Circulation Research

REVIEW

Deciphering Platelets: Are They Cells or an Evolved Form of Extracellular Vesicles?

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ABSTRACT: Platelets are abundant in blood, where they maintain the integrity of the vasculature. Megakaryocytes, the cells responsible for platelet genesis, produce membrane protrusions from which as many as 5000 anucleate platelets can be released into the bloodstream. Platelets lack genomic DNA but contain different molecules, such as RNA, as well as organelles transmitted from the parent megakaryocyte. There is no consensus in the scientific community on whether platelets are cells or not: for example, they are sometimes called cells, small cells, anucleated cells, cell fragments, or megakaryocyte fragments. Extracellular vesicles are particles delimited by a lipid bilayer that are released from cells but cannot replicate on their own. Like platelets, extracellular vesicles lack a nucleus and carry components from their donor cell. Herein, we will explore various viewpoints suggesting that platelets may be cells, albeit not conventional cells, or may be a previously unrecognized type of extracellular vesicle. Beyond a mere debate over terminology, this perspective seeks to help properly define and classify platelets, aiming for better integration into the concept of either cells or extracellular vesicles. This will foster a clearer understanding and drive advances in platelet research.

Key Words: blood platelets ■ extracellular vesicles ■ megakaryocytes ■ organelles

Platelets circulate in the blood of mammals.[1] In addition to their role in promoting hemostasis, accumulating evidence shows they also actively contribute to immunity and inflammation.[2–6] Platelets are produced by large cells called megakaryocytes through intricate mechanisms involving cell membrane protrusions, cytoskeleton rearrangement, and proplatelet formation.[7–11] Studies also suggest that megakaryocyte membrane budding may contribute to platelet genesis.[12,13] During platelet formation, a single megakaryocyte can give rise to hundreds to thousands of platelets.[14] Since platelets lack a nucleus and originate from membrane processes, in contrast to the mechanisms of mitosis or meiosis normally involved in cellular division, it is debatable if they should be considered true cells. Are platelets specialized cells that evolved to generate perfectly suited anucleated corpuscles with enhanced prohemostatic potential? Conversely, if platelets do not qualify as cells, what are they? Would platelets be a type of extracellular vesicle (EV)?

Platelets remain inadequately classified, falling ambiguously between being considered or not as cells or cell fragments. Since EVs are, by definition, derived from cells, even EVs from platelets often lack acknowledgment as significant contributors in the EV field, despite their potential importance. This lack of clear classification, along with insufficient consideration of their role as cells or EVs, contributes to significant gaps in understanding mechanisms regulating platelet biogenesis and their (patho)physiological functions. This article, authored by a balanced group of pro-cell and pro-EV advocates, introduces the basic definitions of cells and EVs and explores the historical discovery of platelets and their phylogenesis. It presents arguments for considering platelets as either cells or EVs and highlights the challenges in distinguishing platelets from EVs and identifying their respective functions according to current knowledge.

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Nonstandard Abbreviations and Acronyms	
EV	extracellular vesicle

DEFINITION OF CELLS

Cells form the basic structural and functional unit of all living organisms. They can be broadly categorized into 2 types: prokaryotic and eukaryotic. Prokaryotic cells, found in bacteria and archaea, lack a true nucleus and membrane-bound organelles. In contrast, eukaryotic cells, which make up plants, animals, fungi, protists, and most algae, are enclosed by a plasma membrane that separates their cytoplasm from the external environment and contain membrane-bound organelles, such as the nucleus that harbors the genetic material (DNA), mitochondria, chloroplasts (in plants and algae), endoplasmic reticulum, and Golgi apparatus, each performing specific functions within the cell.[15] In this article, when discussing cells, we specifically refer to eukaryotic cells.

Cells monitor, respond, and adapt to external changes, maintaining a stable intracellular environment through homeostasis. They can evolve and differentiate over time and can specialize to execute certain functions within a multicellular organism. Entities such as viruses and phages (bacteriophages) also contain genetic material (DNA or RNA), which they pass to their progeny, and can multiply and mutate; however, they are not classified as cells because they need actual cells to replicate, indicating that the presence of genetic material or the capacity to multiply are not enough to be considered a cell.

DEFINITION OF EXTRACELLULAR VESICLES

According to the Minimal Information for Studies of Extracellular Vesicles (2023), EVs are defined as particles that are released from cells, that are delimited by a lipid bilayer, and cannot replicate on their own.[16] EVs are diverse in terms of their biogenesis, biochemical composition, and size.[17] The term exosome refers to EVs that originate from intracellular compartments in multivesicular bodies, while ectosome (also known as microvesicle or microparticle) refers to EVs formed by budding and shedding from the plasma membrane.[16] However, it is recommended to avoid the use of these terms unless specific investigations have confirmed whether the origin of the EVs is from the intracellular or plasma membrane. EVs can be produced by virtually any type of cell across all living organisms and are composed of biomolecules originating from the parent cell, including proteins, coding and noncoding RNA, and organelles, but they do not contain a functional nucleus.[16,18,19] EV membrane surfaces are also decorated with biomolecules they pick up from the environment, which together form the EV molecular corona.[17,20,21] EVs are involved in homeostasis, intercellular communication, membrane repair, and molecule recycling, and they are being explored for potential therapeutic applications. The criteria discussed herein, outlining the pros and cons of classifying platelets as either cells or EVs, are summarized in Figure 1.

DISCOVERY OF PLATELETS AND THEIR PHYLOGENESIS

The discovery of blood platelets dates back to the nineteenth century, coinciding with a burgeoning interest in microscopic blood analysis.[22] In 1865, Schultze[23] observed and described small, colorless spherules forming irregular clumps in blood. In 1878, Georges Hayem observed platelets' role in hemostasis.[24] The first comprehensive description of this role dates from 1881 to 1882 by the Italian pathologist Giulio Bizzozero,[25] who identified platelets by intravital microscopy in living animals. Although Bizzozero did not explicitly call platelets cells, he did recognize them as the third blood component together with red and white blood cells.

Platelets are only present in mammals within the Kingdom Animalia, that is, in ~0.3% of all living organisms.[26] In all other animal species, hemostasis is mediated by nucleated cells called thrombocytes, which play multiple roles, including in immune defense.[27] Mammalian platelets are unique because they are small, anucleate, and rich in membrane reservoirs from their genesis. These characteristics allow platelets to circulate near the periphery of the bloodstream, close to the vessel walls, enabling them to rapidly respond to any vascular damage, to resist blood shear once adhered to the vessel wall, to quickly release their granular contents, and to aggregate.[28,29]

Indeed, mammalian platelets exhibit a more efficient hemostatic function compared with the nucleated thrombocytes of other animal orders.[26,30] The reason for the sudden evolutionary appearance of anucleate platelets, estimated to have occurred around 220 million years ago in the Triassic period, is still unknown, but it might have resulted from an inheritable random mutation in a gene regulating cell division in the hemostatic cell progenitors, leading to polyploidization and thus to the development of the megakaryocyte/platelet lineage.[31] One hypothesis is that the development of platelets conferred an evolutionary advantage by facilitating the evolution of eutherian placentation, which might not otherwise have been possible due to the high risk of bleeding associated with hemochorial implantation and placental detachment at parturition.[32–34] Whether platelets are considered cells or EVs, they clearly emerged later in the course of evolution, providing an evolutionary advantage to their carriers.

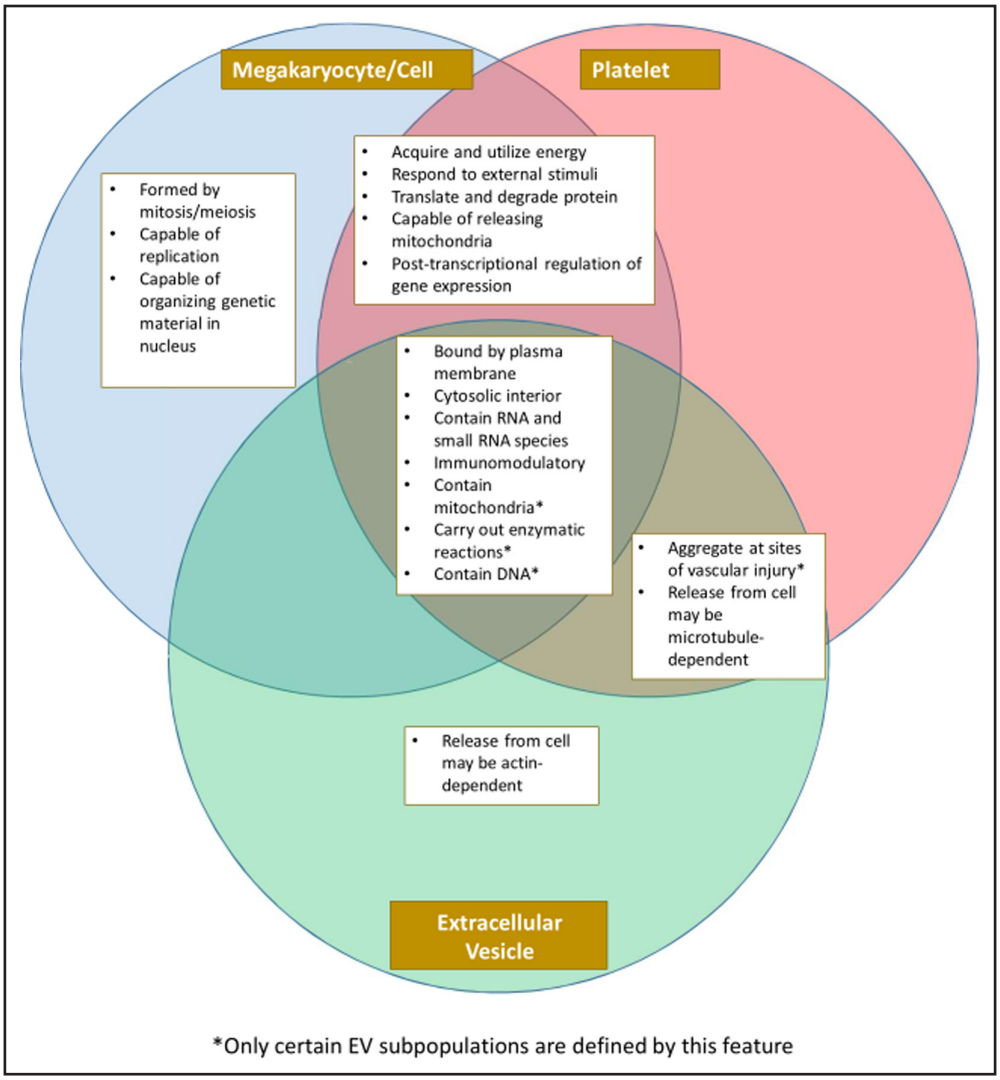

Figure 1. Representation of the criteria discussed to examine similarities between platelets and cells or extracellular vesicles. Venn diagram representation of the particularities that platelets, extracellular vesicles and megakaryocytes share or that distinguishes them. *Only certain extracellular vesicle subpopulations are defined by this feature.

PLATELET GENESIS

When a cell divides, it replicates its genome, and a copy of the genetic material is transferred to both daughter cells. Intriguingly, this process differs greatly for platelet genesis. Thrombopoiesis is the process by which megakaryocytes develop from hematopoietic stem cells along the myeloid branch of hematopoiesis. According to the classical model of hematopoiesis, each mature megakaryocyte is derived from a hematopoietic stem cell that sequentially transitions through the multipotent progenitor, common myeloid progenitor, megakaryocyte-erythroid progenitor, and megakaryocyte progenitor states.[35] After megakaryocytes are terminally differentiated, they undergo maturation, increase in size, become full of platelet-specific granules, expand their cytoplasmic content of cytoskeletal proteins, and develop a highly tortuous invaginated membrane system (demarcation membrane).[36] Megakaryocytes then assemble and release platelets by extending long proplatelet extensions into the bloodstream, a process dependent on cytoskeletal microtubules. By this mechanism, platelet number can be greatly enhanced by megakaryocytes in response to stress such as bleedings (Figure 2).

PRO-CELL ARGUMENTS

While platelets might evolutionarily derive from thrombocytes, which contain a nucleus, they break many rules according to the classical definition of a cell. Indeed, such rule-breaking may be unsurprising given that platelets are the progeny of megakaryocytes, a highly unusual cell type itself. The latter have a highly invaginated demarcation membrane system, are at least double the size of other bone marrow cells, and can have a nucleus with 64 times the genomic content of other cells.

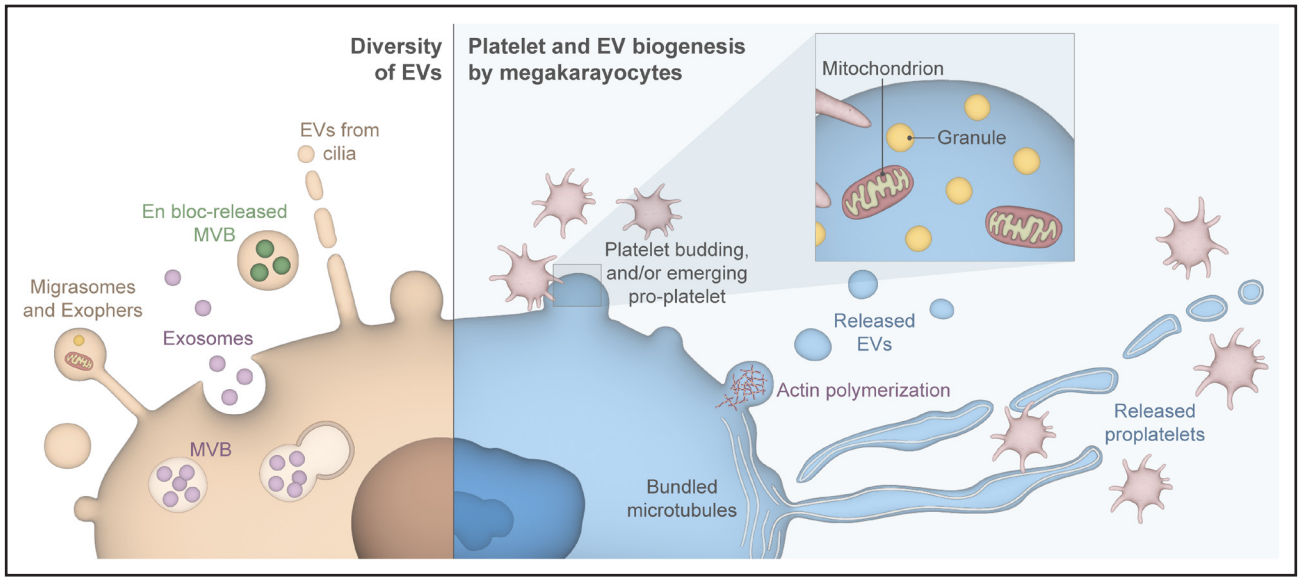

Figure 2. Heterogeneity of the extracellular vesicles (EVs).
Different types of EVs are released from a hypothetical cell (**left**). Smaller vesicles called exosomes are stored in multivesicular bodies (MVBs) and can be secreted from cells. The MVB can be released en bloc and can contain small vesicles. Large EVs, such as migrasomes and exophers, are produced by long membrane extensions and can contain organelles, such as mitochondria. Primary cilia are long membrane protrusions from which EVs can be released. Budding EVs, also known as microvesicles or ectosomes, can be released from the plasma membrane. Megakaryocytes (**right**) produce long membrane protrusions from which pro-platelets and platelets are released. The release of pro-platelets implicates microtubules, while actin polymerization was found to be implicated in EV release from megakaryocytes. Note that other types of EVs yet to identify in megakaryocytes may be released through different mechanisms, as microtubules are necessary in the transport of MVBs to the plasma membrane. Platelet budding and emerging pro-platelets contain granules and mitochondria needed for platelet functions. A nucleus is illustrated at the center of both cells (left and right).

Despite the absence of a nucleus, platelets fulfill a number of the criteria for defining a cell. They can perform an impressive diversity of cellular functions, including communicating with other cell types, respiration, and translation and degradation of proteins.[37] Furthermore, it has been shown that platelets generate progeny through a process involving membrane sprouting under certain experimental conditions.[38,39] If this process is confirmed in vivo and is to be different from EV release, it would indicate that platelets are also capable of multiplying. Moreover, they respond to environmental changes through the engagement of numerous surface receptors, which lead to different steps of platelet activation (adhesion, secretion, spreading, aggregation, and procoagulant transformation),[40] and by doing so can make their own EVs. This EV formation is tunable by the mode of activation,[41,42] and the resulting EVs are heterogeneous in size, morphology, and biogenesis route.[43–45] Platelet-derived EVs are formed during injury and inflammation, and platelet reactivity also increases under pathological conditions, such as cardiovascular diseases or cancer, and during aging. Platelets harbor a plethora of membrane receptors whose engagement depends on the environment and cellular responses, resulting in differential EV production (Figure 3).

The different biogenesis mechanisms underlying the formation of platelets and the release of EVs from megakaryocytes further suggest that platelets may differ from EVs. Platelet production involves a highly intricate complex mechanism, sorting organelles and specifically packaging certain mRNA molecules into daughter platelets.[46] While platelet production is a microtubule-driven process, EV generation by megakaryocytes is resistant to inhibition of microtubule assembly.[47] Instead, EV production is augmented by inhibition of actin polymerization, suggesting that it is driven by actin-dependent processes.[47] This is further supported by data showing that the platelet-derived EV proteome contains pathways related to cytoskeletal regulation through Rho GTPase, a major regulator of actin dynamics.[48]

The release of EVs allows platelets to communicate with cells throughout the body, including cells residing in the bone marrow, lymph, and synovial fluid.[49–51] Notably, platelets communicate with innate immune cells. Platelets interact with neutrophils, thereby enhancing their activity, and with monocytes, reprogramming their inflammatory functions.[52,53] They can also contribute to adaptive immune responses by interacting with dendritic cells, or B and T lymphocytes, via CD40L (cluster of differentiation 40-ligand) and major histocompatibility complex (MHC) class I antigen presentation.[53] Although platelets do not migrate to the same extent as the nucleated inflammatory cells, they do migrate to sites of inflammation or infection to collect and bundle bacteria[54,55] (Figure 3).

Each platelet contains 4 to 7 mitochondria, which play roles in cell metabolism, activation, and ATP

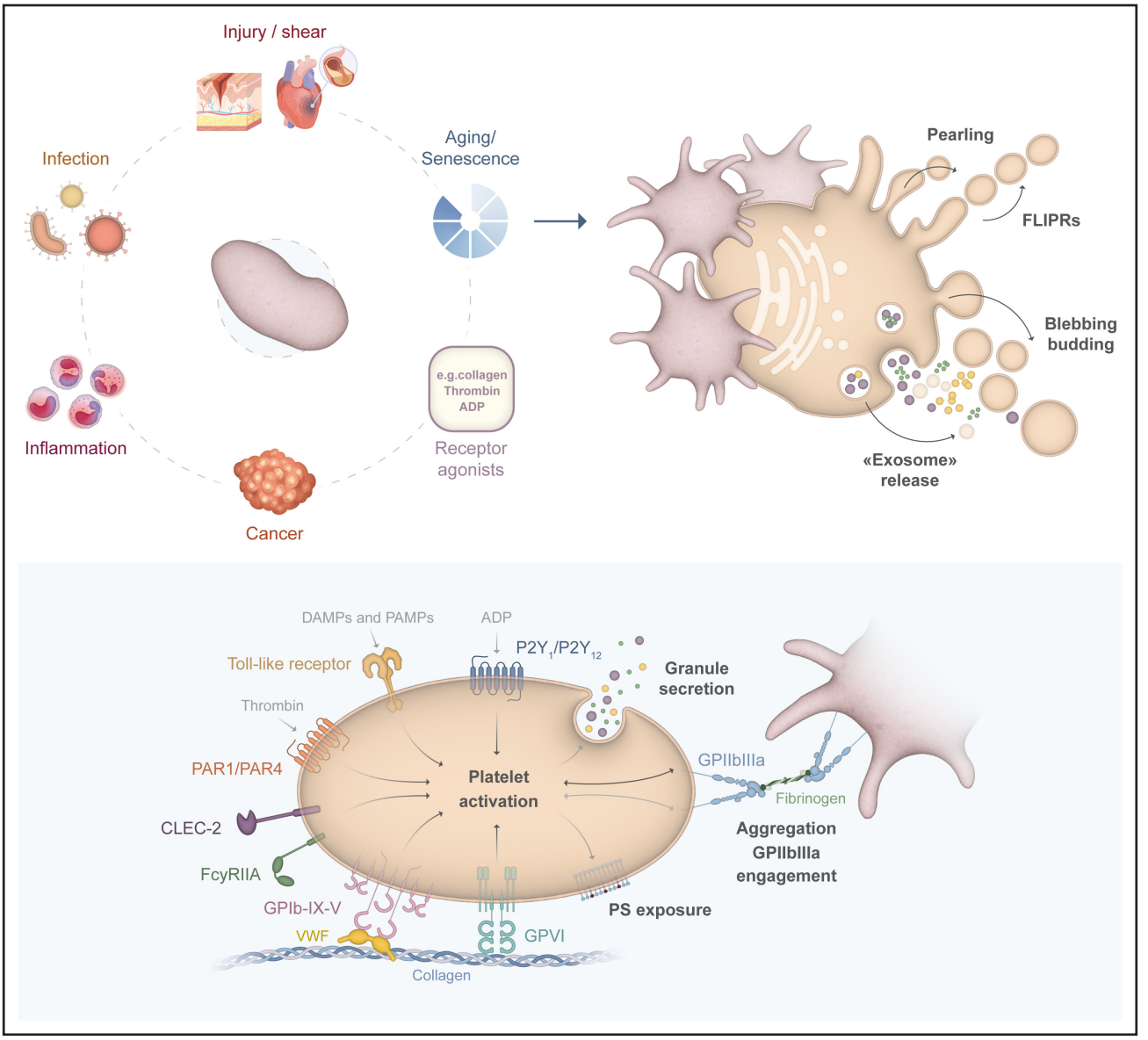

Figure 3. Generation of platelet-derived extracellular vesicles (PEVs).
Platelets serve as extracellular sentinels in circulation responding to multiple pathophysiological conditions and also becoming sensitized to such signals during aging. When platelet surface receptors become differently triggered, intracellular signaling leads to various activation end points: granule secretion, aggregate formation exposure of phosphatidylserine containing procoagulant surface and the generation of PEVs. Depicted are some pathways that have been shown to differently modulate the resulting PEVome (all platelet-derived extracellular vesicles [EVs] and their cargo) including the different routes of biogenesis. Literature based examples of the different PEV formation routes include plasma membrane based pearling and bleeding and formation of flow-induced protrusions (FLIPRs) from pseudopods, whereas the exosome-like EVs are suggested originating from internal granules. Pathogen-associated molecular patterns (PAMP) and damage-associated molecular patterns (DAMP) can activate toll-like receptors (TLR) on platelets. CLEC-2 indicates C-type lectin-like receptor 2; GPVI, glycoprotein VI; and PS, phosphatidylserine.

production and can regulate cell processes and viability.[56,57] Specifically, platelet mitochondria help meet the high metabolic energy demands of platelets, with half of mitochondrial activity being dedicated to ATP production and the reserve energy used for other activities, such as response to oxidative stress.[58] This suggests that platelet mitochondria perform analogous functions to those in nucleated cells and with comparable efficacy, implying that platelets can perform respiration and regulate their viability.

Platelets also have functional ribosomes and polysomes[59,60] and can regulate selective protein expression in response to activation.[61] Platelets have been shown to splice pre-mRNA to mature mRNA to express proteins, such as BCL-3 (B-cell chronic lymphocytic lymphoma-3), and translation alters their thrombotic phenotype and extends the platelet's lifetime.[61] Notably, while it was previously thought that platelets only translated proteins in response to stimuli, recent findings suggest that platelets in the circulation constitutively translate proteins.[62]

Furthermore, this protein translation is essential for maintaining platelet reactivity.[62] Finally, recent studies have suggested that platelets contain genomic DNA fragments inherited from megakaryocytes or captured from the circulating extracellular medium[63]; however, the details of these findings and their implications need further study.

PRO-EV ARGUMENTS

Eukaryotic cells are produced through mitosis or meiosis and contain genetic material enclosed in a nucleus. By this definition, platelets, which are produced by proplatelet formation and membrane budding from megakaryocytes and lack a nucleus at all stages of their maturation process, do not qualify as cells. Although erythrocytes also lack a nucleus and are still considered cells, erythrocytes differ from platelets because they originally possess a nucleus, which is lost during their maturation process.

Giulio Bizzozero, the first researcher to describe platelets in detail, was careful not to refer to them as cells and avoided the use of the suffix "cyte" used for thrombocytes, which is derived from the Greek word "kutos," meaning cell. Instead, he more prudently referred to them as "blood corpuscles" and "discoid corpuscles without a nucleus." Other researchers suggested that these "particles" might be disintegrated leukocytes or precursors of erythrocytes. Although these do not constitute definitive evidence, and Bizzozero never explicitly stated that platelets are not cells, the terminologies chosen by these pioneers do not support the idea that platelets are cells and suggest they might be closer to cell fragments.

If platelets are not cells, they might be more appropriately labeled EVs, which are defined as particles that are released from cells, are delimited by a lipid bilayer, and cannot replicate on their own.[16] This simple and broad definition is meant to be inclusive of all membrane-delimited vesicles and does not specify the exact mechanism by which these particles are released, such as whether it involves microtubules or not. Indeed, microtubules, which are reportedly necessary in platelet genesis, also play a role in transporting multivesicular bodies to the plasma membrane, a key process in exosome sorting. Additionally, the release of EVs from primary cilia in other cells is microtubule-dependent, as microtubules are essential for structuring the axoneme in primary cilia.[64,65] Therefore, the argument that microtubule involvement in platelet genesis can distinguish it from EV release remains to be fully substantiated.

Like some other EVs, platelets possess a cytosolic interior that is simpler than that of cells (poorer content in endoplasmic reticulum, Golgi, and absence of a nucleus) and can transfer RNA molecules to cellular recipients, such as endothelial cells and leukocytes, pointing to a role in intercellular communication in a manner similar to that of EVs.[66] Platelets do possess certain properties that distinguish them from other EV populations, but they clearly meet all essential EV criteria, that is, they are released from cells, possess an intact lipid bilayer, and lack the ability to replicate themselves.

Many readers will be most familiar with small EVs (diameter of ~50–200 nm), previously often referred to as exosomes. However, increasing evidence suggests that some large EV types represent functionally significant but previously unrecognized EV populations. These EVs of micron size include apoptotic bodies,[63] large oncosomes,[67] migrasomes,[68] exophers,[69] en bloc-released large EV clusters,[70] and amphiectosomes.[17] Given that normal human platelets are 1.5–3 microns in diameter,[71] their size range aligns with that of large EVs (Figure 2).

Importantly, both platelets and EVs are characterized by their heterogeneity.[72] Platelet diversity arises partly due to a subpopulation called coated platelets, which retain procoagulant proteins on their surface.[73] This feature of coated platelets closely resembles the surface-adsorbed corona of EVs.[74]

Many cell-like characteristics of platelets also occur in EVs. For example, like platelets, certain large EV populations, such as exophers, apoptotic bodies, and certain platelet EVs, contain organelles, such as mitochondria, and some types of EVs have metabolic activity.[69,75] In addition, both platelets and EVs perform enzymatic reactions.[40,76,77] Platelets are themselves capable of releasing EVs, a feature that has also been shown for some large EV populations, such as the release of small EVs by endothelial EVs and the release of small EVs from secreted amphisomes (amphiectosomes) through a torn bag mechanism.[78,79] Finally, given their sets of receptors capable of detecting subendothelial matrix or damage-associated molecular patterns, platelets are capable of aggregating at sites of vascular injury, something which has been observed for certain EV subpopulations generated following traumatic brain injury.[80–82]

CHALLENGES IN DISTINGUISHING PLATELETS AND EVS

Multiple studies show that platelets and EVs, especially EVs formed from platelets themselves, are difficult to distinguish in different settings. Examples of this challenge are provided, with an emphasis on hemostasis.

ROLE OF PLATELET IN HEMOSTASIS

Because platelets have an important role in hemostasis, individuals with thrombocytopenia are at risk of excessive bleeding or bruising. Platelets rapidly bind to injured blood vessels and form a hemostatic plug that is stabilized by cross-linked fibrin. Moreover, there is a close relationship between platelets and coagulation. For example, exposure of anionic phospholipids, such as phosphatidylserine on the surface of activated platelets,

promotes binding of coagulation factors, formation of coagulation factor complexes, and fibrin formation.[83] Furthermore, platelets are a rich source of coagulation factors and hemostatic proteins.

The procoagulant activity of components with a particulate nature present in plasma was first observed in 1946, when Chargaff and West showed that centrifugation of plasma at 31 000*g* prolongs plasma clotting time.[84] In 1967, Wolf[85] described this nonsoluble fraction as submicron particles, which he called dust, which were at posteriori attributed to EVs. Human blood contains ~10^8 to 10^{10} EVs per mL of plasma,[86,87] a major fraction of which expose molecules that are also present on megakaryocytes and (activated) platelets, indicating that megakaryocytes, platelets, or both significantly contribute to the total blood EV population. EVs from other blood cell lineages also may expose phosphatidylserine, suggesting that they too may participate in procoagulant activities and play a role in hemostasis.[88]

SCOTT SYNDROME

In Scott Syndrome, a rare moderate to severe inherited bleeding abnormality, platelets do not expose phosphatidylserine upon activation due to a defective calcium-dependent phospholipid scramblase.[89] These platelets cannot bind coagulation factors Va and Xa,[90] which may explain the bleeding tendency.[91] However, they are also unable to release phosphatidylserine-exposing EVs in vitro. The relative contribution of the defect in phosphatidylserine exposure on platelets and the inability to release EVs to the bleeding in these patients is unknown. Thus, although Scott Syndrome is sometimes used as an example supporting the role of platelet EVs in hemostasis, it remains unclear whether this function is actually performed by platelets or platelet EVs.

THE PRESENCE OF RESIDUAL PLATELETS AFFECTS EV RESEARCH

A challenge when isolating EVs from human blood, plasma, or serum is that platelets cannot easily be separated from EVs by size, density, or immunophenotype.[92,93] Tetraspanins are generally considered universal markers of EVs and are used to define or capture EVs[94]; however, both platelets and EVs expose tetraspanins, suggesting that platelets may also be isolated by methods designed to immune-capture EVs.

A commonly used protocol that was developed to separate blood cells from the (EV-containing) plasma can remove platelets with an efficacy of ~99% to 99.5%.[95] Consequently, about 0.5% to 1% of platelets are left within the (EV-containing) plasma. Thus, commonly used terms such as "platelet-free plasma" and "platelet-depleted plasma" are misleading for EV researchers, because the plasma may still contain ~500 000 to 1 000 000 platelets per mL.[93] Freeze-thawing of plasma/serum samples might also cause these contaminating platelets to generate new EVs.

Consequences of this cooccurrence are illustrated by the following examples. The removal of residual platelets reduces the concentration of supposedly plasma EV-associated miRNA by 50% to 75%, which brings into question the numerous studies that investigate the role of extracellular noncoding RNA in blood.[96] Additionally, the concentration of platelet EVs has been measured as a potential biomarker for diseases, such as cancer and cardiovascular disease.[97] However, most studies use biobanked samples containing unknown concentrations of residual platelets and platelet fragments. The resulting differences in the reported concentrations of platelet-derived EVs can be orders of magnitude, which complicates the interpretation of these data. It is also worth noting that the choice of anticoagulant or the use of serum during preanalytical processing has an effect on artificial platelet EV concentrations due to platelet activation.[98]

THE PRESENCE OF EVS AFFECTS PLATELET RESEARCH

EVs from platelets and other cells can also interfere in studies on platelets. Platelets are most commonly isolated by gel filtration (size-exclusion chromatography) or centrifugation. However, gel filtration does not separate platelets from EVs. In contrast, centrifugation enables most EVs to be discarded. Thus, the term isolated platelets can be misleading in some studies, depending on the isolation method. For example, "tumor-educated platelets" is a term used for platelets present in the blood of cancer patients.[99] The miRNA profile of these platelets differs from platelets in healthy individuals, which is explained by the uptake of cancer-derived biomolecules. However, because of the methodologies used to isolate tumor-educated platelets in liquid biopsies, up to 20% of the original concentration of blood EVs may still be present in the sample.[100] Consequently, the coding or noncoding RNA being studied might actually be derived from confounding EVs.

In summary, the biophysical similarities and shared receptors, such as tetraspanins, between platelets and EVs make their separation challenging, complicating study interpretations. These similarities support the hypothesis that platelets may differ fundamentally from typical cells. Alternatively, frequent platelet contamination in EV preparations could suggest that platelets are, in fact, a type of EV.

CONSIDERATIONS IN CURRENT AND FUTURE RESEARCH

Despite extensive research, the process of platelet production is still not fully understood, with ongoing debate

over whether platelets are formed through budding from the megakaryocyte membrane or via proplatelet formation.[6,10,12,13,101,102] Indeed, the structures observed budding directly from megakaryocytes may also be either EVs or emerging proplatelets (Figure 2), highlighting the difficulty in distinguishing EVs from platelets even with the most advanced imaging technologies. Determining whether platelets are cells or EVs could help answer key questions about platelet genesis and might help reconcile these different studies.

Platelets are evolutionarily younger than EVs, which are present in all 3 domains of life (bacteria, archaea, and eukaryotes). For example, given that avian thrombocytes generate EVs,[103] we can speculate that EVs existed in dinosaurs, although platelets were likely absent in these animals, as they are distant ancestors of birds.[30] It is hypothesized that platelets may have emerged as specialized EVs contributing to the evolutionary development of mammals. The exact evolutionary mechanism is unknown, but a thrombocyte-like precursor might have developed mechanisms to produce different types of EVs alongside the more primitive EVs. These cells may have initiated the budding of larger EVs, similar to current platelets, which could explain the platelet budding observed in recent studies. Eventually, this process might have led to pro-platelet formation and platelet release, which might be more efficient in increasing platelet production than the budding process. This would make EVs a missing link or intermediate form between cells and platelets. It is also possible that this evolutionary process began with the generation of a different type of cell that used processes other than mitosis or meiosis to generate multiple progenies from 1 mother cell.

What might have driven this evolutionary process, potentially leading to the rise of mammals? There is no definite answer, but virus integration into the genome is generally considered an important factor that contributed to the emergence of mammal species, or evolution in general. For instance, syncytin, a critical component of the placenta, is in fact encoded by an endogenous retroviral gene initially implicated in virus envelope formation.[104,105] Thus, without such infection and integration by a retrovirus, there would be no functional placenta. It would be interesting to determine if a random mutation, or a similar process implicating endogenous viruses, might have triggered the formation of this new type of cell or these more evolved EVs by megakaryocytes. Understanding similarities and differences in the release of EVs versus platelets, by interfering in activation using known platelet inhibitors or in pathways of EV release, such as ceramide synthesis, for example,[106–108] we may identify key mechanisms pertaining to platelet genesis. Moreover, understanding how megakaryocytes, in comparison to other cellular lineages, produce EVs, while considering platelets as an EV subtype, may provide insights into mechanisms that may have promoted the emergence of platelets in mammals and of the key molecular pathways involved in platelet genesis.

Similarly, if platelets are indeed EVs, this could enhance our understanding of the biological and clinical significance of EVs. EVs are generally considered important players in intercellular communication, but their relevance has been questioned, in part due to challenges in separating the effects of EV-donor cells from those of EVs themselves. The main argument supporting the functional relevance of EVs is the fact that they are produced by all types of cells from all living domains and that it would be counterproductive for cells to expel molecules if they cannot be utilized by another cell, as well as the identification of well-controlled molecular mechanisms promoting EV release. However, if platelets are EVs, this would offer a solid demonstration of the significance of EVs, as having a crucial role in mammalian evolution. Moreover, the effectiveness of platelet storage and transfusion would provide the best demonstration of a clinical application employing EVs.

FINAL REMARKS

While it is unclear if platelets are cells or EVs, they represent a special category. Thus, can we simply consider platelets as a unique class? While many researchers in the fields of hemostasis and thrombosis may consider megakaryocytes and, by extension, platelets as unique for various reasons, these reasons are often subjective, reflecting their primary research interests. However, biologists from other fields may have a different perspective, finding other specialized cells equally unique. For instance, neurons are remarkable for their ability to transmit electrochemical signals, while spermatozoa are unique in carrying half the genomic DNA of other cells and traveling between individuals to deliver male chromosomes to the female ovule. Thus, simply calling platelets unique risks creating gaps in knowledge, as platelets must be integrated into the frameworks of existing categories, such as cells or EVs, to ensure a comprehensive understanding across these fields.

In summary, although platelets exhibit unique features that distinguish them from both cells and EVs, they unequivocally fulfill only the current minimal criteria defining EVs, not cells. Furthermore, EVs may represent a missing link between cells and platelets, bridging gaps in our understanding of their shared characteristics and distinct roles. This novel perspective on platelet classification opens the door for thoughtful and objective discussions, encouraging readers to critically evaluate their true identity. This approach has the potential to inspire renewed research efforts in both platelet and EV biology, driving scientific discovery and advancing clinical applications.

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Disclosures

None.

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