Title: Exosomes: nanoparticles involved in cardioprotection?

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Exosomes: nanoparticles involved in cardioprotection?

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Abstract

Exosomes are nano-sized lipid vesicles released from cells. They are capable of transferring proteins, mRNA and miRNA between cells, and therefore represent a potential means of intercellular communication. Exosomes can be pro-angiogenic and may have cardioprotective properties. In contrast, their larger cousins, microvesicles, appear to have generally detrimental effects, being pro-thrombotic and pro-inflammatory. Exosomes are released from multivesicular bodies via an exocytic pathway, and have the potential for cell-specific targeting. This normal process is "hijacked" during various pathological conditions such as cancer, viral infection, and amyloidopathies. We assess the evidence for a role of exosomes and microvesicles in normal cardiovascular physiology, as well as during cardiovascular disease. In addition to offering a potential source of cardiovascular biomarkers, exosomes may offer a non-immunogenic means of manipulating the heart.

Keywords: exosomes, microvesicles, cardiovascular disease, heart, cardioprotection

Abbreviations used: CHD, coronary heart disease; CVD, Cardiovascular disease; EPC, endothelial progenitor cells; MVB multivesicular bodies; MSC, mesenchymal stem cells; Shh, sonic hedgehog.

Introduction

Cardiovascular disease (CVD) is the number one cause of death globally. Figures from The World Health Organization show an estimated 17.3 million deaths from CVD in 2008 alone with a predicted rise to almost 25 million deaths per annum by 2030. Although mortality from CVD has declined in the USA over the past 10 years, there is still an average of 2,150 Americans who die from CVD each day [1]. Myocardial infarction remains a major cause of mortality and morbidity. The restoration of blood and oxygen to the ischemic myocardium under threat of infarction is of paramount importance, but reperfusion paradoxically exacerbates the cellular damage incurred during the severe ischemic insult [2]. Although modern acute coronary care has significantly improved survival rates after a heart attack, the sequelae of ischemia and reperfusion injury frequently include hypertrophy and heart failure. In response, the heart may undergo various metabolic and structural adaptations including the growth of new vessels (angiogenesis). Factors that can influence or interrupt either the initial damage or the pathological response are being actively sought after, as novel means of treatment. Ideally, such treatments should be safe, effective, specific, and for ease of delivery should be non- or minimally invasive.

Exosomes are extracellular, lipid bilayer vesicles that range from 30 to 100nm in diameter [3, 4]. They arise within endosomal compartments called multivesicular bodies (MVBs), which bud internally to form intraluminal vesicles. Upon fusion with the plasma membrane, MVBs release their contents into the extracellular fluid at which point the vesicles are referred to as exosomes (**Figure 1**). Secreted exosomes have been isolated from numerous cell lines as well as most bodily fluids including saliva, urine and plasma [4-7]. Although originally ignored as cell "debris", it is increasingly evident that exosome release is regulated and occurs via an energy-dependent pathway. Exosomes are believed to ferry proteins, mRNA and miRNA cargos through the bloodstream and other body fluids, shielding them from enzymatic degradation – a process which some retroviruses may "hi-jack" in order to travel beneath the immune system's "radar" [8]. With the recent discovery that exosomes can deliver their cargos to recipient cells, it has become apparent that they therefore represent a potential mode of intercellular communication throughout the entire circulatory system [3, 4]. This has caused immense interest in the potential of both exosomes and microvesicles to act as therapeutic agents or as biomarkers of diverse pathological states including Alzheimer's disease, viral infection, cancer, and, increasingly, cardiovascular disease [3, 4, 9]. Exosomes appear to be present in extraordinary numbers (~10^{10} / ml) in the plasma of healthy individuals [5], suggesting a role beyond pathology. As high as this figure is, it is probably not unreasonable, considering that the platelets represent a major source of plasma exosomes, and a normal blood platelet count is already on the order of 10^8 / ml. Despite this seemingly astronomical number, exosomes are so minute that the volume contained within 10^{10} spherical exosomes of 50 nm diameter would be only ~5 nl or 0.0005% of 1 ml. When prepared for transmission electron micrography, exosomes exhibit a specific - biconcave or "cup-shape" - morphology (**Figure 2**), although it is important to recognize that this is likely to be an artifact of drying during preparation, and that exosomes spheroid in solution.

Exosomes differ from the larger microvesicles (sometimes called microparticles) that are also found in plasma, in more than just their size. Microvesicles are believed to form by budding off - or "shedding" - directly from the plasma membrane (**Figure 1**), and appear to have properties distinct from those of exosomes [3, 9, 10]. In the cardiovascular system microvesicles have predominantly detrimental effects, including pro-thrombotic, pro-inflammatory, and endothelial-disrupting actions

[11, 12]. Numerous studies have found a strong association between elevated numbers of circulating microvesicles and cardiovascular disease [12, 13]. For example, an increase in pro-coagulant microparticles has been measured in the blood of patients with acute coronary syndromes [14], and an increase in circulating endothelial microparticles has been shown to be an independent risk factor for future cardiovascular events[15]. A recent FACS analysis of blood from 78 patients with ST segment elevation myocardial infarction (STEMI) undergoing primary percutaneous coronary intervention (PPCI) found that microvascular obstruction correlated with intracoronary levels of microparticles originating from both endothelial cells and platelets [16]. Microvesicles do have some practical advantages over exosomes in that they are more straightforward to isolate, are easily quantified using flow cytometry, and contain significantly more protein per vesicle (due to their size). This makes microvesicles, an appealing "pond" in which to fish for novel biomarkers. In contrast, exosomes are below the detection limit of flow cytometry, and require specialized equipment such as nanoparticle tracking or light scattering for their quantification. However, exosomes represent a totally distinct population of vesicles from that of microvesicles, and may provide unique information on cellular status – healthy or otherwise. Also, although research is at an early stage, they also possess characteristics making them preferable to microvesicles as novel means of delivering therapeutics, as will be described.

On the optimal method of exosome purification

A "hot topic" in the exosome field remains the optimal method of exosome purification, and secondly, their characterization (for detailed reviews see [4, 17, 18]). Consequently, this topic bears brief discussion here. There are essentially four main approaches to purification of exosomes, which are based on immune-affinity capture, size filtration, size exclusion, or ultracentrifugation. While immune-affinity is regarded as having the advantage of specificity when an appropriate epitope is available, yields are often quite low [18]. In contrast, filtration through a series of filters down to 100 nm pore size followed by centrifugation to concentrate, while yielding quite high protein content, risks impurity due to the fragmentation of larger microparticles into smaller vesicles under filtration pressure [17]. This may explain why the product of platelets from septic patients purified in such a manner were found to worsen cardiac function in isolated muscles [19]. The use of ultrafiltration has been less-well explored, but methods such as cross-flow filtration are an efficient way to concentrate exosomes away from smaller protein contaminants whilst avoiding the hazards of passage through small apertures at high pressure [20]. The most generally accepted method is to use a well-defined series of serial centrifugation steps which remove cells and microvesicles, followed by concentration by ultracentrifugation and subsequent density gradient purification [18]. It is worth noting that the optimal method for microvesicle purification is no more well-established, though efforts to standardize method of collection, centrifugation and transport are being made [21].

Exosome content

Given the variety of methods used to isolate exosomes, characterization of the exosome population used is essential prior to their analysis. Proteomic analysis of exosomes has identified common characteristic marker proteins on their surface and in their lumen [4]. Typical exosomal marker proteins include the tetraspanins CD9, CD63, and CD81, and heat-shock proteins such as HSP70 and HSP90 [4]. In addition, exosomes typically contain cytoplasmic proteins such as actin, annexins, and glycolytic enzymes such as glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and enolase [4]. They also contain molecules involved in MVB biogenesis such as Alix, TSG101 and Rab proteins [4], which

have shown to be involved in exosome release. It should be appreciated that these exosomes could transport many, as yet unidentified, proteins and in view of the cardioprotective effects that have been observed (described below), it could be hypothesized that some would be prosurvival. Understanding of the mechanism of exosome biogenesis and release is evolving rapidly. Just a few years ago, evidence favored the release of exosomes by a mechanism independent of the ESCRT (endosomal sorting complex required for transport) machinery, but requiring the sphingolipid ceramide [22]. With the recent description of a role for syndecan, syntenin, Alix and ESCRTs in the control exosome formation [23], the balance of evidence is now swinging towards support of an ESCRT-regulated mechanism of membrane budding of exosomes [23, 24].

A scale diagram using representations of crystallographic structures of some major exosomal molecules, puts into perspective just how restricted the space is within exosomes (**Figure 2**). However, *en masse*, exosomes might deliver a significant quantity of proteins to effect changes in recipient cells. Indeed, experiments have shown that exosomes can transfer signaling ligands such as those of the Notch family from tumors to endothelial cells in quantities sufficient to alter their morphology [25].

Some of the proteins mentioned above are already known to influence cardioprotection. Indeed, the relationship of heat-shock protein and cardio-protection was well established in the 1990's [26]. Many heat shock proteins (including αB-crystallin, HSP60 and HSP70) are secreted in exosomes [27, 28], and in some instances can be transferred to adjacent cells to confer protection against oxidative stress [29]. Interestingly, circulating HSP70 levels are negatively correlated with symptoms of cardiovascular disease [30], suggesting exosomal HSP70 may be beneficial. HSP60 is also secreted from cardiomyocytes in exosomes [31], although the implications of this are unclear, and circulating HSP60 levels have been associated with autoimmune disease [30]. Other secreted proteins implicated in CVD or myocardial ischemia and reperfusion have been identified in exosomes. For example, the inflammatory cytokine TNFα, which can induce contractile dysfunction, hypertrophy, fibrosis and cell death, has been detected in exosomes released from hypoxic cardiomyocytes [32]. Interestingly, the TNF receptor has also been identified in plasma exosomes [33]. Other molecules important in the cardiovascular system identified in exosomes include PPARγ [34], PTEN [35], annexins[4], DPPIV[4], EGFR [36] and a host of metabolic enzymes [4]. The p22 and gp91 subunits of phagocyte-like NADPH oxidase as well as NAD(P)H oxidase activity has been detected in exosomes derived from platelets of septic individuals, although the significance of this is not known [37]. A recent high-profile publication demonstrated an evolutionarily conserved role for exosomes in the secretion of Wnt proteins, and in this case the presentation of Wnt on exosomal surfaces was shown to contribute to biological Wnt signaling [38]. The "Exocarta" database [39] catalogues the proteins that have been identified in exosomes and also the number of studies in which they have been identified [4], as an important means of assessing the robustness of this localization. However, it is important to keep in mind that the majority of studies thus far have been performed on exosomes released from malignant cells, which may have an abnormal composition.

Plasma exosomes, like microvesicles, originate primarily from platelets and megakaryocytes, and to a lesser degree endothelial cells, erythrocytes, and leukocytes [10, 12, 13]. The exosomal proteome differs according to the type of cell that released it [4], such that platelet or endothelial exosomes can be identified by their expression of typical cellular markers such as CD31 (PECAM-1) or CD62P (P-

selectin) respectively [10]. Furthermore, studies indicate that cellular stress can alter exosomal protein and RNA content [40], suggesting that exosomes represents a snapshot of the physiological state of the cell - a kind of "status update", released by cells into the circulation.

In some respects, it is easier to envisage how transfer of even minute quantities of miRNA might have more extensive effects relative to the transfer of protein. Hence, the discovery that mRNA and miRNA are also localized within exosomes has generated much interest, although the extent to which plasma miRNA is contained within exosomes is still controversial. Some reports suggest that exosomes contain the majority of plasma miRNA[41], while others suggest that plasma miRNAs are mainly found in complexes with carrier proteins such as argonaute [42] or high density lipoproteins [43]. In any event, numerous studies have demonstrated the ability of exosomes (and microvesicles) to transfer miRNA to recipient cells [44, 45]. In a seminal paper in the field, exosomes from mast cells were shown conclusively to contain both miRNA and mRNA, and to be able to transfer mRNA to recipient cells for translation into new proteins [44]. Importantly, the RNA was shown to be contained within the exosomes, since it was protected from treatment with RNAse or trypsin [44]. The miRNA or mRNA content of exosomes may also reflect the cell they originate from. The miRNA content of dendritic cell exosomes varies with cell maturation [46]. Similarly, exosomes from mast cells exposed to oxidative stress contain different mRNAs to those from control cells [47]. Furthermore, exosomes from stressed cells conferred resistance against oxidative stress to recipient cells [47]. Another fascinating report has detailed the use of purified exosomes as an *in vivo* "transfection reagent" for the delivery into the brains of mice of miRNA, which had been loaded into the exosomes by electroporation [48].

Exosomal miRNA content may be relevant to CVD. Patients with acute myocardial infarction have increased serum levels of miR-1 and miR-133a, while *in vitro* experiments suggest that exosomes from cardiac cells can release miR-133a and transfer it to recipient cells where it modulates gene expression [49]. Curiously, miR-133a normally suppresses hypertrophy by restraining expression of the inositol 1,4,5'-triphosphate receptor II (IP(3)RII) calcium channel. Cellular levels of miR-133a have been found to decrease during the hypertrophic response to pressure overload [50]. Clearly much work remains to be done to clarify to role of exosomes and miRNA in CVD.

In addition to proteins and RNA, exosomes appear to be enriched in sphingomyelins, though levels of cholesterol and phosphatidyl choline appear to depend on cellular source [51]. They can contain bioactive lipids such as phospholipases, arachidonic acid and derivatives such as prostaglandins [52], which may exert effects related to inflammation.

Exosomes and cardioprotection

The early promise of stem cells in cardiac regeneration generated much excitement for their potential to improve function by differentiating into new cardiomyocytes. Despite some waning of this initial optimism, improvement of cardiac function and survival is consistently observed after injection of stem cells, apparently due to their release of paracrine factors. An intriguing possibility which is emerging is that some of these paracrine effects may be mediated by exosomes. For example, exosomes purified from the conditioned medium of $CD34^+$ stem cells are pro-angiogenic both *in vitro* and *in vivo* [53]. Similarly, recent evidence suggests that human embryonic stem cell-derived MSC, rather than differentiating into cardiomyocytes when injected into recipient hearts, actually mediate their cardioprotective properties by the paracrine release of exosomes [54]. The

highly purified, protective component from MSC-conditioned medium was found to contain vesicles of the same density, size, and appearance by electron microscopy as exosomes, and they expressed exosomal marker proteins (CD9, CD81, Alix)[54]. The exosomes were highly cardioprotective when introduced either into Langendorff perfused isolated rat hearts or intravenously into anaesthetized mice immediately before reperfusion. The mechanism by which they confer cardioprotection appears to involve activation of the same cardioprotective kinase pathways as preconditioning [54-57]. Furthermore, the cross-species cardioprotection that was observed points to an evolutionarily conserved pathway.

Exosomes have also been isolated from *in vitro*-cultured, murine cardiac progenitor cells (CPC) and recent data suggests that they are equally able to protect the myocardium from IR injury after intra myocardial delivery [58]. On a practical note, it is important that in experiments such as these, cells are cultured in serum that has been pre-treated (by ultracentrifugation for example) to deplete it of exosomes. Secondly, it is essential to compare cardioprotection against vehicle containing any carrier retained during purification, since in our experience, vehicle such as BSA or polyethylene glycol can cause an apparent decrease in infarct size at high concentration. The question now being pursued in these studies is the identity of the cardioprotective ligands.

The group of Camussi have undertaken extensive work demonstrating that microvesicles released by mesenchymal stem cells (MSC) or endothelial progenitor cells (EPC) can prevent acute kidney injury when injected intravenously following kidney ischemia [59, 60]. Interestingly, the isolation protocol used is similar to the standard ultracentrifugation protocol for exosome purification, and may result in a mixture of microvesicles and exosomes. These results highlight the importance of careful characterization and definition of the population of vesicles after isolation. Microvesicles isolated from ischemic mouse hind limbs have also been found to promote angiogenesis when injected into naïve ischemic limbs [61]. In contrast, microvesicles purified from plasma after a preconditioning protocol were recently shown *not* to affect cardiac ischemia and reperfusion injury when injected intravenously into rats immediately before reperfusion [62]. Recently it has been suggested that plasma exosomes are cardioprotective in a Langendorff perfused heart system [63]. Given the many beneficial aspects of exosomes outlined here, their induction after hypoxia [40, 64], and their ability to transmit cardioprotective signals, an attractive hypothesis is that exosomes contribute to the humoral transmission of cardioprotective state induced by cardioprotective modalities such as remote ischemic preconditioning. If our hypothesis is validated, this would in turn suggest they may harbor novel cardioprotective molecules.

The mechanism by which exosomes exert cardioprotection is almost entirely unknown. It appears to involve a direct interaction with cells in the heart, rather than blood components, because cardioprotection has been observed both *in vitro* and *in vivo*. At least in specific cases, exosomes have been demonstrated to be capable of direct transfer of RNA [44-46] (**Figure 3B**) or protein (**Figure 3A**)[65]. For example, the Notch ligand, Delta-like 4 (Dll4), can be transferred between endothelial cells via exosomes [25]. However, the observation that cardioprotection is rapidly induced after exosome perfusion, suggests a more immediate mechanism. Remarkably, exosomes/microvesicles derived from cancer cells are capable of transferring activated EGF receptors directly from malignant cells to endothelial cells [36] (**Figure 3C**), which then stimulates angiogenesis via autocrine production of VEGF. Alternatively, proteins on the surface of exosomes may interact directly with plasma-membrane

receptors in the myocardium and activate their downstream intracellular signaling pathways (**Figure 3D**). The majority of the hundreds of G-protein coupled receptors studied to date activate intracellular signaling pathways which converge on PI-3kinase/Akt and ERK/MAPK, or JAK/STAT - the so-called "RISK pathway" or "SAFE pathway" of cardioprotection respectively. Interestingly, the paracrine cardioprotective effect of MSC cells requires PI-3kinase/Akt [66], supporting this hypothesis.

Exosomes and cardiovascular disease

Though the potential therapeutic application of exosomes in CVD is only just beginning to be explored, results thus far are exciting. Ischemic heart disease develops as a result of coronary atherosclerotic plaque formation leading to a reduced coronary blood flow. Over a period of time coronary collateral vessels and microvascular angiogenesis develop as a response to myocardial ischemia. It is thought that angiogenesis helps preserve the functionality of ischemic myocardium. Therapeutic coronary angiogenesis and collateralization have tremendous potential as treatment strategies for patients with ischemic heart disease. There is increasing evidence of exosomes having an important role in angiogenesis. Human $CD34^+$ stem cell-derived exosomes induce angiogenic activity *in vitro* and *in vivo* [53]. Intravenous delivery of exosomes from MSC cells suppressed lung inflammation, inhibited vascular remodeling and inhibited the development right ventricular hypertrophy in a mouse model of hypoxic pulmonary hypertension [67]. Exosomes from dendritic cells can modulate immune responses, and injection of exosomes derived from donor bone marrow dendritic cells was found to modulate response to heart transplantation [68].

In addition to being a major risk factor for CVD, vascular complications are a major contributor to the morbidity of diabetes mellitus. The potential of exosome to shuttle miRs and proteins to the ischemic limb in peripheral vascular disease has barely been examined, but a recent study detected a significant increase in miR-15a and miR-16 levels in the serum of patients with critical limb ischemia. In addition to being conjugated to argonaute-2, the miRNAs were found to be within exosomes [69], suggesting their potential as markers of critical limb ischemia. Since miR-15a appears to inhibit angiogenesis [70], it will be interesting to determine whether exosomes actually contribute to the ischemic injury in this case. In other situations, the injection of pro angiogenic exosomes can be beneficial. For example, the previously mentioned vesicles isolated by the group of Camussi [60], have been shown to induce neovascularization in a murine model of hind limb ischemia [71], stimulating angiogenesis by means of miRNA or mRNA transfer [72]. Protection was lost after RNAse treatment or depletion of pro-angiogenic miR-126 and miR-296 [60, 71]. Human cardiomyocyte progenitor cells have also been shown to release exosomes which can stimulate the migration of microvascular endothelial cells [73]. In an interesting recent study, human umbilical vein endothelial cells subjected to shear stress were shown to release vesicles (exosomes and/or microvesicles) enriched in miR-143/145 – which controlled target gene expression in co-cultured smooth muscle cells [45]. Importantly, the released vesicles reduced atherosclerotic lesion formation in a mouse model of atherosclerosis [45].

Many of the studies performed so far utilize vesicles purified from cells cultured *in vitro*, which may have a different lipid composition, protein content or other characteristics from those released *in vivo*. Thus while they demonstrate a potentially useful therapeutic effect, they don't address the fundamental question of the *in vivo* relevance of the native endogenous vesicles. This is difficult to determine without effective and specific tools to prevent microvesicle or exosome release *in vivo*.

There are some exciting leads in this area, with the Rab proteins being implicated in exosome release [74], however the exact contingent of Rab proteins involved are suspected to be cell-type specific, and requires much more investigation. Similarly, the existence of a mechanism controlling the cell-specific delivery of exosomes is not well established. A recent report suggests that selectivity of uptake is regulated by specific interactions between tetraspanins and integrins and can be controlled by altering the expression of different tetraspanins proteins [75]. This raises the possibility that the capacity for "targeting" of exosomes can be harnessed for delivery to specific cells or organs but again, much work remains to be done in this area. This and other basic aspects of exosome biology will be necessary to understand before their clinical application can be seriously considered.

The future potential of exosomes in cardiovascular research

Two obvious avenues for the potential exploitation of exosomes in the cardiovascular field are in their use as biomarkers, and in the potential for harnessing their capacity for delivery of biologics (**Figure 4**). A major advantage of using secreted vesicles such as exosomes for the proteomic identification of plasma biomarkers is that simply by purifying them, many possible cellular proteins of interest are separated from the morass of highly abundant serum proteins [4]. This greatly simplifies subsequent proteomic analysis [4]. Although research is still at a very early stage, the view is emerging that exosomes represent a "snapshot" of the (primarily cytosolic and plasma membrane) cellular proteome of their cell of origin [4]. In terms of biomarker screening, a simple approach may be to analyze exosomes secreted in the urine, which may reflect the protein or miRNA changes that occur with CVD. This possibility has been demonstrated by the finding that there is a dramatic increase in the levels of exosomal miR-1 in the urine of rats and humans after acute myocardial infarction [76].

A second avenue for exploitation might lie in the development of reagents for the delivery of biologics to cells using exosomes – which are essentially "natural liposomes". The capacity for exosomes to be experimentally manipulated for the delivery of specific proteins has been demonstrated by engineering $CD34^{+}$ stem cells to release exosomes containing the pro-angiogenic factor, sonic hedgehog (Shh). Injection of the modified $CD34^{Shh}$ cells into the border zone of mice after myocardial infarction reduced infarct size, increased capillary density, and improved long-term functional recovery [65]. Functional transfer of Shh protein was demonstrated to occur *in vitro*[65], but may prove difficult to confirm *in vivo*. Similarly, exosomes produced by dendritic cells have been isolated and loaded with specific siRNA, then used to deliver knock down *BACE1*, a therapeutic target in Alzheimer's disease, in the brains of mice [48]. Targeting in this case was achieved by expressing a neuron-specific peptide.

Exosomes have a number of characteristics that appear to make them preferable to microvesicles for the purpose of therapeutics, including lower immunogenicity [3]. On the other hand , there is evidence that some exosomes express the pro coagulant protein, tissue factor (TF) and may be pro coagulant, e.g.: exosomes purified from mesenchymal-like cancer cells [77], hypoxic glioblastoma cells [78], and bronchial epithelial cells, particularly after pressure-stress [79]. An earlier study described a pro coagulant activity in exosomes from mast cells [80], however since these vesicles were isolated without an intermediate centrifugation step to remove microvesicles, it is likely that pro coagulant microvesicles contributed to the activity observed [80]. In contrast to these examples, exosomes from various sources including tumors, immune cells, and body fluids have been found to be

immunosuppressive [3]. These contrasting data emphasize once again the importance of carefully defining the vesicle population being examined, and, of the peril of generalizing in a field where many of the basic characteristics are still being elucidated and defined.

Finally, a further avenue for exploitation might be in developing the means to stimulate the body's own production and transport of exosomes that have been programmed for survival, and which may therefore be of direct benefit to patients with CVD.

Conclusion

Interest in exosomes is exploding, with more papers on exosomes published since 2010 than in all previous years combined, and yet, much about their basic biology remains to be determined. By analogy with astronomy, exosomes represent a kind of "dark matter" of the body – invisible to direct microscopy, but whose existence can be inferred by the effects they have on other cells. The question now is just how pervasive is their influence? Certainly, they have been strongly implicated in cancer, and in certain, specific situations such as retroviral budding and transmission, but do these exosomes represent the "dark side" of a normal physiological process? If exosomes, present in plasma at such extraordinary concentrations, are able to transfer protein and RNA to recipient cells in significant quantities, they might represent a new paradigm of intercellular signal transmission. As such, exosome biology could represent a new frontier, at the nanoscale, which will advance our search for novel approaches to cardiovascular signaling and cardioprotection. Though the basic aspects of exosome biology are only just beginning to be explored, it seems certain that in the near future they will be generating a level of interest disproportionate to their size.

Figure legends

Figure 1. Microvesicles are released via plasma membrane shedding, in contrast to the directed release of exosomes from multivesicular bodies (MVB) that fuse with the plasma membrane. Exosomes form by invagination of the MVB membrane. Like microvesicles, they engulf cytosolic contents, and therefore might be thought of as "status updates" released from the cell.

Figure 2. (Left) A diagram of a 50 nm exosome with lipid bilayer with 3D structures of typical associated molecules: CD81 (the extracellular domain only is shown) [81] ; hsp70 (currently the most commonly identified exosomal protein in the Exocarta database of exosomal proteomic studies)[4] ; and siRNA. All elements are drawn to scale, to illustrate the compactness of the exosome. (Right) Electron micrograph of exosomes from rat plasma (SD unpublished data).

Figure 3. Some of the various ways in which exosomes may affect recipient cells. (A) Protein ligands in the exosome's membrane may activate receptors and downstream signaling pathways in recipient

cells. (B) Exosomes may contain activated receptors which are transferred. Exosomes contain protein (C), or miRNA and/or mRNA (D), which may be transferred to recipient cells.

Figure 4. Potential uses for exosomes in the cardiovascular field. Exosomes represent potential sources of diagnostic biomarkers. Alternatively, exosomes may potentially be harvested from plasma (autologous) or cell culture (exogenous) and exploited for their signaling capacity or for the delivery of biologics.

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Disclosures
None

References

1. Go AS, Mozaffarian D, Roger VL, et al. Heart disease and stroke statistics--2013 update: A report from the american heart association. *Circulation*. 2013;127:e6-e245
2. Yellon DM, Hausenloy DJ. Myocardial reperfusion injury. *N Engl J Med*. 2007;357:1121-1135
3. Thery C, Ostrowski M, Segura E. Membrane vesicles as conveyors of immune responses. *Nat Rev Immunol*. 2009;9:581-593
4. Mathivanan S, Ji H, Simpson RJ. Exosomes: Extracellular organelles important in intercellular communication. *J Proteomics*. 2010;73:1907-1920
5. Dragovic RA, Gardiner C, Brooks AS, Tannetta DS, Ferguson DJ, Hole P, Carr B, Redman CW, Harris AL, Dobson PJ, Harrison P, Sargent IL. Sizing and phenotyping of cellular vesicles using nanoparticle tracking analysis. *Nanomedicine*. 2011;7:780-788
6. Caby MP, Lankar D, Vincendeau-Scherrer C, Raposo G, Bonnerot C. Exosomal-like vesicles are present in human blood plasma. *Int Immunol*. 2005;17:879-887
7. Lasser C, Alikhani VS, Ekstrom K, Eldh M, Paredes PT, Bossios A, Sjostrand M, Gabrielsson S, Lotvall J, Valadi H. Human saliva, plasma and breast milk exosomes contain rna: Uptake by macrophages. *J Transl Med*. 2011;9:9
8. Gould SJ, Booth AM, Hildreth JE. The trojan exosome hypothesis. *Proc Natl Acad Sci U S A*. 2003;100:10592-10597
9. Fevrier B, Raposo G. Exosomes: Endosomal-derived vesicles shipping extracellular messages. *Curr Opin Cell Biol*. 2004;16:415-421
10. Heijnen HF, Schiel AE, Fijnheer R, Geuze HJ, Sixma JJ. Activated platelets release two types of membrane vesicles: Microvesicles by surface shedding and exosomes derived from exocytosis of multivesicular bodies and alpha-granules. *Blood*. 1999;94:3791-3799
11. Amabile N, Rautou PE, Tedgui A, Boulanger CM. Microparticles: Key protagonists in cardiovascular disorders. *Semin Thromb Hemost*. 2010;36:907-916
12. Dignat-George F, Boulanger CM. The many faces of endothelial microparticles. *Arterioscler Thromb Vasc Biol*. 2011;31:27-33
13. Viera AJ, Mooberry M, Key NS. Microparticles in cardiovascular disease pathophysiology and outcomes. *J Am Soc Hypertens*. 2012;6:243-252
14. Mallat Z, Benamer H, Hugel B, Benessiano J, Steg PG, Freyssinet JM, Tedgui A. Elevated levels of shed membrane microparticles with procoagulant potential in the peripheral circulating blood of patients with acute coronary syndromes. *Circulation*. 2000;101:841-843
15. Nozaki T, Sugiyama S, Koga H, Sugamura K, Ohba K, Matsuzawa Y, Sumida H, Matsui K, Jinnouchi H, Ogawa H. 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*. 2009;54:601-608
16. Porto I, Biasucci LM, De Maria GL, Leone AM, Niccoli G, Burzotta F, Trani C, Tritarelli A, Vergallo R, Liuzzo G, Crea F. Intracoronary microparticles and microvascular obstruction in patients with st elevation myocardial infarction undergoing primary percutaneous intervention. *Eur Heart J*. 2012;33:2928-2938
17. Gyorgy B, Szabo TG, Pasztoi M, Pal Z, Misjak P, Aradi B, Laszlo V, Pallinger E, Pap E, Kittel A, Nagy G, Falus A, Buzas EI. Membrane vesicles, current state-of-the-art: Emerging role of extracellular vesicles. *Cell Mol Life Sci*. 2011;68:2667-2688
18. Thery C, Amigorena S, Raposo G, Clayton A. Isolation and characterization of exosomes from cell culture supernatants and biological fluids. *Curr Protoc Cell Biol*. 2006;Chapter 3:Unit 3 22
19. Azevedo LC, Janiszewski M, Pontieri V, Pedro Mde A, Bassi E, Tucci PJ, Laurindo FR. Platelet-derived exosomes from septic shock patients induce myocardial dysfunction. *Crit Care*. 2007;11:R120
20. Lamparski HG, Metha-Damani A, Yao JY, Patel S, Hsu DH, Ruegg C, Le Pecq JB. Production and characterization of clinical grade exosomes derived from dendritic cells. *J Immunol Methods*. 2002;270:211-226

21. Lacroix R, Judicone C, Poncelet P, Robert S, Arnaud L, Sampol J, Dignat-George F. Impact of pre-analytical parameters on the measurement of circulating microparticles: Towards standardization of protocol. *J Thromb Haemost*. 2012;10:437-446
22. Trajkovic K, Hsu C, Chiantia S, Rajendran L, Wenzel D, Wieland F, Schwille P, Brugger B, Simons M. Ceramide triggers budding of exosome vesicles into multivesicular endosomes. *Science*. 2008;319:1244-1247
23. Baietti MF, Zhang Z, Mortier E, Melchior A, Degeest G, Geeraerts A, Ivarsson Y, Depoortere F, Coomans C, Vermeiren E, Zimmermann P, David G. Syndecan-syntenin-alix regulates the biogenesis of exosomes. *Nat Cell Biol*. 2012;14:677-685
24. Wehman AM, Poggioli C, Schweinsberg P, Grant BD, Nance J. The p4-atpase tat-5 inhibits the budding of extracellular vesicles in c. Elegans embryos. *Curr Biol*. 2011;21:1951-1959
25. Sheldon H, Heikamp E, Turley H, Dragovic R, Thomas P, Oon CE, Leek R, Edelmann M, Kessler B, Sainson RC, Sargent I, Li JL, Harris AL. New mechanism for notch signaling to endothelium at a distance by delta-like 4 incorporation into exosomes. *Blood*. 2010;116:2385-2394
26. Heads RJ, Latchman DS, Yellon DM. The molecular basis of adaptation to ischemia in the heart: The role of stress proteins and anti-oxidants in the ischemic and reperfused heart. *EXS*. 1996;76:383-407
27. Lancaster GI, Febbraio MA. Exosome-dependent trafficking of hsp70: A novel secretory pathway for cellular stress proteins. *J Biol Chem*. 2005;280:23349-23355
28. Gastpar R, Gehrmann M, Bausero MA, Asea A, Gross C, Schroeder JA, Multhoff G. Heat shock protein 70 surface-positive tumor exosomes stimulate migratory and cytolytic activity of natural killer cells. *Cancer Res*. 2005;65:5238-5247
29. Sreekumar PG, Kannan R, Kitamura M, Spee C, Barron E, Ryan SJ, Hinton DR. Alphab crystallin is apically secreted within exosomes by polarized human retinal pigment epithelium and provides neuroprotection to adjacent cells. *PLoS ONE*. 2010;5:e12578
30. Henderson B. Integrating the cell stress response: A new view of molecular chaperones as immunological and physiological homeostatic regulators. *Cell Biochem Funct*. 2010;28:1-14
31. Gupta S, Knowlton AA. Hsp60 trafficking in adult cardiac myocytes: Role of the exosomal pathway. *Am J Physiol Heart Circ Physiol*. 2007;292:H3052-3056
32. Yu X, Deng L, Wang D, Li N, Chen X, Cheng X, Yuan J, Gao X, Liao M, Wang M, Liao Y. Mechanism of tnf-alpha autocrine effects in hypoxic cardiomyocytes: Initiated by hypoxia inducible factor 1alpha, presented by exosomes. *J Mol Cell Cardiol*. 2012;53:848-857
33. Zhang J, Hawari FI, Shamburek RD, Adamik B, Kaler M, Islam A, Liao DW, Rouhani FN, Ingham M, Levine SJ. Circulating tnfr1 exosome-like vesicles partition with the ldl fraction of human plasma. *Biochem Biophys Res Commun*. 2008;366:579-584
34. Looze C, Yui D, Leung L, Ingham M, Kaler M, Yao X, Wu WW, Shen RF, Daniels MP, Levine SJ. Proteomic profiling of human plasma exosomes identifies ppargamma as an exosome-associated protein. *Biochem Biophys Res Commun*. 2009;378:433-438
35. Putz U, Howitt J, Doan A, Goh CP, Low LH, Silke J, Tan SS. The tumor suppressor pten is exported in exosomes and has phosphatase activity in recipient cells. *Sci Signal*. 2012;5:ra70
36. Al-Nedawi K, Meehan B, Kerbel RS, Allison AC, Rak J. Endothelial expression of autocrine vegf upon the uptake of tumor-derived microvesicles containing oncogenic egfr. *Proc Natl Acad Sci U S A*. 2009;106:3794-3799
37. Janiszewski M, Do Carmo AO, Pedro MA, Silva E, Knobel E, Laurindo FR. Platelet-derived exosomes of septic individuals possess proapoptotic nad(p)h oxidase activity: A novel vascular redox pathway. *Crit Care Med*. 2004;32:818-825
38. Gross JC, Chaudhary V, Bartscherer K, Boutros M. Active wnt proteins are secreted on exosomes. *Nat Cell Biol*. 2012;14:1036-1045
39. Simpson RJ, Mathivanan S. Exocarta. http://www.exocarta.org. Accessed March 13, 2013

40. de Jong OG, Verhaar MC, Chen Y, Vader P, Gremmels H, Posthuma G, Schiffelers RM, Gucek M, van Balkom BWM. Cellular stress conditions are reflected in the protein and rna content of endothelial cell-derived exosomes. *Journal of Extracellular Vesicles*. 2012;1
41. Gallo A, Tandon M, Alevizos I, Illei GG. The majority of microrna s detectable in serum and saliva is concentrated in exosomes. *PLoS ONE*. 2012;7:e30679
42. Turchinovich A, Weiz L, Langheinz A, Burwinkel B. Characterization of extracellular circulating microrna. *Nucleic Acids Res*. 2011;39:7223-7233
43. Vickers KC, Palmisano BT, Shoucri BM, Shamburek RD, Remaley AT. Microrna s are transported in plasma and delivered to recipient cells by high-density lipoproteins. *Nat Cell Biol*. 2011;13:423-433
44. Valadi H, Ekstrom K, Bossios A, Sjostrand M, Lee JJ, Lotvall JO. Exosome-mediated transfer of mrnas and microrna s is a novel mechanism of genetic exchange between cells. *Nat Cell Biol*. 2007;9:654-659
45. Hergenreider E, Heydt S, Treguer K, Boettger T, Horrevoets AJ, Zeiher AM, Scheffer MP, Frangakis AS, Yin X, Mayr M, Braun T, Urbich C, Boon RA, Dimmeler S. Atheroprotective communication between endothelial cells and smooth muscle cells through mirnas. *Nat Cell Biol*. 2012;14:249-256
46. Montecalvo A, Larregina AT, Shufesky WJ, et al. Mechanism of transfer of functional microrna s between mouse dendritic cells via exosomes. *Blood*. 2012;119:756-766
47. Eldh M, Ekstrom K, Valadi H, Sjostrand M, Olsson B, Jernas M, Lotvall J. Exosomes communicate protective messages during oxidative stress; possible role of exosomal shuttle rna. *PLoS ONE*. 2010;5:e15353
48. Alvarez-Erviti L, Seow Y, Yin H, Betts C, Lakhal S, Wood MJ. Delivery of sirna to the mouse brain by systemic injection of targeted exosomes. *Nat Biotechnol*. 2011;29:341-345
49. Kuwabara Y, Ono K, Horie T, Nishi H, Nagao K, Kinoshita M, Watanabe S, Baba O, Kojima Y, Shizuta S, Imai M, Tamura T, Kita T, Kimura T. Increased microrna-1 and microrna-133a levels in serum of patients with cardiovascular disease indicate myocardial damage. *Circ Cardiovasc Genet*. 2011;4:446-454
50. Drawnel FM, Wachten D, Molkentin JD, et al. Mutual antagonism between ip(3)rii and mirna-133a regulates calcium signals and cardiac hypertrophy. *J Cell Biol*. 2012;199:783-798
51. Record M, Subra C, Silvente-Poirot S, Poirot M. Exosomes as intercellular signalosomes and pharmacological effectors. *Biochem Pharmacol*. 2011;81:1171-1182
52. Subra C, Grand D, Laulagnier K, Stella A, Lambeau G, Paillasse M, De Medina P, Monsarrat B, Perret B, Silvente-Poirot S, Poirot M, Record M. Exosomes account for vesicle-mediated transcellular transport of activatable phospholipases and prostaglandins. *J Lipid Res*. 2010;51:2105-2120
53. Sahoo S, Klychko E, Thorne T, Misener S, Schultz KM, Millay M, Ito A, Liu T, Kamide C, Agrawal H, Perlman H, Qin G, Kishore R, Losordo DW. Exosomes from human cd34(+) stem cells mediate their proangiogenic paracrine activity. *Circ Res*. 2011;109:724-728
54. Lai RC, Arslan F, Lee MM, Sze NS, Choo A, Chen TS, Salto-Tellez M, Timmers L, Lee CN, El Oakley RM, Pasterkamp G, de Kleijn DP, Lim SK. Exosome secreted by msc reduces myocardial ischemia/reperfusion injury. *Stem Cell Res*. 2010;4:214-222
55. Arslan F, Lai RC, Smeets MB, Akeroyd L, Choo A, Aguor EN, Timmers L, van Rijen HV, Doevendans PA, Pasterkamp G, Lim SK, de Kleijn DP. Mesenchymal stem cell-derived exosomes increase atp levels, decrease oxidative stress and activate pi3k/akt pathway to enhance myocardial viability and prevent adverse remodeling after myocardial ischemia/reperfusion injury. *Stem Cell Res*. 2013;10:301-312
56. Davidson SM, Hausenloy D, Duchen MR, Yellon DM. Signalling via the reperfusion injury signalling kinase (risk) pathway links closure of the mitochondrial permeability transition pore to cardioprotection. *Int J Biochem Cell Biol*. 2006;38:414-419

57. Hausenloy DJ, Yellon DM. Survival kinases in ischemic preconditioning and postconditioning. *Cardiovasc.Res.* 2006;70:240-253
58. Chen L, Wang Y, Pan Y, Zhang L, Shen C, Qin G, Ashraf M, Weintraub N, Ma G, Tang Y. Cardiac progenitor-derived exosomes protect ischemic myocardium from acute ischemia/reperfusion injury. *Biochem Biophys Res Commun*. 2013
59. Gatti S, Bruno S, Deregibus MC, Sordi A, Cantaluppi V, Tetta C, Camussi G. Microvesicles derived from human adult mesenchymal stem cells protect against ischaemia-reperfusion-induced acute and chronic kidney injury. *Nephrol Dial Transplant*. 2011;26:1474-1483
60. Cantaluppi V, Gatti S, Medica D, Figliolini F, Bruno S, Deregibus MC, Sordi A, Biancone L, Tetta C, Camussi G. Microvesicles derived from endothelial progenitor cells protect the kidney from ischemia-reperfusion injury by microrna-dependent reprogramming of resident renal cells. *Kidney Int*. 2012;82:412-427
61. Leroyer AS, Ebrahimian TG, Cochain C, Recalde A, Blanc-Brude O, Mees B, Vilar J, Tedgui A, Levy BI, Chimini G, Boulanger CM, Silvestre JS. Microparticles from ischemic muscle promotes postnatal vasculogenesis. *Circulation*. 2009;119:2808-2817
62. Jeanneteau J, Hibert P, Martinez MC, Tual-Chalot S, Tamareille S, Furber A, Andriantsitohaina R, Prunier F. Microparticle release in remote ischemic conditioning mechanism. *Am J Physiol Heart Circ Physiol*. 2012;303:H871-877
63. Bell R, Beeuwkes R, Botker HE, et al. Trials, tribulations and speculation! Report from the 7th biennial hatter cardiovascular institute workshop. *Basic Res Cardiol*. 2012;107:300
64. King HW, Michael MZ, Gleadle JM. Hypoxic enhancement of exosome release by breast cancer cells. *BMC Cancer*. 2012;12:421
65. Mackie AR, Klyachko E, Thorne T, Schultz KM, Millay M, Ito A, Kamide CE, Liu T, Gupta R, Sahoo S, Misener S, Kishore R, Losordo DW. Sonic hedgehog-modified human cd34+ cells preserve cardiac function after acute myocardial infarction. *Circ Res*. 2012;111:312-321
66. Angoulvant D, Ivanes F, Ferrera R, Matthews PG, Nataf S, Ovize M. Mesenchymal stem cell conditioned media attenuates in vitro and ex vivo myocardial reperfusion injury. *J Heart Lung Transplant*. 2011;30:95-102
67. Lee C, Mitsialis SA, Aslam M, Vitali SH, Vergadi E, Konstantinou G, Sdrimas K, Fernandez-Gonzalez A, Kourembanas S. Exosomes mediate the cytoprotective action of mesenchymal stromal cells on hypoxia-induced pulmonary hypertension. *Circulation*. 2012
68. Peche H, Heslan M, Usal C, Amigorena S, Cuturi MC. Presentation of donor major histocompatibility complex antigens by bone marrow dendritic cell-derived exosomes modulates allograft rejection. *Transplantation*. 2003;76:1503-1510
69. Spinetti G, Fortunato O, Caporali A, et al. Microrna-15a and microrna-16 impair human circulating proangiogenic cell functions and are increased in the proangiogenic cells and serum of patients with critical limb ischemia. *Circ Res*. 2013;112:335-346
70. Yin KJ, Olsen K, Hamblin M, Zhang J, Schwendeman SP, Chen YE. Vascular endothelial cell-specific microrna-15a inhibits angiogenesis in hindlimb ischemia. *J Biol Chem*. 2012;287:27055-27064
71. Ranghino A, Cantaluppi V, Grange C, Vitillo L, Fop F, Biancone L, Deregibus MC, Tetta C, Segoloni GP, Camussi G. Endothelial progenitor cell-derived microvesicles improve neovascularization in a murine model of hindlimb ischemia. *Int J Immunopathol Pharmacol*. 2012;25:75-85
72. Deregibus MC, Cantaluppi V, Calogero R, Lo Iacono M, Tetta C, Biancone L, Bruno S, Bussolati B, Camussi G. Endothelial progenitor cell derived microvesicles activate an angiogenic program in endothelial cells by a horizontal transfer of mrna. *Blood*. 2007;110:2440-2448
73. Vrijsen KR, Sluijter JP, Schuchardt MW, van Balkom BW, Noort WA, Chamuleau SA, Doevendans PA. Cardiomyocyte progenitor cell-derived exosomes stimulate migration of endothelial cells. *J Cell Mol Med*. 2010;14:1064-1070

74. Ostrowski M, Carmo NB, Krumeich S, et al. Rab27a and rab27b control different steps of the exosome secretion pathway. *Nat Cell Biol*. 2010;12:19-30; sup pp 11-13
75. Rana S, Yue S, Stadel D, Zoller M. Toward tailored exosomes: The exosomal tetraspanin web contributes to target cell selection. *Int J Biochem Cell Biol*. 2012;44:1574-1584
76. Cheng Y, Wang X, Yang J, Duan X, Yao Y, Shi X, Chen Z, Fan Z, Liu X, Qin S, Tang X, Zhang C. A translational study of urine mirnas in acute myocardial infarction. *J Mol Cell Cardiol*. 2012;53:668-676
77. Garnier D, Magnus N, Lee TH, Bentley V, Meehan B, Milsom C, Montermini L, Kislinger T, Rak J. Cancer cells induced to express mesenchymal phenotype release exosome-like extracellular vesicles carrying tissue factor. *J Biol Chem*. 2012;287:43565-43572
78. Svensson KJ, Kucharzewska P, Christianson HC, Skold S, Lofstedt T, Johansson MC, Morgelin M, Bengzon J, Ruf W, Belting M. Hypoxia triggers a proangiogenic pathway involving cancer cell microvesicles and par-2-mediated heparin-binding egf signaling in endothelial cells. *Proc Natl Acad Sci U S A*. 2011;108:13147-13152
79. Park JA, Sharif AS, Tschumperlin DJ, Lau L, Limbrey R, Howarth P, Drazen JM. Tissue factor-bearing exosome secretion from human mechanically stimulated bronchial epithelial cells in vitro and in vivo. *J Allergy Clin Immunol*. 2012;130:1375-1383
80. Al-Nedawi K, Szemraj J, Cierniewski CS. Mast cell-derived exosomes activate endothelial cells to secrete plasminogen activator inhibitor type 1. *Arterioscler Thromb Vasc Biol*. 2005;25:1744-1749
81. Kitadokoro K, Bordo D, Galli G, Petracca R, Falugi F, Abrignani S, Grandi G, Bolognesi M. Cd81 extracellular domain 3d structure: Insight into the tetraspanin superfamily structural motifs. *EMBO J*. 2001;20:12-18

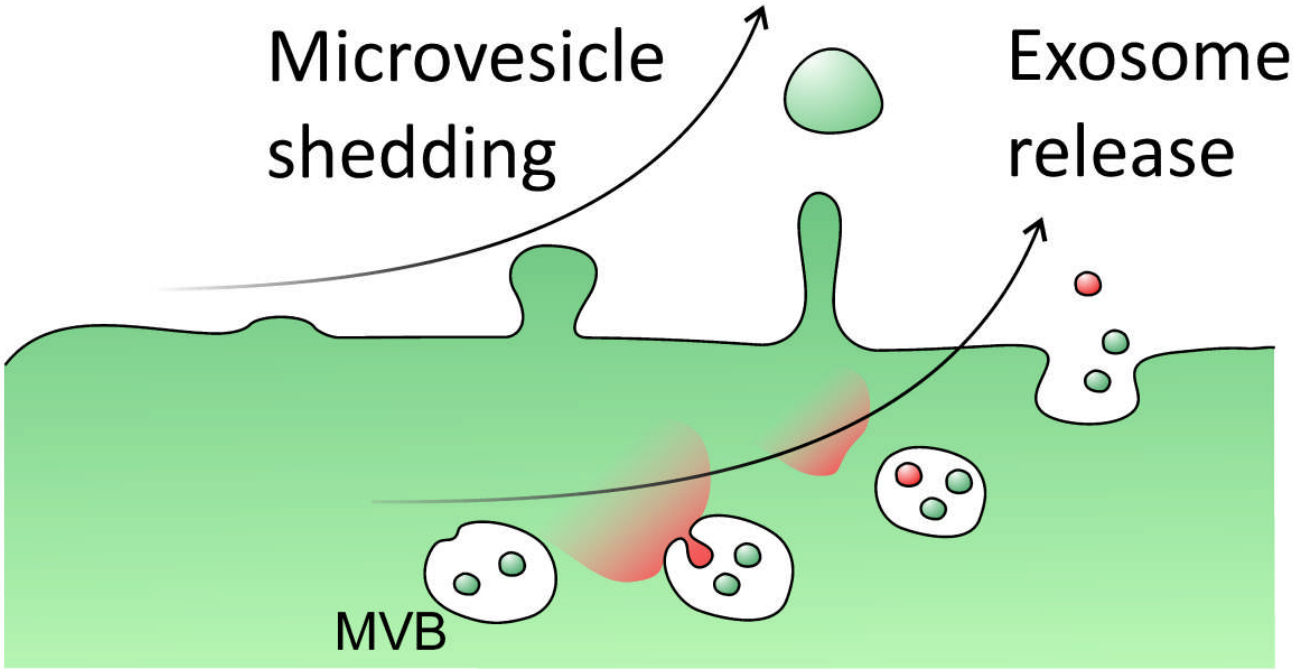

Figure 1. Microvesicles are released via plasma membrane shedding, in contrast to the directed release of exosomes from multivesicular bodies (MVB) that fuse with the plasma membrane. Exosomes form by invagination of the MVB membrane. Like microvesicles, they engulf cytosolic contents, and therefore might be thought of as "status updates" released from the cell.

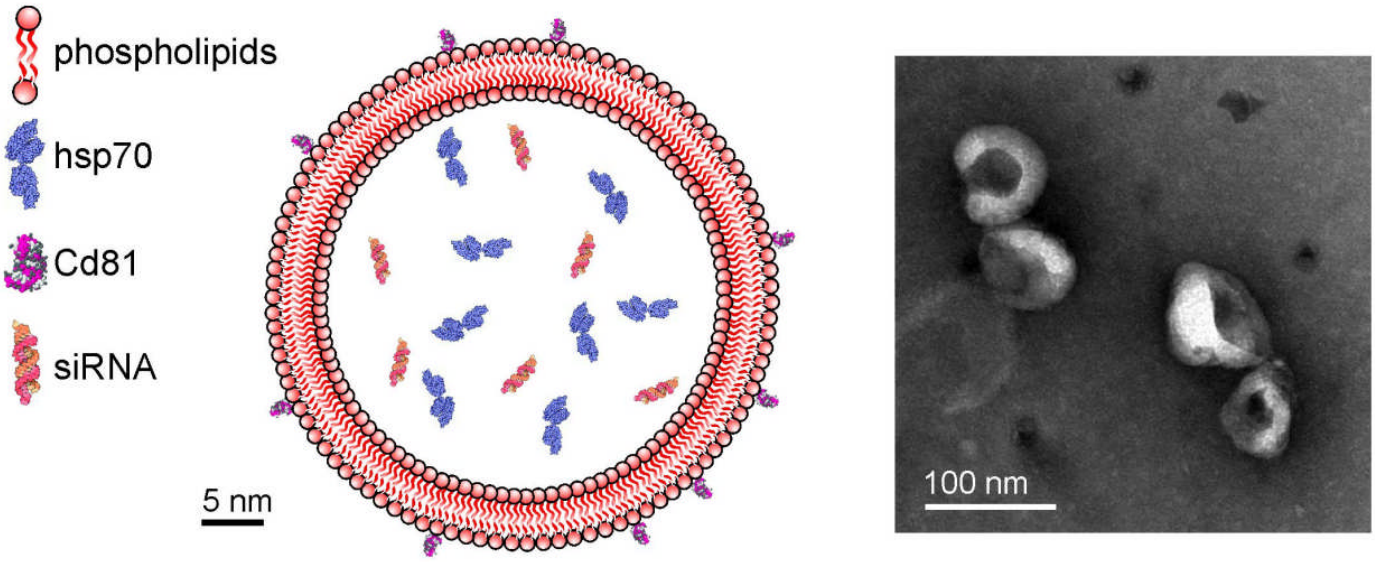

Figure 2. (Left) A diagram of a 50 nm exosome with lipid bilayer with 3D structures of typical associated molecules: CD81 (the extracellular domain only is shown) [71] ; hsp70 (currently the most commonly identified exosomal protein in the Exocarta database of exosomal proteomic studies)[4] ; and siRNA. All elements are drawn to scale, to illustrate the compactness of the exosome. (Right) Electron micrograph of exosomes from rat plasma (SD unpublished data).

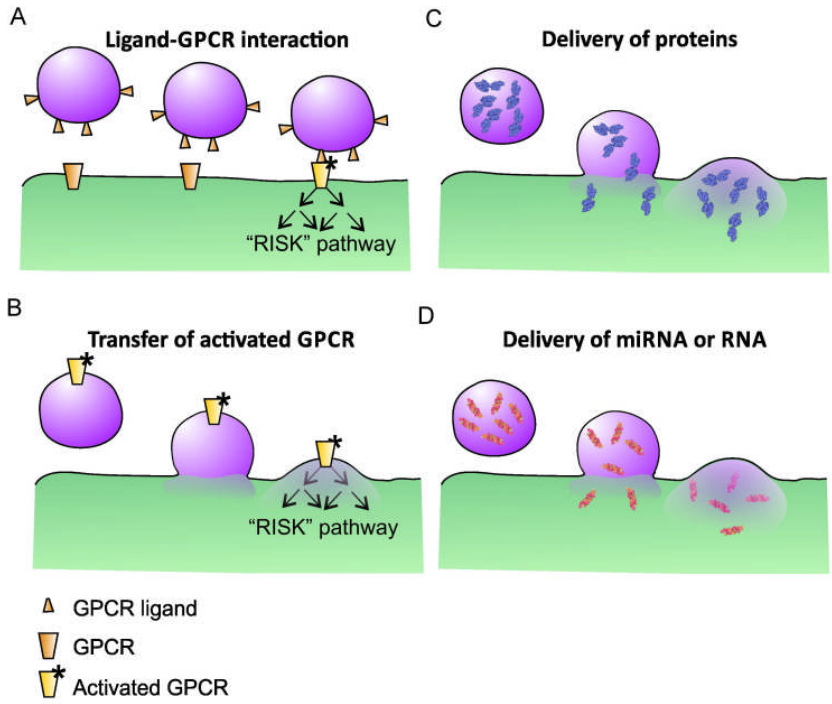

Figure 3. Some of the various ways in which exosomes may affect recipient cells. (A) Protein ligands in the exosomes membrane may activate receptors and downstream signaling pathways in recipient cells. (B) Exosomes may contain activated receptors which are transferred. Exosomes contain protein (C), or miRNA and/or mRNA (D), which may be transferred to recipient cells.

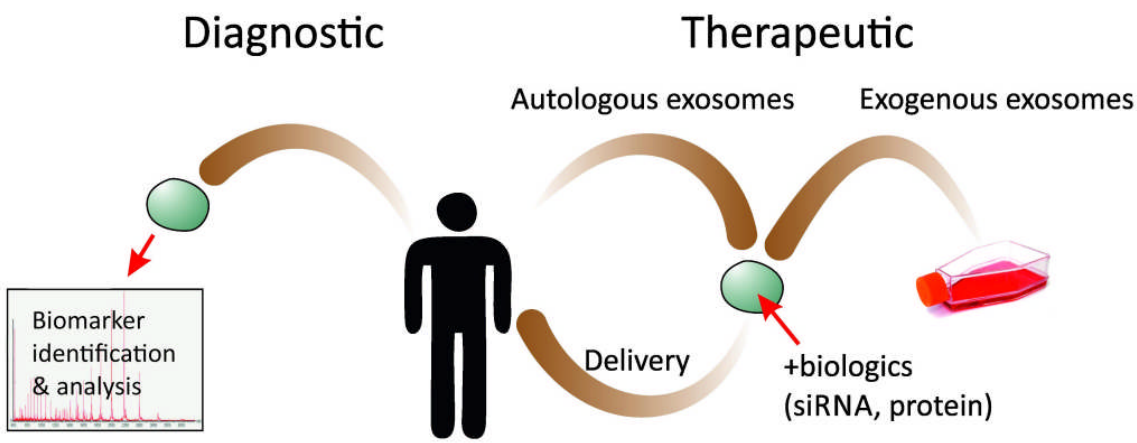

Figure 4. Potential uses for exosomes in the cardiovascular field. Exosomes represent potential sources of diagnostic biomarkers. Alternatively, exosomes may potentially be harvested from plasma (autologous) or cell culture (exogenous) and exploited for their signaling capacity or for the delivery of biologics.