Microparticles and Exercise in Clinical Populations

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Abstract

Microparticles (MPs) are shed membrane vesicles released from a variety of cell types in response to cellular activation or apoptosis. They are elevated in a wide variety of disease states and have been previously measured to assess both disease activity and severity. However, recent research suggests that they also possess bioeffector functions, including but not limited to promoting coagulation and thrombosis, inducing endothelial dysfunction, increasing pro-inflammatory cytokine release and driving angiogenesis, thereby increasing cardiovascular risk. Current evidence suggests that exercise may reduce both the number and pathophysiological potential of circulating MPs, making them an attractive therapeutic target. However, the existing body of literature is largely comprised of *in vitro* or animal studies and thus drawing meaningful conclusions with regards to health and disease remains difficult. In this review, we highlight the role of microparticles in disease, comment on the use of exercise and dietary manipulation as a therapeutic strategy, and suggest future research directions that would serve to address some of the limitations present in the research to date.

Introduction

Microparticles (MPs) are shed membrane vesicles, usually ranging in size from 0.1 to 1 μm. They are distinct from exosomes, which tend to be smaller (<0.1 μm) and have a different method of formation (1) – this review will focus solely on MPs, their pathophysiology within clinical populations and the potential of exercise as a therapeutic strategy.

Causes of formation

MPs are released from the cell membrane during apoptosis or activation, elicited by a variety of stimuli. For example, the activation stimuli could be inflammation, oxidative stress or mechanical/haemodynamic fluctuations depending on the parent cell in question. After their formation, MPs express surface proteins and antigens that are suggestive of their cellular origin, through which they can be identified by laboratory techniques (the most common MP cellular

sources and their corresponding surface antigens are listed in Table 1). The MP membrane might also include negatively charged phospholipids, the majority of which are phosphatidylserine (PS) which is exposed on the outer layer (2). For a list of possible detection methods and a comparison of their minimum detectable vesicle sizes, see Van Der Pol et al (3).

MP Cellular Source	**Surface antigen/s used for determination**
All cells	Phosphatidylserine*
Leukocyte	CD11a, CD45
Granulocyte	CD11b, CDF66
Platelet	CD31 (PECAM-1), CD40L, CD41/a, CD42b, CD61
Monocyte	CD11b, CD14, CD16
Endothelial cell	CD31, CD51, CD54 (ICAM-1), CD62E (E-Selectin), CD62P (P-Selectin), CD105 (endoglin), CD144 (VE-Cadherin), CD146 (S-Endo 1)
Neutrophil	CD66b
Erythrocyte	CD235a
Lymphocyte	CD3, CD4, CD36

Table 1. The most common cellular sources of MPs, along with the corresponding antigens exposed on their outer surface. As many cellular sources can be represented by several cell surface markers, differences may occur in the literature when studies have used different markers for the same MP derivation, which could lead to inaccuracies. CD = Cluster of Differentiation, PECAM = platelet-endothelial cell adhesion molecule, ICAM = intercellular adhesion molecule. *The value of measuring PS (by assessing the degree of ligation with its detector reagent Annexin-V) to quantify 'all MPs' has been questioned; as many as 80% of MPs do not bind with Annexin-V *in vitro* and therefore do not express PS on their outer surface (4). PS-negative MPs which do not bind with Annexin-V demonstrate reduced pro-coagulant activity compared to their PS-positive counterparts (4) however their functional significance remains unclear and warrants further investigation.

Mechanisms of formation

A resting, inactivated phospholipid membrane will display phospholipid asymmetry, i.e. different phospholipids displayed on the outer and inner layer (PS is displayed on the inner layer in a healthy membrane(5)). This asymmetry is maintained by the enzymes gelsolin, aminophospholipid translocase, floppase, scramblase and calpain (for a full review of these enzymes' kinetics and how they pertain to MP formation, see Piccin, Murphy, & Smith, 2007)(6). Cellular activation or apoptosis causes the endoplasmic reticulum to release calcium into the cytosol, which alters the actions of

these enzymes, resulting in a restructuring of the cytoskeleton and a reversal of the phospholipid asymmetry and therefore externalisation of PS. This causes outward 'blebbing' of the cell membrane and ultimately fissure, resulting in a released vesicle that might express both PS and surface proteins related to its parental cell on its outer membrane. This process is displayed in Figure 1.

Less is known about how MPs are acutely cleared from the circulation. Firstly, clearance must exceed production, meaning that the activation/apoptotic stimulus must either be removed or decreased to a large enough extent to allow the return of cell quiescence, thus reducing MP formation. Beyond reduced production, proposed mechanisms for the removal of MPs from the circulation include direct binding of phagocytes to either PS or opsonisation proteins (e.g. complement) on the MP surface (7), IgM-mediated phagocytosis by macrophages (8), and destruction by circulating phospholipases (9). Particle size may influence the method employed to clear MPs (8), however this requires further investigation. Conversely, MPs may also adhere to the endothelium or form thrombi due to their reported expression of adhesion molecules (e.g. P-Selectin) (10) or Tissue Factor (TF) (11), respectively, meaning they are not removed from the circulation but are not detectable using standard techniques, creating the illusion of their absence. Further investigation is necessary to elucidate how MPs are acutely removed from the circulation.

Sources

MPs can be derived from many different cellular sources as shown in Table 1, including leukocytes, platelets, erythrocytes and endothelial cells (12). The stimuli for the release of MPs from these cells differ depending on the cell, as various conditions will initiate activation and/or apoptosis of each cell type. Whilst MPs are present in healthy populations (13), the primary aim of this review is to explain the pathophysiological role of MPs in clinical populations and the potential impact of exercise.

Methods of Detection

There are several different laboratory techniques that are regularly used for the identification of circulating MPs in the literature. Broadly, these include: flow cytometry; transmission electron microscopy; nanoparticle tracking analysis, and resistive pulse sensing. Whilst flow cytometry generally has a higher minimum detectable threshold than other techniques and can be time and labour intensive, it provides the most information with regards to MP size, complexity and cellular surface marker expression, and therefore remains the 'gold standard' technique most applicable to clinical research (3,14). Flow cytometry also provides high throughput whilst remaining relatively cheap, making it desirable when compared to other techniques (15). More novel techniques for detection of MPs include Raman microspectroscopy, micro nuclear magnetic resonance, and small-angle X-ray scattering. However, whilst these techniques may offer new insights in MP research, they are very specialised and not yet commercially available (16). Lastly, it must also be noted that sample collection and preparation techniques, including needle gauge and anticoagulant used for sample collection, tourniquet use, centrifugation protocol, freezing and thawing protocol, and buffer filtration may influence the detection of total (17,18) and phenotype-specific MPs (19) and thus should be considered when interpreting results. The lack of uniformity in MP collection and analysis protocols used in the literature makes the results difficult to interpret.

Microparticles in Disease

Elevated MP levels have been found in a variety of disease states (20), leading to the investigation into their use as prognostic markers to both comment on the current pathophysiological condition and predict future outcomes. There now exists a steadily growing body of literature that suggests that MPs can also display biological effector functions, i.e. they are able to influence other cells or systems (21), primarily in a pathophysiological manner.

Biomarker functions

As MPs are released upon cell stress, they are elevated in a variety of disease states and might be used as biomarkers of disease severity. MPs are elevated in a number of chronic systemic inflammatory conditions (22) including rheumatoid arthritis (23) and systemic lupus erythematosus (24), cardiovascular diseases (25) including stroke and acute coronary syndrome patients (26,27), various forms of cancer including colon, prostate, breast, ovarian and gastric cancer (28,29), HIV (30), and various forms of renal disease including pre-dialysis chronic kidney disease, patients receiving varying dialysis modalities and renal transplant recipients (31,32). Many other conditions have been associated with increased MP levels – their rather unspecific nature of release (i.e. upon an activation or stress stimuli) means that a wide variety of stimuli can elicit MP shedding from a large number of cell types. For this reason, elevations in total or phenotype-specific MP counts may not be unique for each disease (33). Therefore, it may be more pertinent to 'profile' trends in the changes of many MP surface markers in different disease states to identify a panel of a combination of markers, the changes of which can be much more sensitive to disease severity or risk than the measurement of one MP phenotype or marker alone (34). This profiling method has been successful in strengthening the use of MPs as biomarkers in conditions such as various liver diseases (35), malaria (36) and atherosclerosis (37). However, this approach is not always successful in delineating different diseases, for instance in various forms of cancer (34). In this case, combining the identification of surface makers with measures of micro RNA content can increase the biomarker sensitivity of MPs (38,39) and improve diagnostic power.

Bioeffector functions

More recently MPs have also been considered as biologically active with effector functions rather than simply biomarkers of disease (21). The majority of this research has occurred either *in vitro* or *ex vivo*, owing to the difficulty of isolating the effects of MPs in an *in vivo* setting and the ethical issues involving MP infusion in human participants due to their potential pathophysiological impact.

Several studies have used *in vivo* study designs to investigate MP infusion in animals, for instance to explore the mechanism behind MP-associated coagulation (40) and thrombus formation (41) in mice, however the primary purpose of this review is to comment on the current state of the literature concerning MPs in diseased human populations.

Endothelial MPs (EMPs) can induce endothelial activation and dysfunction (42) by reducing endothelium-dependent vasodilation in response to acetylcholine (43,44) and decreasing the release of the vasodilation-inducing nitric oxide (NO) (44,45) when incubated *in vitro* with rat aortic rings. This can reduce the ability of the vasculature to respond to fluctuations in haemodynamic pressure, inducing cardiac stress and left ventricular hypertrophy (46), and increasing cardiovascular mortality (47). Similarly, angiotensin II, which promotes vasoconstriction via activation of the renin angiotensin system and thus increases cardiovascular risk (48) can increase EMP release when incubated *in vitro* with murine endothelial cells (49), indicating endothelial damage. Increased circulating count of MPs of all cellular derivations has been positively associated with the circulating concentration of several reactive oxygen species (ROS), including plasma glutathione peroxidase and superoxide (50,51). When EMPs are incubated *in vitro* with human umbilical vein endothelial cells (HUVECs), the detrimental changes seen in angiogenesis (e.g. a reduction in total capillary length) were alleviated the presence of superoxide dismutase (52), implicating ROS production as a potential mechanism by which MPs can impair vascular function.

EMPs released from HUVECs in response to the pro-inflammatory cytokine TNF-α have a high calcium content and can induce osteogenesis and calcification when incubated with vascular smooth muscle cells (53). Similarly, platelet MPs (PMPs) incubated with rat aortic rings can promote angiogenesis via increased vascular endothelial growth factor (VEGF) activity (54) whilst EMPs incubated with HUVECs can increase PI3K activity, which plays a critical role in angiogenesis (55). Whilst angiogenesis is important for maintaining vascular health and homeostasis (56), excessive or dysregulated angiogenesis has been implicated in many conditions, including cancer (via loss of

tumour growth suppression), some autoimmune disorders, atherosclerosis, pulmonary hypertension and inflammatory bowel disease, among others (57). These effects on the vasculature may increase cardiovascular risk and thus risk of mortality (58,59). Lastly, PS externalised on MPs can bind with the pro-thrombotic and pro-coagulant TF to initiate and promote thrombosis and coagulation (60–62) increasing the risk of embolism and driving atherosclerosis (63). Elevated MP counts might therefore be predictive of mortality in a variety of conditions (64–66).

Whilst the 'quantity of MPs (i.e. concentration in the circulation) is important, their 'quality' (i.e. sourced from healthy or dysfunctional parent cells, their protein and RNA contents and composition) also impacts their transfer of information and thus bioeffector functions (67). EMPs released from a healthy endothelium help to maintain a protective low-grade procoagulant activity by increasing platelet clot stability (68), whilst EMPs released from damaged endothelial cells (e.g. due to atherosclerosis) can further induce endothelial dysfunction in a 'vicious cycle' manner by promoting atherogenesis (21,25). MPs isolated from human atherosclerotic plaque contain the metalloprotease TNF-α converting enzyme which increases TNF-α shedding from HUVECs, whilst this enzyme is not found in MPs isolated from healthy human internal arteries (37). Similarly, EMPs isolated from acute myocardial infarction, when incubated with rat aortic rings, caused a significant reduction in acetylcholine-induce endothelium dependent relaxation which was not seen when EMPs isolated from non-ischaemic patients were incubated at the same concentration (43) suggesting a difference in the quality of these MPs. Similarly, MPs were found at similar levels in the circulation of cardiac surgery patients and healthy controls, however the MPs from the patient group expressed significantly more TF, and thus promoted thrombogenesis to a greater extent in an *in vivo* model (11). Furthermore, the mRNA and micro RNA composition of EMPs as well as the ability of EMPs to transfer these RNAs to their target cells differs in certain disease conditions, for instance in coronary heart disease (69). Similarly, activation status may alter the micro RNA composition of MPs (70,71).

Consequently, any intervention which reduces the level of circulating MPs and positively alters their composition in clinical populations might be a therapeutic strategy, which could ultimately reduce morbidity and mortality. However, caution must be exercised when interpreting findings from *in vitro* studies. Whilst *in vitro* studies provide useful and direct information regarding how a particular variable impacts MP kinetics, they cannot account for the plethora of other factors that may influence these parameters in an *in vivo* setting. This is particularly pertinent in patients that suffer from systemic conditions that may alter a wide array of factors that could be expected to alter MP kinetics.

It should be noted that beneficial effects of MPS are also reported in the literature. MPs deliver RNAs, growth factors and cell surface receptors to target cells and as such are necessary for cellular communication (72). MP functions that are detrimental when aberrantly regulated (e.g. accelerated thrombosis) are a necessary response to vascular injury and important in wound healing. Additionally, platelet-derived MPs can inhibit apoptosis of polymorphonuclear leukocytes, potentially mediated by the influence of TGF-β1 (73). Similarly, shedding of endothelial cell MPs prevents an accumulation of caspase 3 and thus promotes cell survival via prevention of premature endothelial cell detachment and apoptosis (74). Some leukocyte derived MPs stimulate NO production (75) and can release anti-inflammatory effectors such as Annexin 1 (76) which can prevent endothelial leukocyte adhesion and thus endothelial dysfunction (77). However, the vast majority of the research to date focusses on the deleterious effects of MPs (67) and thus they are considered to be largely pathophysiological in nature.

Signalling Mechanisms

MPs exert their bioeffector functions by implementing a variety of immunologic signalling mechanisms. MPs may bring about activated T-cell apoptosis by exposing Fas ligand (FasL – a death receptor ligand) (78) which can contribute to immune suppression and has been implicated in indirectly promoting tumour growth (79). MPs also mediate antigen presentation via the exposure of

MHC class I and II molecules (80) which they can present to dendritic cells to facilitate immune surveillance (81). Similarly, the lipid component of MPs can activate Toll Like Receptor 4 on macrophages, stimulating antigen presentation (82). Additionally, MPs promote inflammation by transferring transport receptors to target cells (83) – for instance MPs from activated leukocytes increase tyrosine phosphorylation of endothelial cells, thus inducing their activation and increasing TF and IL-6 production (84). The pro-atherosclerotic role of MPs is mediated in part by the ability of MPs to transfer Intercellular Adhesion Molecule-1 (ICAM-1) to endothelial cells, thus increasing monocyte adhesion to the endothelium and promoting atherosclerotic plaque progression (85). MPs can also alter protein structure and function by transferring genetic information to their target cells, for instance mRNA and microRNA, which subsequently alter post-transcriptional regulation (83,86). Lastly, MPs may promote virus survival and growth via transfer of chemokine receptors, For instance, MPs released from HIV-infected cells can transfer CCR5 and CXCR4 to cells lacking these receptors, therefore making them susceptible to HIV (87). The immunologic signalling mechanisms of extracellular vesicles are discussed in greater depth by van der Pol et al (83) and are summarised in Figure 2.

Exercise and Microparticles

The beneficial effects of regular, moderate intensity aerobic and resistance exercise are well documented in the general population, and include improved body composition (88), increased physical capacity (89), reduced cardiovascular disease risk (90), reduced systemic inflammation (91), enhanced immune function (92) and reduced mortality (93). Exercise may also influence MP release via haemodynamic mechanisms. Aerobic exercise elicits increased blood flow to meet the extra oxygen demands of the working muscles, which can modify haemodynamic activation of both freely circulating cells and cells adhered to the endothelium via alterations in shear stress. Shear stress is a product of blood viscosity and flow rate; therefore aerobic exercise-induced increased blood flow can increase shear stress (94), which has been implicated in MP formation and release via

modulation of cell membrane quiescence (95–97) due to mechanic and haemodynamic cellular activation. When considering platelets, increased shear stress may increase GPIb-dependent binding to endothelial Von Willebrand Factor, which can initiate PMP formation and thrombosis (96). The mechanism by which increased shear stress elicits increased MP shedding from other cell types is less clear and warrants further investigation. Additionally, both reduced physical activity and enforced physical inactivity can cause endothelial dysfunction (impaired flow-mediated dilation) accompanied by increased circulating resting EMP levels (13,98). Whilst acute aerobic exercise can also transiently increase MP formation as explained above, regular aerobic exercise training has been shown to improve endothelial function in cardiovascular disease populations (99,100) and therefore may be expected to reduce resting MP levels. Acute aerobic exercise may also increase cellular activation by transiently increasing catecholamine (e.g. norepinephrine) levels (101), thus increasing MP shedding by lowering membrane quiescence. Lastly, acute aerobic exercise can increase leukocyte apoptosis (102), potentially triggered by increases in cellular oxidative stress caused by increased reactive oxygen species production (103). As MPs are released by apoptotic cells (104), this exercise-induced apoptosis also increases MP production.

Healthy Population

There has been a great deal of recent research investigating the effects of acute and chronic exercise on MP kinetics in healthy participants undertaking aerobic exercise. However, the findings seem to be conflicting; some studies report increased post-exercise MP counts of platelet origin, particularly after strenuous exercise (105–109), which suggests a pro-thrombotic effect due to the high TF expression usually found on platelet-derived MPs (110,111). Mechanical activation of platelets and thus accelerated MP shedding is cited as the cause of this. Conversely, other studies have found no change in EMP or PMP levels following high-intensity (100% peak power output) cycling (112) or even shown a reduction in circulating EMPs following cycling of various intensities (55-100% peak power output) (113). This disparity may be caused by training status; the studies mentioned above

which found increased MPs used healthy but sedentary (i.e. exercise frequency of ≤ 1/week) participants, whilst those that found decreased MPs used trained participants (either author-defined as 'fit' (112) or trained triathletes and cyclists (113)). This hypothesis is supported by studies investigating chronic regular aerobic exercise training, which display both an attenuation in the acute exercise-induced increase in MPs (neutrophil and platelet derived) (108,109) and a reduction in resting EMP counts (97,114) following prolonged training (e.g. 3 times/week for 6 months). Therefore, in the general population, it seems that whilst acute aerobic exercise may increase circulating MP counts, regular aerobic exercise training can either attenuate or abolish this effect and reduce resting circulating MP levels. This may be due to an adaptation effect caused by the repeated exposure of the endothelium to high SS elicited by aerobic exercise, which would prevent endothelial leukocyte adhesion and endothelial cell activation and/or apoptosis. Regular exercise training also improves endothelial function and increases resting NO availability (115), which may partially mitigate the increased SS caused by increased blood flow and thus prevent MP formation as explained above (52).

Clinical Populations

There is less research concerning exercise and MPs in clinical populations, especially considering the importance of health improvements when compared to the already 'healthy population'. When compared to healthy controls, patients with vascular disease referred for stress echocardiography (incremental intravenous Dobutamine infusion until 85% of age-predicted maximum heart rate was reached) displayed a diminished post-test increase in leukocyte, granulocyte and monocyte MPs, whilst platelet, erythrocyte and endothelial MPs increased as normal (116). Similarly, a single bout of high intensity interval cycling (intervals completed at 100% peak power output) did not affect platelet or endothelial MP counts in coronary heart disease patients (112). It is unclear why patients with cardiovascular deficiencies would exhibit reduced MP release, as they would be expected to display reduced exercise-induced vasodilation due to arterial stiffening (117) and potentially

increased shear stress-mediated MP release. A possible explanation is that CVD patients display reduced cardiac contractile power due to a reduction in stroke volume mediated by left ventricular hypertrophy and a reduced ejection fraction. Therefore, the haemodynamic response to aerobic exercise may be blunted in CVD patients (118), blunting the subsequent MP response. However, following 12 weeks of either continuous or interval aerobic exercise training, coronary artery disease patients displayed no change in resting EMP levels, despite showing improvements in endothelial function as measured by flow mediated dilation (119). In the same study, baseline EMP levels were inversely correlated with increases in peak VO_2 consumption, suggesting that pre-existing elevated EMP levels (perhaps suggesting the presence of endothelial dysfunction) may prevent subsequent aerobic training adaptation. A possible explanation, similar to above, is that cardiovascular disease-associated contractile and endothelial impairments create a vascular 'dormancy' which reduces MP increases in response to exercise. This increase is seen as a normal physiological response (116), the absence of which may prevent the chronic training-associated improvements in MP levels seen in the general population. However, renal transplant recipients displayed reduced circulating EMP levels following 6 months of aerobic exercise training compared to non-exercise controls (120). Renal transplant recipients are considered at heightened risk of cardiovascular events (121) and display impaired flow mediated dilation (122) and increased prevalence of left ventricular hypertrophy (123), demonstrating that exercise can improve MP levels in a population displaying cardiovascular decrements. This was accompanied by a reduced endothelial progenitor cell concentration, which may signify either reduced vascular repair capacity or reduced vascular damage (which is more likely considering the reduced EMP levels), therefore reducing the repair stimulus and subsequent progenitor cell response.

There is a clear lack of uniformity concerning the effects of exercise on MP levels and composition in clinical populations in the current body of literature. This may be in part due to the different methods of isolation used. Flow cytometry was the most commonly used technique to measure MPs in the studies mentioned above, however the processing and isolation protocols used were not

uniform which may have impacted the MP counts. Whilst it is evident that regular exercise training is effective in reducing cardiovascular risk in both healthy and diseased populations, the disparity seen in the MP literature may suggest that one size does not fit all. For instance, it is unclear why acute aerobic exercise, particularly of a very high intensity, would elicit an increase in circulating MP counts in sedentary healthy individuals (105,106) but not in the cardiovascular disease population (112). It is possible that MP release is increased following exercise in cardiovascular disease patients but they are not measurable in the circulation, for instance because they have formed clots or adhered to endothelial cells, thereby promoting their pathophysiological influence. As previously mentioned, little is known about the clearance of MPs from the circulation (124) and therefore this may require further investigation. Similarly, patients from different disease populations display differing MP responses to comparable exercise interventions. More research is necessary to investigate the effects of different exercise regimens on MP kinetics in various patient populations in order to tailor rehabilitation programmes more effectively to patients depending on their comorbid conditions. For instance, cardiac rehabilitation programmes in coronary heart disease patients typically consist of regular moderate intensity aerobic exercise (125) however this type of training does not seem to influence MP levels in this population (119). Similarly, the lack of changes in MP kinetics seen after high intensity training requires further investigation. As the primary cause of MP formation during exercise is suggested to be cellular activation caused by haemodynamic activation, it is unclear why this type of training would not elicit increased MP release in certain populations (112,119). Lastly, resistance training is an effective training modality for reducing cardiovascular risk in diseased populations (126) however the impact on MP kinetics is under-researched. Research investigating the role of resistance training in modulating MP levels and reducing cardiovascular risk will allow more well-rounded exercise programmes to be designed for specific patient populations.

Interestingly, MPs may also play a role in the adaption to exercise training. MPs and exosomes released during aerobic exercise have been proposed to contain proteins and nucleic acids (for instance heat shock protein 70) (127) that are hypothesised to mediate organ crosstalk and promote

systemic adaption to aerobic exercise (128). As such, it is been hypothesised that small extracellular vesicles released from the muscle during aerobic exercise may mediate many of the systemic adaptations to endurance exercise that prevent or lessen the severity of health conditions such as obesity and Type 2 Diabetes Mellitus (129). However, this concept requires further investigation.

Diet and Body Composition

Diet, body composition and gender also influence MP levels, and these relationships may be modulated by exercise. Inactive, obese males displayed reduced circulating EMP levels following moderate-to-high intensity cycling compared to a non-exercise control trial completed in a randomised counterbalanced manner, whilst overweight females displayed increased EMP levels compared to their non-exercise control trial (130). The cause of this gender disparity is unknown; the authors suggest a possible modulating effect of oestrogen with regards to cardiovascular disease risk. Indeed, in healthy individuals, increased EMP and PMP counts have been observed in females, the levels of which may be altered by the menstrual cycle stage (i.e. luteal versus follicular phase) and the associated fluctuations in oestrogen and progesterone (131). Additionally, other cardiovascular risk factors such as central obesity, elevated total cholesterol and reduced high-density lipoprotein cholesterol may be more prevalent in females (132). MP kinetics may therefore be another mechanism by which cardiovascular risk differs based on gender. Furthermore, females display elevated endothelial progenitor cell counts compared to males (133), suggesting stimulated repair mechanisms in response to greater activation or damage, which would explain the increased EMP levels. Regardless of gender, excessive adipose tissue has been associated with elevated circulating PMP levels compared to age-matched non-obese controls (134). This elevation was partially reduced by a 12 week calorie-restricted diet (1200 kcal/day for women, 1700 kcal/day for men) which reduced BMI by roughly 10%, and was reduced to a greater degree by a 12 week programme of calorie restriction and regular aerobic exercise (3 times/week, 60 minutes, 12-14 RPE) which reduced BMI by roughly 12%. Diet coupled with exercise was also more efficacious in reducing

fat tissue mass, visceral and subcutaneous fat area, and total and LDL cholesterol, offering other possible explanations for the reduced PMP count beyond simply reducing BMI. The increased MP levels seen in obesity may be partially caused by the MP response to a high fat and/or carbohydrate diet. Postprandial hypertriglyceridaemia and hyperglycaemia caused by high dietary fat and carbohydrate intake can induce vascular dysfunction (i.e. impaired vasodilatation), possibly mediated by increased oxidative stress and NO inactivation (135), or increased adhesion molecule (VCAM-1, ICAM-1) expression, increasing leukocyte infiltration (136). As such, EMP and total MP are elevated in response to high fat and carbohydrate meals (137,138), offering a possible explanation for the increased coagulation and thrombotic activity of the TF pathway seen during hypertriglyceridaemia and hyperglycaemia (139). However, this response may be ameliorated by exercise. Moderate intensity cycling (60-75% VO_2 to elicit an energy expenditure of 4-6 kcal/kg) completed 1 hour before ingestion of a high-fat meal blunted the postprandial increase in EMP levels that was seen in the non-exercise control trial (140). However, 100 mins of cycling at 70% VO_2 peak completed the previous evening did not affect the increase in EMPs seen in response to a high fat meal ingested the following morning (141). This suggests a more direct effect on MPs rather than indirect via lipid alterations, as moderate exercise completed the day before the consumption of a high fat meal can attenuate postprandial lipaemia (142). MPs would also be affected if they were dependent on blood lipid levels.

It is unclear whether or not the increase in MP counts often seen after consumption of a high fat meal represents a clinically significant effect that could elicit pathophysiological consequences. In some disease states, such as diabetes or coronary artery disease, the disparity between the MP counts of the disease population and the healthy population is comparable with the magnitude of the increase seen between pre- and post-prandial conditions (141,143). Whilst this suggests the potential to exert pathophysiological effects, the transient nature of the post-prandial MP increase may prevent the development of any clinically significant health decrements. Further research is

required to investigate the impact of regular high fat meal consumption on MP kinetics and the possible downstream pathophysiological consequences.

Clinical Implications and Further Research

In summary, elevated MP levels and altered composition are seen in a number of disease states, having a number of pathophysiological effector functions. Exercise may help to reduce MP levels and thus diminish their pathophysiological potential but more research is needed to elucidate these effects, particularly in clinical populations that display elevated cardiovascular risk. Studies investigating chronic exercise training in clinical populations are needed to investigate MP levels and composition, and how they relate to measures of systemic inflammation, thrombotic potential, vascular damage and various cardiovascular risk factors. Additionally, the effects of resistance exercise with regards to MPs are under-researched, as resistance exercise can be a powerful therapeutic tool for reducing morbidity and maintaining physical function in clinical populations (144). Increases in blood pressure and associated reductions in arterial compliance (145) caused by skeletal muscle contraction during resistance exercise could create an environment that promotes MP shedding, an interesting topic for future research. The evidence to date is encouraging, and suggests that, whilst acute exercise can increase circulating MP counts, regular exercise training can diminish this effect and eventually reduce overall resting MP counts, partially preventing their pathophysiological effects. This effect has been demonstrated within as little as 5 weeks of regular aerobic exercise training (109). However, given the widespread systemic effects of exercise and the numerous pathways eliciting MP release from various cell types, more research must be done to better understand how exercise affects the number and bioeffector function of microparticles.

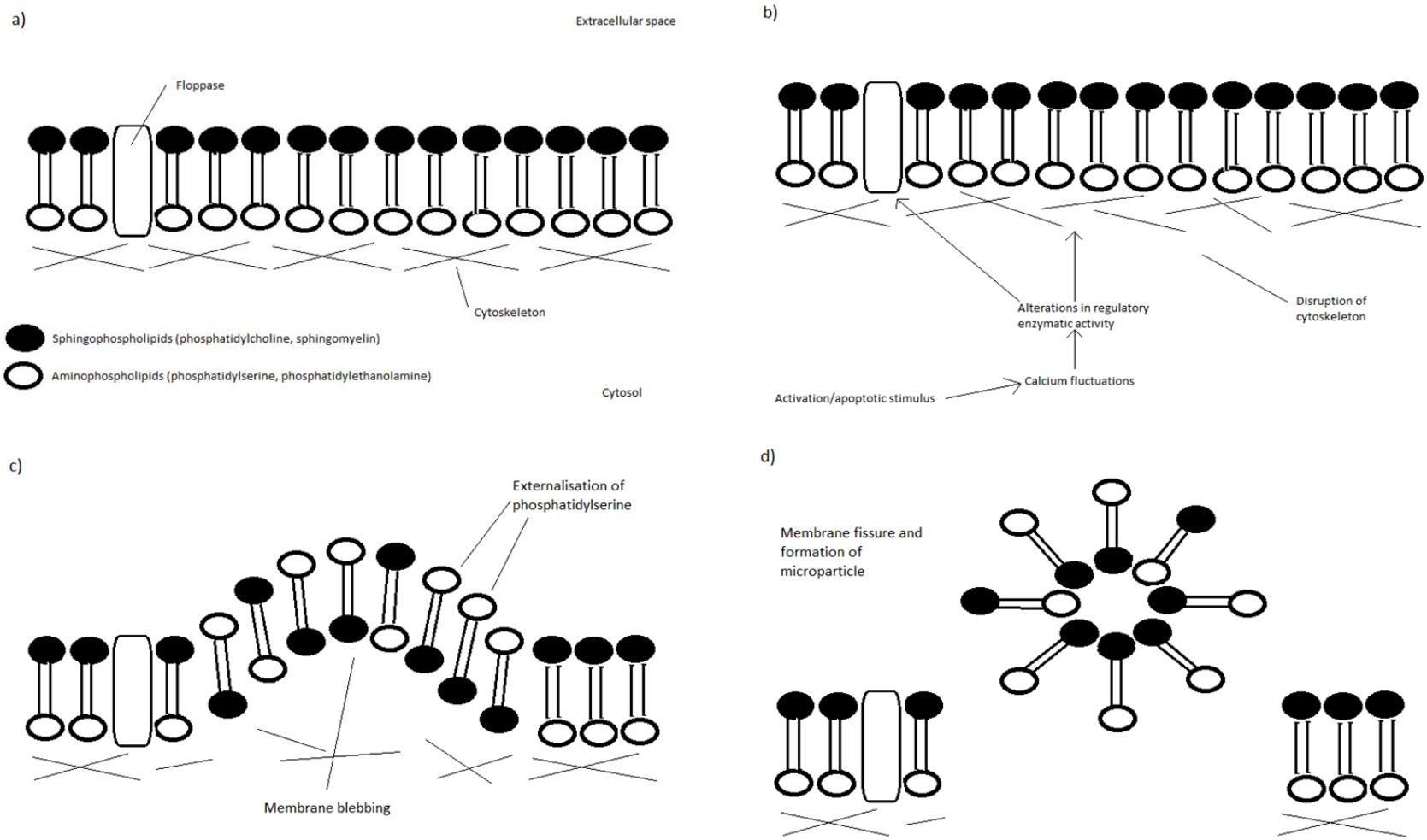

Figure 1. The steps involved in the process of MP formation. a) Demonstrating a healthy membrane, with phospholipid asymmetry and the presence of the regulatory enzyme floppase (interchangeable in this diagram with the other regulatory enzymes mentioned). b) Activation or apoptotic stimuli causes fluctuations of cytosolic calcium, altering the activity of the regulatory enzymes and causing cytoskeletal disruption. c) Membrane blebbing, and loss of phospholipid asymmetry resulting in externalisation of phosphatidylserine. d) Fissure of the membrane, resulting in the formation of an MP which is now a distinct vesicle from its original membrane. This MP will express surface antigens representative of its parent cell, which can be assessed to identify the origin of the MP. The MP size and number of phospholipids present in the membrane in Figure d) is not truly representative; the purpose of this diagram is to illustrate the formation process. In reality, the MP is of far greater size relative to the phospholipids, which are also present in far greater abundance in the MP membrane.

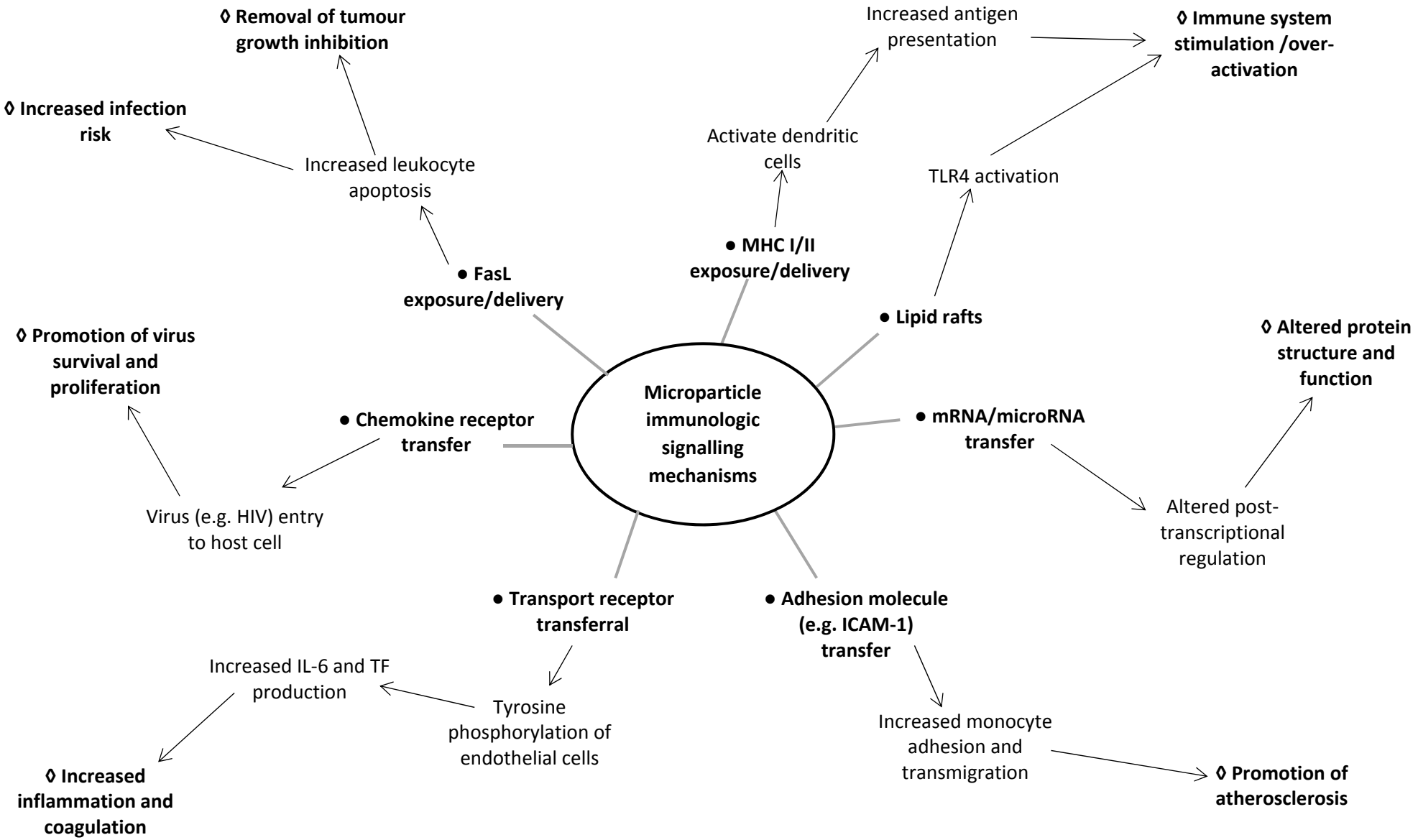

Figure 2. Examples of the immunologic signalling mechanisms MPs utilise to facilitate their bioeffector functions. Included are the typical pathways that these mechanisms initiate, and the typical end result. ● = signalling mechanism, ◊ = typical physiological consequence.

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