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REVIEW ARTICLE

Beyond the thrombus: Platelet-inspired nanomedicine approaches in inflammation, immune response, and cancer

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Abstract

The traditional role of platelets is in the formation of blood clots for physiologic (e.g., in hemostasis) or pathologic (e.g., in thrombosis) functions. The cellular and subcellular mechanisms and signaling in platelets involved in these functions have been extensively elucidated and new knowledge continues to emerge, resulting in various therapeutic developments in this area for the management of hemorrhagic or thrombotic events. Nanomedicine, a field involving design of nanoparticles with unique biointeractive surface modifications and payload encapsulation for disease-targeted drug delivery, has become an important component of such therapeutic development. Beyond their traditional role in blood clotting, platelets have been implicated to play crucial mechanistic roles in other diseases including inflammation, immune response, and cancer, via direct cellular interactions, as well as secretion of soluble factors that aid in the disease microenvironment. To date, the development of nanomedicine systems that leverage these broader roles of platelets has been limited. Additionally, another exciting area of research that has emerged in recent years is that of platelet-derived extracellular vesicles (PEVs) that can directly and indirectly influence physiological and pathological processes. This makes PEVs a unique paradigm for platelet-inspired therapeutic design. This review aims to provide mechanistic insight into the involvement of platelets and PEVs beyond hemostasis and thrombosis, and to discuss the current state of the art in the development of platelet-inspired therapeutic technologies in these areas, with an emphasis on future opportunities.

KEYWORDS
cancer, extracellular vesicles, immune response, inflammation, nanomedicine, platelets

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1 | INTRODUCTION

Platelets are blood cells derived from megakaryocytes, and are primarily responsible for facilitating physiological (hemostasis) and pathological (thrombosis) blood clots via specific mechanisms of adhesion, aggregation, procoagulant function, clot retraction, and granule secretion.[1–6] Significant research have been focused on elucidating these mechanisms, and developing therapeutics to modulate them for the treatment of hemorrhage by making clots (hemostatic therapy) and thrombosis by preventing or breaking clots (antithrombotic therapy).[7–9] Nanomedicine, a field involving the design of nanoparticles with unique biointeractive surface modifications and payload encapsulation for disease-targeted drug delivery, has become an important component of such therapeutic development.[10–12] Beyond their traditional role in forming good and bad clots, platelets have been implicated in several other pathologies, including innate and adaptive immune response against pathogens, inflammation, and cancer, via direct cellular interactions, as well as via secretion of soluble factors that aid in the disease microenvironment.[13–21] To date, the development of nanomedicine systems that leverage these broader roles of platelets have been limited, but this presents tremendous opportunities for therapeutic innovation. Another exciting and emerging area of scientific investigation is that of platelet-derived extracellular vesicles (PEVs) that can directly and indirectly influence various physiological and pathological processes, including recent findings in COVID-19 patients.[22–24] This makes PEVs a unique paradigm for platelet-inspired therapeutic design. In this review, we aim to provide mechanistic insight into the involvement of platelets in physiological and pathological processes beyond hemostasis and thrombosis and discuss how our current understanding of these mechanisms can be leveraged to develop platelet-inspired therapeutic technologies. We also discuss anticipated challenges and future opportunities in this area.

2 | PLATELETS IN IMMUNE RESPONSES AND INFLAMMATION

Beyond their well-recognized role in hemostasis thrombosis and thromboinflammation, platelets have emerged as critical players in innate and adaptive immune responses against pathogens (Figure 1).[25,26] Initial defense against pathogens in the circulation is established by two major lines of defense.[27] On a cellular level, different microbes are recognized by pathogen-associated molecular pattern receptors known as the toll-like receptors (TLRs), whereas outside the cell the complement system amplifies the defense against such pathogens. These two systems work in concert to mediate the initial response against a pathogen that crosses over into the circulation. Platelets contain the transcripts of all known TLRs in humans and, interestingly, they are upregulated in women.[28] Functionally, TLRs can be classified as surface receptors (TLR2, TLR4, and TLR5), recognizing surface protein components of pathogens (e.g., bacteria, viruses), or endosomal TLRs (TLR3, TLR7, TLR8, and TLR9) specifically recognizing nucleic acids of these pathogens. TLR2 and TLR4 are functional receptors in platelets and follow almost similar activation signaling cascades as in nucleated cells.[29–33] However, contrary to nucleated cells, in which cytokines are synthesized and released, activation of these receptors in platelets leads to the release of prepackaged proteins from platelet granules (e.g., P-selectin, CD40L, von Willebrand factor [VWF]) or the activation of surface integrins. These processes lead to increased platelet aggregation, increased binding of platelets to fibrinogen, and, in the case of TLR2 activation, formation of platelet-leukocyte aggregates relevant to thromboinflammatory pathologies. Activation of platelet TLR4 also leads to neutrophil activation, ultimately contributing to accelerated release of neutrophil DNA (neutrophil extracellular trap formation or NETosis), that promotes prothrombotic pathology.[34–36] Platelet TLR2 can also contribute to NETosis, although not to the same extent as platelet-TLR4. Both of these receptors use the PI3K/AKT signaling cascade for intracellular messaging and stimulation of granule release. The direct interaction with immune cells, in addition to the moderate aggregation, mediated by platelet TLR2 and TLR4 suggests that these receptors balance immunity and thrombosis, and this balance can be critical during platelet interaction with bacterial pathogens in the circulation. Platelets also express functional endosomal TLRs (TLR3, TLR7, and TLR9) and their activation in platelets serves as an important first stage of pathogen detection to alert the innate immune cell response.[37–41] Outside of the cell, the innate immune response to pathogens is mediated by the complement cascade, where a sophisticated concert of mechanisms involving more than 30 proteins ultimately leads to the lysis of bacteria or infected cells. The complement cascade is activated by three different pathways, the classical, alternative, or lectin pathway, with each converging at complement 3 (C3). Interestingly, platelets contain a few of the complement cascade factors including C3, C4, and C1 inhibitor stored in their alpha granules.[42] Activation of platelet endosomal TLR7 can lead to the release of C3 from platelet granules and, once in the circulation, this C3 can mediate NETosis. Additional factors from platelets also regulate NETosis after TLR7 stimulation, suggesting that intravascular NETosis is not only initiated but also controlled by platelets. Platelet CD40L (CD154) has also been reported to modulate adaptive immunity via inducing dendritic cell maturation, isotype switching in B cells and augmenting T-cell responses.[43] Therefore, platelet-mediated mechanisms are important therapeutic targets in modulating innate and adaptive immune responses.

Platelets also play a significant role in the inflammatory responses across several pathological conditions. For example, atherosclerosis is a vascular inflammatory disease in which the involvement of platelets in mediating leukocyte recruitment and signaling in the early stages of the pathology has now been recognized as a critical mechanistic event, beyond the obvious involvement of platelets in plaque rupture and thrombosis.[44–49] P-selectin, mobilized to the surface of activated platelets, is a major participant in the recruitment of inflammatory cells via interaction with P-selectin glycoprotein ligand-1 (PSGL-1) on leukocytes.[50] Platelets also interact with the inflamed endothelium via platelet GPIbα binding to

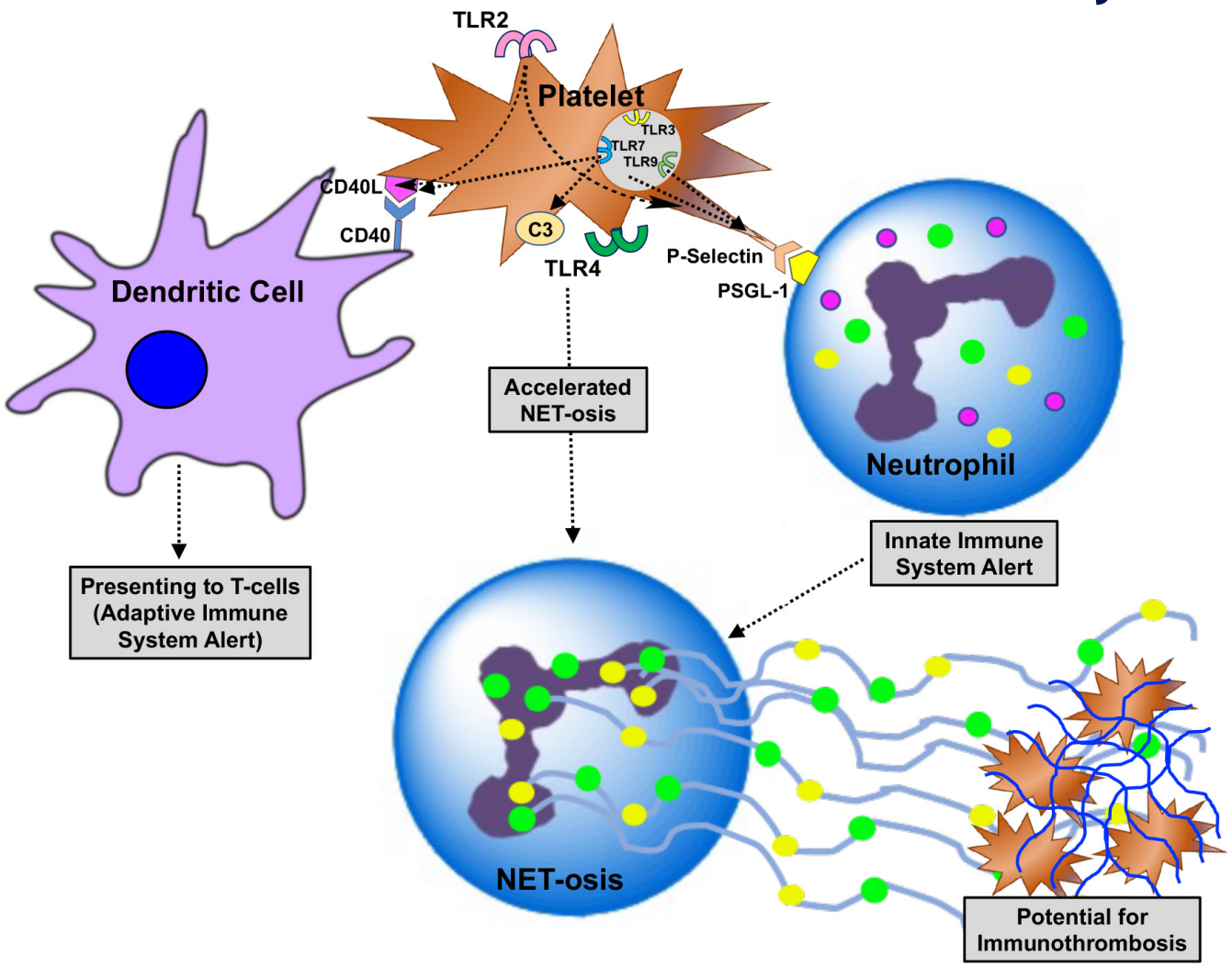

FIGURE 1 Platelet involvement in immune response: Platelets contain surface-expressed as well as endosomal toll-like receptors (TLRs) that recognize pathogen-associated molecules, leading to platelet presentation of surface moieties (e.g., CD40L, P-selectin, integrins) that facilitate recruitment and interaction with immune cells (e.g., neutrophils, dendritic cells). Platelets can also release complement molecules (e.g., C3). The combination of these mechanisms can lead to innate immunothrombotic events like neutrophil activation and extracellular trap formation (NET-osis), as well as adaptive immune events like modulating dendritic cell and T-cell responses

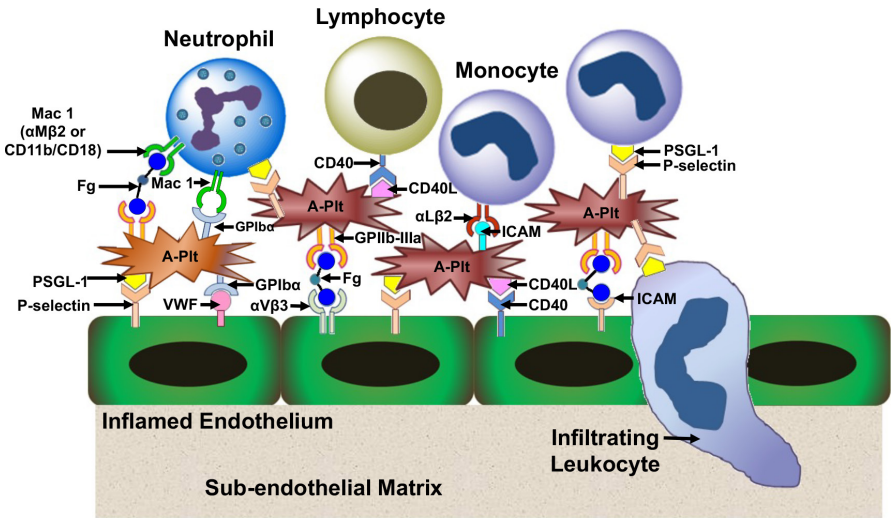

FIGURE 2 Platelet interactions with endothelium and leukocytes in inflammatory diseases: Platelets can directly bind to inflamed endothelium via PSGL-1-to-P-selectin and GPIbα-to-VWF interactions; platelets can also mediate the recruitment and binding of leukocytes to inflamed endothelium via multiple interactions like CD40L-to-CD40, GPIbα-to-Mac 1, PSGL-1-to-P-selectin, and fibrinogen (Fg)-bridging platelet surface GPIIb-IIIa to leukocyte surface integrins

endothelium-secreted VWF, as well as via binding to various integrins and cell adhesion molecules (CAMs) on the surface of endothelial cells, such as fibrinogen-mediated interaction with endothelial integrin αvβ3, platelet CD40L binding to endothelial CD40, platelet interactions with platelet-endothelial CAM, and intercellular cell adhesion molecule (ICAM), etc.[51–54]

Activated platelets also secrete pro-inflammatory biomolecules like interleukin-1β, Regulated Upon Activation Normal T-cell Expressed and Secreted (RANTES, also called CCL5), monocyte chemoattractant protein-1, macrophage colony stimulating factor, which promote inflammatory cell recruitment.[55,56] Activated platelets also secrete platelet-derived growth factor (PDGF) and transforming growth factor beta (TGF-β) that stimulate smooth muscle cell migration, proliferation, and collagen synthesis.[57] In addition, activated platelets, along with macrophages, produce matrix-metalloproteases (MMPs) that degrade extracellular matrix to aid infiltration of smooth muscle cells and inflammatory cells. CXCL4 or platelet factor 4 (PF-4), released from active platelet α-granules, is an important chemoattractant for neutrophils, monocytes, and fibroblasts, and also a stimulant of monocyte differentiation into macrophages.[58] Platelet-leukocyte interactions in the vascular inflammatory niche is also rendered by lymphocyte function-associated antigen 1 and macrophage 1 antigen (also called $\alpha_M\beta_2$ or CD11b/CD18) binding to platelet ICAM-2 and GPIbα, respectively.[59,60]

Figure 2 shows a schematic of various interactions between the platelet-leukocyte-endothelium triad. These interactions, along with platelet-secreted pro-inflammatory factors, have been implicated in inflammatory conditions including atherosclerosis, asthma, allergic rhinitis, eczema, psoriasis, inflammatory bowel disease, and rheumatoid arthritis. Platelets have also been implicated in inflammatory response of the host to allografts (e.g., during ischemia-reperfusion of the donor organ). Additionally, incompatible antigens coded by the major histocompatibility complex are recognized by platelets and drive antibody-meditated rejection of allografts.[61] Platelet aggregation at the site of allografts has been identified as an early marker of transplant rejection. Platelets facilitate interactions between the vascular endothelium of the donor graft and leukocytes in the recipient. They also mediate the recruitment and arrest of leukocytes in this host response to donor organ. Although platelets do not contain a nucleus, they contain mRNA in their cytoplasmic granules and are capable of *de novo* synthesis of proteins including P-selectin and other inflammatory factors.[62] Platelets can also internalize proteins and RNA from the donor and transfer them to the recipient after they are released back into circulation in their activated state.[63] Such inflammatory signaling and cell recruitment can result in increased recognition and expression of donor antibodies by the recipient's immune system. It has been demonstrated that platelets accumulate at VWF-positive endothelial cells at the intracapillary area in allogeneic, but not syngeneic, grafts in a murine model of heart transplantation.[64] Accordingly, platelet depletion as well as inhibition resulted in improved microvascular perfusion in this model. It was also shown that activated platelets expressing P-selectin, and releasing serotonin and PF4, accumulate at the renal allograft following passive transfer of donor-specific antibodies in a model of renal transplant in immunodeficient mice.[65] This function of platelets was further demonstrated in a model of antibody-mediated rejection of renal allografts.[66,67] Platelet P-selectin also supports the activation of complement cascade and the generation of potent anaphylatoxins C3a and C5a, amplifying the inflammatory and thrombotic environment. The immune and inflammatory mechanisms of platelets indicate the potential of platelet-targeted therapies in the management of such pathologies.

3 | PLATELET-INSPIRED NANOMEDICINE FOR TREATING IMMUNE AND INFLAMMATORY DISEASES

Several antiplatelet agents have been investigated for their therapeutic effect in immune and inflammatory pathologies.[68] Depleting platelets or blocking platelet receptors with antibodies has demonstrated therapeutic promise in experimental models of immune-mediated inflammatory diseases. However, this approach also presents the risk of adverse systemic effects. More localized delivery of immunosuppressants can be potentially achieved by nanomedicine systems that exploit platelet-inspired binding mechanisms. To this end, antibody-conjugated nanoparticles have been explored in models of immune-mediated inflammation. For example, immunoliposomes decorated with selectin-targeting antibodies have been studied for the targeted delivery of dexamethasone in murine models of arthritis and glomerulonephritis.[69,70] Radiolabeled platelets have been used to diagnose early stages of transplant rejection in humans.[71] This suggests that platelet-mimetic synthetic nanoparticles could be potentially used for targeting transplant sites to deliver anti-inflammatory and immunosuppressant drugs as well as molecular imaging agents to improve localized therapeutic and diagnostic efficacy while avoiding systemic risks. In our research focused on the development of platelet-inspired nanomedicine, we have demonstrated the ability to mimic various platelet interactions (e.g., adhesion to VWF and collagen, aggregation mediated by fibrinogen, binding mediated by P-selectin, anchorage to fibrin, heterotypic binding to activated neutrophils) via decoration of liposomal nanoparticle platforms with combinations of biointeractive peptides.[11,72,73] We envision that such surface-engineered nanoparticle platforms could be potentially customized for targeted delivery of appropriate therapeutic agents in the management of immune and inflammatory pathologies.

4 | PLATELET ROLE IN CANCER

Cancer metastasis, or the spreading of tumor cells from the primary disease site to nearby and distal organs of the body, is a highly complex process involving malignant cell detachment from primary tumor and their epithelial-to-mesenchymal transformation (EMT), migratory invasion of the cells into neighboring tissues as well as

their intravasation into proximal blood and lymph vessels, transport of such cells via circulation while avoiding immune surveillance, their extravasation from circulation and arrest in distal tissue beds, development of metastatic microenvironment, and colonization and growth of the cells at these distal sites.[74–76] In 1865, the French clinician Armand Trousseau reported his observation that migratory thrombophlebitis is an indicator of occult malignancy, and since then a compelling body of experimental and clinical evidence has established crucial mechanistic roles of platelets in cancer metastasis.[77] Specifically, platelets are implicated in playing a three-pronged role in cancer metastasis: (1) facilitation and maintenance of the EMT process, (2) shielding of circulating tumor cells (CTCs) from immune surveillance/neutralization and shear stress, and (3) secretion of pro-metastatic factors in the tumor microenvironment both at primary site and at distal sites (Figure 3).[78–94]

Cancer cells express surface-level tissue factor, allowing for localized thrombin generation that can activate nearby platelets.[78] Activated platelets can also undergo direct or mediated binding interactions with cancer cells, for example, platelet P-selectin-based direct binding to cancer cell CD44, fibrinogen-mediated binding between platelet GPIIb-IIIa and integrin $\alpha_V\beta_3$ on cancer cells and cancer-associated angiogenic endothelial cells, and VWF-mediated binding between platelet GPIbα and GPIbα-like motifs on cancer cells.[80–84] These binding interactions can allow activated platelets to "cloak" cancer cells from immune surveillance in circulation, as well as enable adhesion of the cloaked cells to vascular walls and tissue beds at distal sites.[82,85,86] Platelets carry TGF-β within alpha granules that is released on platelet activation and can subsequently activate the TGF-β/Smad pathway in cancer cells.[87] Additionally, direct contact between platelets and cancer cells can result in activation of the NF-κB pathway.[88] Both of these pathways can promote the transcription of pro-metastatic genes that contribute to EMT processes. Platelets also secrete signaling molecules such as PDGF and vascular endothelial growth factor, as well as proteases (e.g., MMP-2, MMP-9) that can contribute to EMT mechanisms, intravasation, extravasation, and angiogenesis.[89] Platelet CLEC-2 has also been implicated in cancer metastasis by virtue of its interaction with the sialylated membrane glycoprotein Podoplanin that is found on several types of invasive cancers, including squamous cell carcinoma, brain tumor, osteosarcoma, and melanoma.[90,91] Antibody-mediated blocking of Podoplanin or therapeutic inhibition of platelet CLEC-2 have shown promising effect in reducing or inhibiting tumor progression.[92] Additionally, platelet GPVI has recently been implicated in promoting cancer metastasis and has been identified as a potential therapeutic target.[93,94] Therefore, therapeutic inhibition or modulation of platelet-cancer interactions hold great promise in developing innovative cancer therapies, and platelet-inspired nanomedicine can play an important role in such development.

5 | PLATELET-INSPIRED NANOMEDICINE APPROACHES FOR TREATING CANCER

Several preclinical and clinical studies in cancer treatment have been conducted with therapeutic strategies to reduce platelet count, prevent alpha-granule release, block P-selectin or GPIIb/IIIa mediated

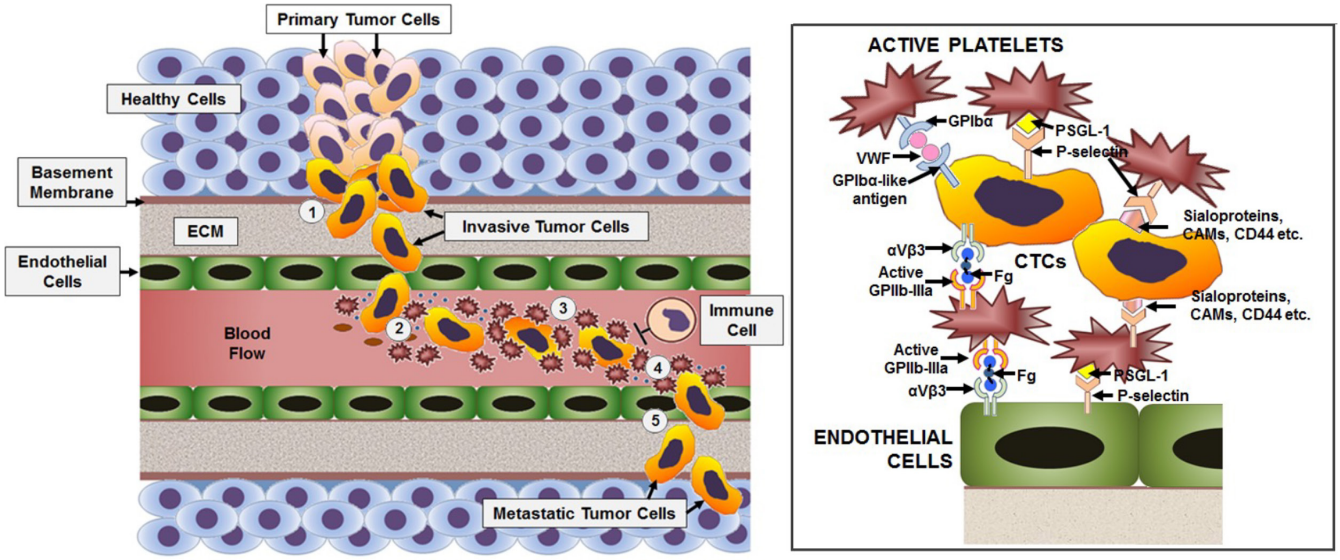

FIGURE 3 Platelet role in cancer metastasis: (1) Platelet-secreted molecules can an aid in epithelial-to-mesenchymal transformation (EMT) of metastatic cancer cells; (2) platelet-derived proteases can facilitate intravasation of metastatic cells; (3) bidirectional communication between platelets and circulating tumor cells (CTCs) can result in platelet activation, and direct as well as mediated binding of activated platelets to CTCs to form a "cloak" that enables immune cell evasion; (4) platelets can aid adhesion and extravasation of CTCs at distal sites; and (5) platelets can secrete cytokines and growth factors that enable the development of metastatic microenvironment. Enlarged box shows selected examples of platelet interactions with cancer cells (e.g., VWF-mediated binding between platelet GPIbα and analogous antigens on cancer cells, fibrinogen (Fg)-mediated binding between platelet GPIIb-IIIa, and cancer cell surface integrins, direct binding of cancer cell CD44 to platelet P-selectin)

binding interactions, and inhibit platelet activation.[95–101] Although these have shown promising results, the systemic nature of these strategies may be associated with bleeding risks and other side effects. Therefore, using nanomedicine platforms that mimic or leverage platelet-cancer interactions to deliver specific therapeutic payloads in a targeted manner can allow for enhancing treatment benefit while avoiding systemic side effects. The promise of such an approach is evident in studies involving chemotherapy drug (e.g., doxorubicin) encapsulation in actual platelets for tumor-targeted delivery in lymphoma.[102,103] However, studies have also shown that doxorubicin can directly impact platelet activation and functions leading to thrombotic and thrombocytopenic side effects.[104,105] This provides a strong rationale to use platelet-mimetic nanoparticles as potential carrier platforms for doxorubicin and other drugs to avoid effects on endogenous platelets while enabling targeted delivery to cancer cells.

In our research in this area, we have demonstrated that the pro-metastatic breast cancer line MDA-MB-231 (human) and 4T1 (mouse) have high expression of platelet-interactive surface functionality (e.g., P-selectin mediated binding to PSGL-1, fibrinogen-mediated binding to β_3 integrins), and decorating liposomal nanoparticles with ligand combinations mimicking such interactions enabled targeted delivery of chemotherapy (doxorubicin) to these cells *in vitro*.[106,107] Several research groups in recent years have also explored an interesting strategy of coating anticancer drug loaded nanoparticles with lipid membrane extracted from platelets (a process termed biointerfacing), with the rationale that the some of the tumor-interactive mechanisms of the platelets will be retained in the coated membrane to enable tumor-targeted drug delivery. In this framework, platelet membrane-coated nanoparticles have been used to deliver immune checkpoint inhibitors, chemotherapy drugs, and photothermal therapy agents to cancer, with promising results.[108,109] In another analogous approach, doxorubicin-loaded nanoparticles were coated with platelet-derived membrane as well as decorated with TNF-related apoptosis-inducing ligand, and these systems showed promising efficacy in tumor-targeting and reducing metastasis.[110] In another recent approach, detergent-treated platelets lacking adhesion and aggregation capabilities were shown to act as "platelet decoys" that can inhibit platelet arrest on matrix proteins *in vitro* and reduce tumor metastasis in mouse model *in vivo*.[111] Altogether, these studies highlight the potential of platelet-derived and platelet-inspired nanomedicine approaches for cancer therapy. Figure 4 shows the various platelet-inspired design approaches that are currently being studied for therapeutic applications in cancer and in other pathological settings.

6 | PLATELET EXTRACELLULAR VESICLES: A NEW PARADIGM FOR PLATELET-INSPIRED NANOMEDICINE

In recent years, exciting research has emerged in the area of extracellular vesicles (EVs) regarding their physiologic and pathologic roles, as well as their potential utilization for therapeutic delivery.[112–120] The terminology of "extracellular vesicle" broadly covers microparticles/microvesicles (100–1000 nm in diameter) originating from membrane budding/blebbing processes, and exosomes (30–100 nm in diameter) originating from cellular endocytic mechanisms (Figure 5).[112–114] PEVs are well-established regulators of intracellular communication and contain diverse cargo including microRNAs, cytokines, and organelles like mitochondria, which reflect their cell of origin.[115–120] Once released from their parent cell, PEVs can communicate with cells both nearby and at distant sites through their access to blood, lymph, and synovial fluid. EV distribution in body tissues is thought to be naturally defined by surface proteins inherited from the parent cell. This natural ability of EVs to efficiently package and carry cargo and achieve a targeted biodistribution makes them attractive candidates for therapeutic applications. Specifically, infusion of EVs created from cultured cells is a burgeoning area of intertest in nanomedicine.[121,122] In this framework, PEVs are emerging as an exciting area of platelet-derived nanomedicine platform.[123]

PEVs derived from the platelet mother cell, megakaryocytes, compose more than 80% of the EVs in blood. Because they are abundant and naturally occurring in healthy people, their risk of immunogenicity upon infusion is very low. In addition, PEVs are a component of current platelet transfusions because platelets release vesicles into the solution during storage. As such, there is very low risk of immunogenicity for PEVs in both single and repeat dosing. PEVs are produced as a by-product of platelet activation. They are natural carriers of proteins, organelles, and nucleic acids and hold tremendous promise as direct cell messengers for novel therapeutics because of their natural targeting to bone marrow stem cells, amenability to large-scale/commercial manufacture, and potential low immunogenicity.[124,125] In addition, there is compelling evidence that PEVs interact with other cells to alter their fate and functionality. For example, PEVs can transfer the surface protein CXCR4 to CXCR4-null cells, causing the otherwise resistant recipient cells to be susceptible to HIV infection.[126] In addition, we have recently found that PEVs have the capacity to functionally reprogram hematopoietic stem and progenitor cells in the bone marrow by delivering cargo that restores megakaryopoiesis.[127] In another study, PEVs were found to promote hemostasis and prevent hemorrhagic shock.[128] Recent research has also indicated that PEVs stemming from tumor-induced platelet activation can participate in bidirectional communication between PEVs and cancer cells.[129] In addition to carrying signaling molecules such as TGF-β, PDGF, and vascular endothelial growth factor, PEVs have been shown to express a higher density of surface proteins such as P-selectin and CD41, as well as lipids like phosphatidylserine, making them highly prothrombotic and procoagulant, and contributing to cancer-associated thrombosis.[130] Altogether, these studies suggest that PEVs alone have the capacity to target, bind, and alter specific cells for unique bioactive effects.

Beyond surface-mediated interactions and specific targeting, PEVs are also potentially suited for therapeutic delivery to specific cells and tissues. For example, the ability of PEVs to recruit to the bone marrow can make them potential delivery platforms that target

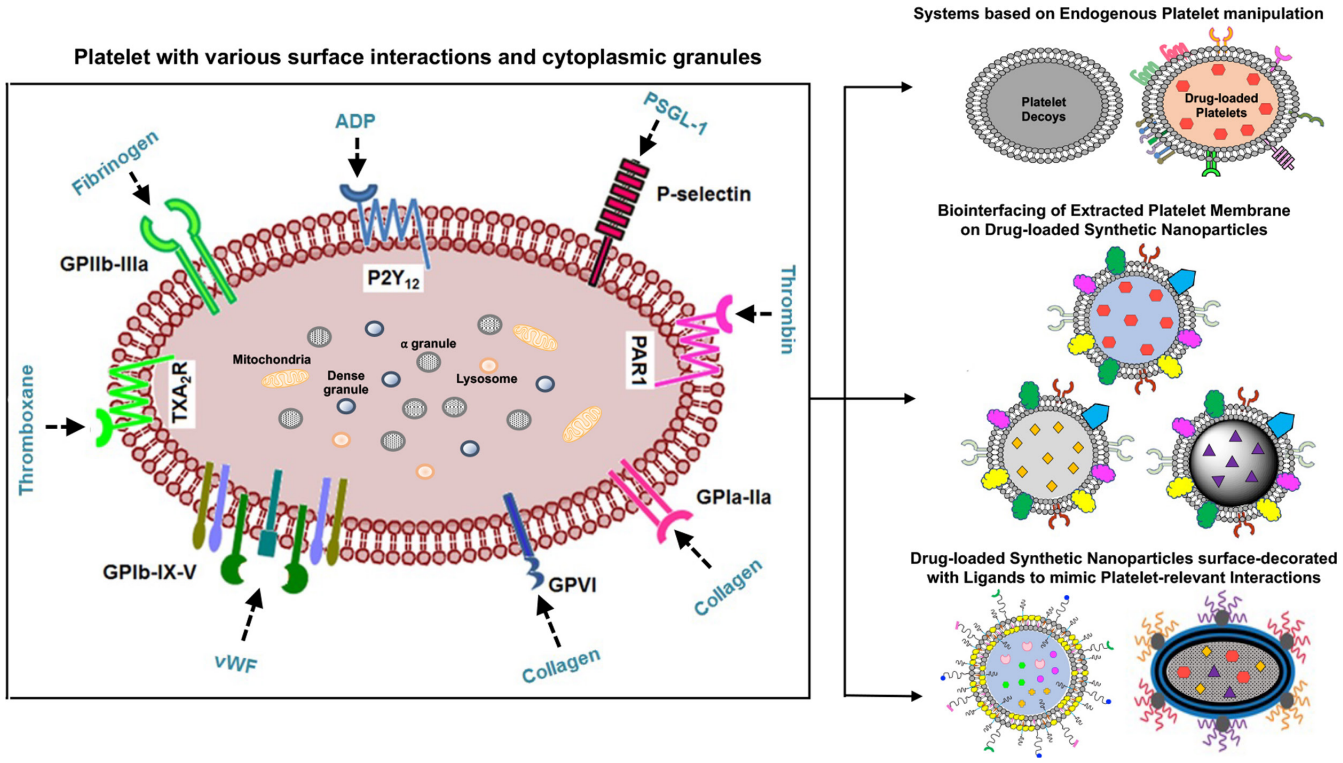

FIGURE 4 Current state of the art in the design of platelet-inspired therapeutic platforms: Platelets bear a variety of surface-motifs that interact with specific agonists and ligand molecules, and a variety of cytoplasmic granules that secrete their content upon platelet activation; platelet-inspired therapeutic platforms are designed to similarly achieve specific surface interactions and release encapsulated drug payloads. Systems based on direct platelet manipulation include using "ghost platelets" as decoys to inhibit native platelet interactions with other cells and tissues, as well as using platelets as carriers for drugs loaded within their cytoplasm; systems based on the concept of "biointerfacing" use membrane extracted from platelets to coat drug-loaded synthetic nanoparticles, with the rationale that the extracted membrane will still bear certain biointeractive motifs to enable targeted drug delivery. Systems based on fully synthetic approaches use surface-decoration of drug-loaded synthetic nanoparticles with specific ligands (e.g., antibodies, antibody fragments, peptides) that mimic platelet-specific interactions with pathologic cells and tissues to enable cell-specific and site-specific drug delivery

resident bone marrow cells, such as gene therapy delivery to hematopoietic stem and progenitor cells. Gene therapies are differentiated from drugs in that they aim to cure the disease instead of treating the symptoms. However, gene therapies require delivery vehicles to transport genes into the nucleus of target cells, which is a major bottleneck to their translational advancement. Historically, gene therapy approaches involve removal of target cells from the patient, delivery of the genetic material to the cells *in vitro*, and then returning the cells back to the patient's body. Although viral vectors (e.g., adenovirus, adeno-associated virus, retrovirus, lentivirus) constitute the majority of current gene delivery platforms, their direct *in vivo* administration present considerable risks including indiscriminate tissue biodistribution, risk of insertional mutagenesis, oncogene activation, and immunogenicity/toxicity. This has prompted research into exploring nonviral gene delivery approaches using nanoparticles manufactured from unique lipids and polymeric materials.[131] In this context, EVs are emerging as a unique bio-derived member of such nonviral platforms. For example, small RNAs (siRNA and miRNA), small linear DNA, and plasmid DNA have been successfully loaded into EVs for a variety of delivery applications.[132–134] Because EVs can transfer their cargo to alter the function of recipient cells via surface receptor signaling, plasma membrane fusion, and internalization, using PEVs carrying native cargo or encapsulating intended cargo can provide unique opportunities for targeted delivery of drugs and genetic material for specific therapeutic effects. In addition, future studies will explore if PEVs can be further engineered to express or remove specific biomarkers of interest, to further refine biodistribution, cell recognition, and communication for drug delivery.

7 | DISCUSSION

Platelets have emerged as one of the most versatile cellular entities, with significant mechanistic involvement in hemostasis, thrombosis, inflammation, immune response and cancer. Platelets play these mechanistic roles by virtue of a variety of surface interactions with other cells and tissues, secretion of various cytoplasmic granule contents, and transfer of intracellular cargo via unique mechanisms including extracellular vesicle production. These versatile roles of platelets present a strong rationale for the development of platelet-inspired nanomedicine strategies for targeted treatment of these diseases. To this end, a robust volume of past

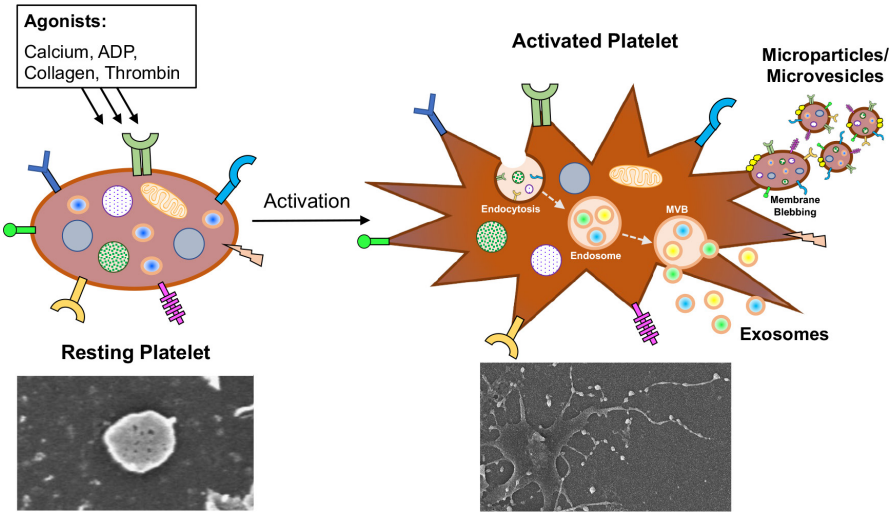

FIGURE 5 Platelet activation and production of platelet-derived extracellular vesicles (PEVs): Platelets undergo activation by a variety of agnonists; platelet activation leads to its shape and cytoskeletal changes, as well as the production of microparticles/microvesicles (100–1000 nm diameter) via membrane budding/blebbing processes, and exosomes (30–100 nm in diameter) via endocytic mechanisms, collectively known as PEVs. Representative scanning electron microscopy image of a resting discoid platelet and that of an activated "star-shaped" platelet secreting EVs are shown in the figure

and ongoing research has focused on the design of platelet-derived and platelet-inspired microparticle and nanoparticle platforms for therapeutic applications in hemostasis and thrombosis areas, but similar development in the areas of immune response modulation, inflammation, and cancer have been limited. Considering the persistent issues of systemic or off-target side effects that arise in the current pharmacological management of such pathologies, drug delivery using platelet-inspired nanomedicine platforms may provide highly innovative pathways to enhance therapeutic efficacy while maintaining systemic safety. Although innovation and preclinical evaluation of these approaches are under way at a promising speed, their clinical translation would require successful achievement of several critical milestones. For systems that use manipulation of donor platelets (e.g., membrane extraction, cytoplasmic content depletion, intracellular drug loading), there will be potential challenges in platelet availability, storage, functional heterogeneity, and reproducible manufacturing that are already evidenced in the existing framework of platelet transfusion products in the hemostatic management of patients.[135] One potential solution to such challenges can be the utilization of donor-independent platelets (e.g., production of platelets from stem cells using specific bioreactor systems). This approach is also under way in the preclinical stage, but requires optimization of the cost, scale, and time needed for such platelet production along with establishing functional efficacy and reproducibility, to be suitable for clinical translation.[136] On the other hand, for systems that use a fully synthetic approach of drug-loaded ligand-decorated platelet-mimetic nanoparticles, the cost, scalability, and production time may be optimized effectively because pf several decades of experience with nanoparticle technologies in the pharmaceutical sector (e.g., liposomal doxorubicin formulation Doxil approved in 1995, lipid nanoparticle-based mRNA vaccine Comirnaty for COVID-19 approved in 2021).[137] However, a potential challenge with such systems can be unwanted immune response from nanoparticles and off-target side effects.[138] Therefore, rigorous preclinical *in vitro* and *in vivo* evaluation of efficacy and pharmacology/toxicology parameters will be needed, along with meticulous navigation of the regulatory framework, to enable the establishment of unique nanomedicine strategies that leverage the role of platelets beyond hemostasis and thrombosis.

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CONFLICT OF INTEREST

Anirban Sen Gupta is an inventor on issued patents US 9107845B2, US9636383B2, US 10426820B2, and US 10,434149B2, all focused on composition and use of "synthetic platelets" technology. Anirban Sen Gupta is also a cofounder of Haima Therapeutics, where these patents are licensed for further translation and commercialization aspects. Anirban Sen Gupta is also an inventor on issued patent US 9107963 (Heteromultivalent Nanoparticle Compositions) for platelet-inspired drug delivery platform. Kellie R. Machlus is a consultant for Keros Therapeutics and STRM.BIO. Cian Desai and Milka Koupenova have nothing to disclose.

AUTHOR CONTRIBUTIONS

Cian Desai contributed to writing sections on platelet involvement in cancer and associated nanomedicine approaches. Milka Koupenova contributed to writing sections on platelet role in immune response. Kellie R. Machlus contributed to writing sections on platelet extracellular vesicles and editing other sections in the paper. Anirban Sen Gupta wrote sections on platelet mechanisms in immune response, inflammation and cancer, all sections on platelet-inspired nanomedicine technologies spanning the pathologies described here, prepared all schematic figures, and compiled the complete paper.

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