-MP) in splenectomized patients with ITP. Thromb. Res. **122**, 599–603 CrossRef PubMed

132 Al Faraj, A., Gazeau, F., Wilhelm, C., Devue, C., Guérin, C.L., Péchoux, C., Paradis, V., Clément, O., Boulanger, C.M. and Rautou, P.E. (2012) Endothelial cell-derived microparticles loaded with iron oxide nanoparticles: feasibility of MR imaging monitoring in mice. Radiology **263**, 169–178 CrossRef PubMed

133 Rautou, P.E. and Mackman, N. (2012) Del-etion of microvesicles from the circulation. Circulation **125**, 1601–1604 CrossRef PubMed

134 Rand, M.L., Wang, H., Bang, K.W., Packham, M.A. and Freedman, J. (2006) Rapid clearance of procoagulant platelet-derived microparticles from the circulation of rabbits. J. Thromb. Haemost. **4**, 1621–1623 CrossRef PubMed

135 Gemmell, C.H., Yeo, E.L. and Sefton, M.V. (1997) Flow cytometric analysis of material-induced platelet activation in a canine model: elevated microparticle levels and reduced platelet life span. J. Biomed. Mater. Res. **37**, 176–181 CrossRef PubMed

136 Helmond, S.E., Catalfamo, J.L. and Brooks, M.B. (2013) Flow cytometric detection and procoagulant activity of circulating canine platelet-derived microparticles. Am. J. Vet. Res. **74**, 207–215 CrossRef PubMed

137 Johnson, L., Coorey, C.P. and Marks, D.C. (2014) The hemostatic activity of cryopreserved platelets is mediated by phosphatidylserine-expressing platelets and platelet microparticles. Transfusion **54**, 1917–1926 CrossRef PubMed

138 Ayers, L., Kohler, M., Harrison, P., Sargent, I., Dragovic, R., Schaap, M., Nieuwland, R., Brooks, S.A. and Ferry, B. (2011) Measurement of circulating cell-derived microparticles by flow cytometry: sources of variability within the assay. Thromb. Res. **127**, 370–377 CrossRef PubMed

139 van den Goor, J.M., Nieuwland, R., Rutten, P.M., Tijssen, J.G., Hau, C., Sturk, A., Eijsman, L. and de Mol, B.A. (2007) Retransfusion of pericardial blood does not trigger systemic coagulation during cardiopulmonary bypass. Eur. J. Cardiothorac. Surg. **31**, 1029–1036 CrossRef PubMed

140 Rank, A., Nieuwland, R., Crispin, A., Grutzner, S., Iberer, M., Toth, B. and Pihusch, R. (2011) Clearance of platelet microparticles *in vivo*. Platelets **22**, 111–116 CrossRef PubMed

141 Yuana, Y., Bertina, R.M. and Osanto, S. (2011) Pre-analytical and analytical issues in the analysis of blood microparticles. Thromb. Haemost. **105**, 396–408 CrossRef PubMed

142 van der Pol, E., Coumans, F.A., Grootemaat, A.E., Gardiner, C., Sargent, I.L., Harrison, P., Sturk, A., van Leeuwen, T.G. and Nieuwland, R. (2014) 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 CrossRef PubMed

143 Lacroix, R., Judicone, C., Mooberry, M., Boucekine, M., Key, N.S. and Dignat-George, F. (2013) Standardization of pre-analytical variables in plasma microparticle determination: results of the International Society on Thrombosis and Haemostasis SSC Collaborative workshop. J. Thromb. Haemost. **11**, 1190–1193 CrossRef

144 Lacroix, R., Judicone, C., Poncelet, P., Robert, S., Arnaud, L., Sampol, J. and Dignat-George, F. (2012) Impact of pre-analytical parameters on the measurement of circulating microparticles: towards standardization of protocol. J. Thromb. Haemost. **10**, 437–446 CrossRef PubMed

145 Lee, R.D., Barcel, D.A., Williams, J.C., Wang, J.G., Boles, J.C., Manly, D.A., Key, N.S. and Mackman, N. (2012) Pre-analytical and analytical variables affecting the measurement of plasma-derived microparticle tissue factor activity. Thromb. Res. **129**, 80–85 CrossRef PubMed

146 Lacroix, R., Robert, S., Poncelet, P., Kasthuri, R.S., Key, N.S. and Dignat-George, F. (2010) Standardization of platelet-derived microparticle enumeration by flow cytometry using calibrated beads: results of ISTH SSC collaborative workshop. J. Thromb.Haemost. **8**, 1216–1224 CrossRef PubMed

147 Robert, S., Poncelet, P., Lacroix, R., Arnaud, L., Giraudo, L., Hauchard, A., Sampol, J. and Dignat-George, F. (2009) Standardization of platelet-derived microparticle counting using calibrated beads and a Cytomics FC500 routine flow cytometer: a first step towards multicenter studies? J. Thromb. Haemost. **7**, 190–197 CrossRef PubMed

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