*Minireview*

# Platelet-mimetic strategies for modulating the wound environment and inflammatory responses

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## Abstract

Platelets closely interface with the immune system to fight pathogens, target wound sites, and regulate tissue repair. Natural platelet levels within the body can be depleted for a variety of reasons, including excessive bleeding following traumatic injury, or diseases such as cancer and bacterial or viral infections. Platelet transfusions are commonly used to improve platelet count and hemostatic function in these cases, but transfusions can be complicated by the contamination risks and short storage life of donated platelets. Lyophilized platelets that can be freeze-dried and stored for longer periods of time and synthetic platelet-mimetic technologies that can enhance or replace the functions of natural platelets, while minimizing adverse immune responses have been explored as alternatives to transfusion. Synthetic platelets typically comprise nanoparticles surface-decorated with peptides or ligands to recreate specific biological characteristics of platelets, including targeting of wound and disease sites and facilitating platelet aggregation. Recent efforts in synthetic platelet design have additionally focused on matching platelet shape and mechanics to recreate the marginalization and clot contraction capabilities of natural platelets. The ability to specifically tune the properties of synthetic platelet-mimetic materials has shown utility in a variety of applications including hemostasis, drug delivery, and targeted delivery of cancer therapeutics.

**Keywords:** Platelets, hemostasis, artificial platelets, platelet-mimetic, nanoparticles, bionanoscience

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## Introduction

Platelets provide several key functions during the process of hemostasis and tissue repair including targeting wound sites, facilitating hemostasis, and modulating the repair and regeneration process following injury. In addition to their role in hemostasis, platelets play a large role in the body's innate immune response to pathogens and other contaminants. Furthermore, platelets also interface with cancer cells and have been shown to contribute to cancer metastasis. The development of non-immunogenic platelet-mimetic technologies has the potential to greatly improve treatment of bleeding and may also have significant potential for targeted drug delivery for treatment of cardiovascular disease and cancer. Recently, researchers have used various platelet-mimetic strategies for such applications. In this review, we present an overview of platelet biology and immune-related functions, and then discuss recent platelet-mimetic strategies. Finally, we discuss how these strategies allow for the design of materials that interface with the immune system.

## Platelets in coagulation and hemostasis

Platelets are small, anuclear blood cells that play a primary role in coagulation and hemostasis following tissue injury. Platelets are derived from megakaryocytes, which become polyploid and form a demarcation membrane system (DMS) as they mature. This DMS allows for storage of membranes that can be released into proplatelet protrusions that then develop into mature platelets upon release and circulation through the bloodstream.[1,2] Their unique structural and biological components enable platelets to promote hemostasis and wound repair following injury (Figure 1). Platelets circulate in an inactive state; following initiation of the coagulation cascade, platelets become active, which stimulates adhesion of platelets to the injured vessel wall, platelet aggregation, and release of growth factors from platelet granules. In their inactive, or quiescent, state, platelets are stiffer than red blood cells. This stiffness causes them to be pushed towards the vessel wall during circulation in a process known as margination, which in turn gives them the proximity necessary to interact with and aggregate

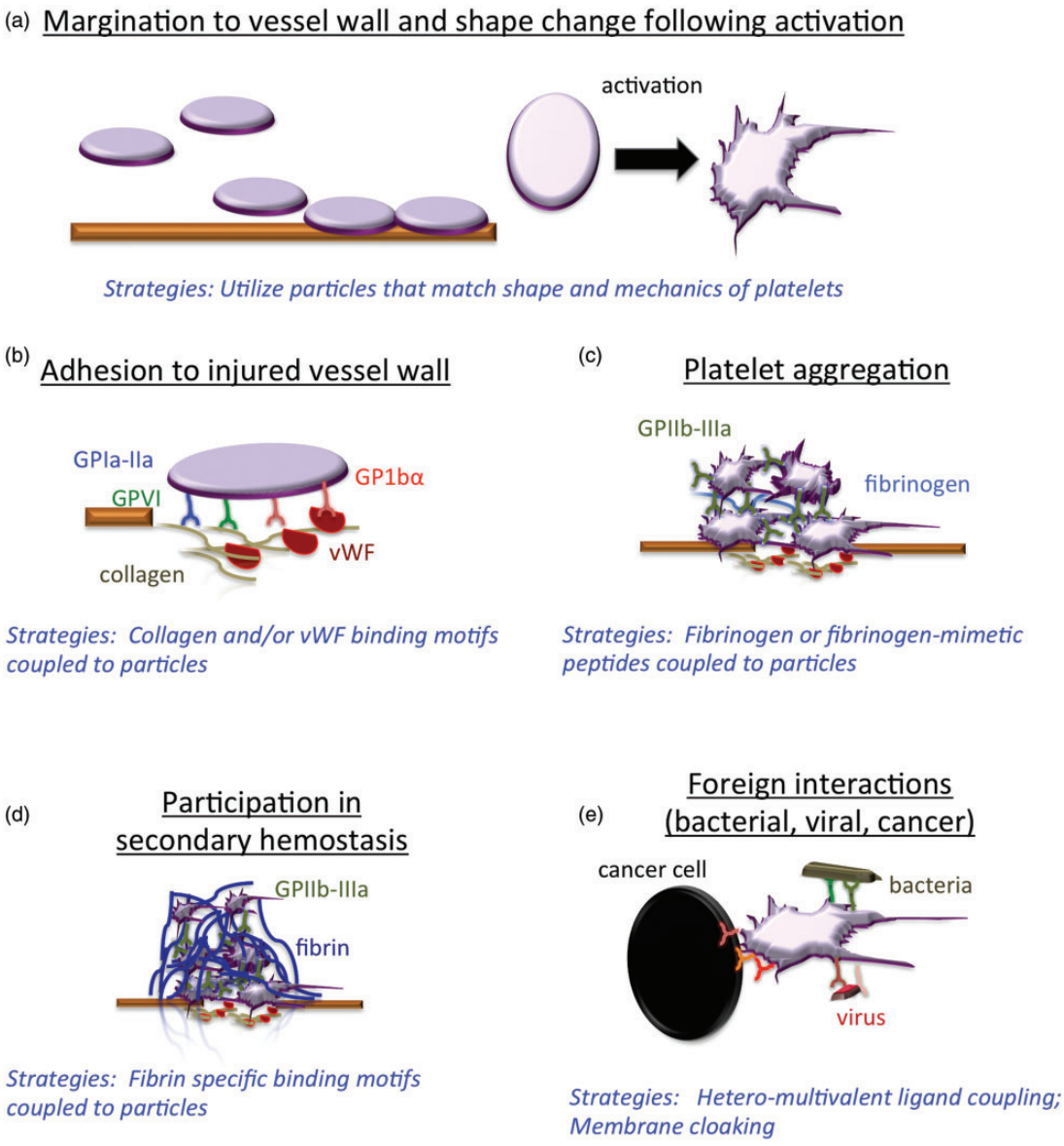


**Figure 1** Schematic overview of natural platelet functions and synthetic strategies for mimicking those functions, including shape change following activation (a), adhesion to injured vascular endothelium (b), aggregation (c), participation in secondary hemostasis (d), and interactions with foreign entities, such as cancer cells, bacteria and viruses (e). (a) When natural platelets become activated, they change shape and marginate towards vessel walls, where they can adhere and carry out hemostatic functions. One strategy for platelet-mimetic technologies is to create particles that can match this ability to change shape and carry out margination by utilizing particles that match the shape and mechanics of platelets. (b–d) Conjugating vWF, collagen-binding motifs, and/or fibrinogen to particles provides another strategy for mimicking natural platelet activity; the coupled proteins/binding motifs allow the particles to mimic platelet features such as targeting and aggregation at injury sites and participation in both primary and secondary hemostasis. (e) Platelet-mimetic technologies can also be used to mimic the immune functions of natural platelets. This has been achieved via heteromultivalent ligand coupling, which allows the particles to target pathogens or diseased cells, and through membrane cloaking in which drug delivery vehicles are encased in natural platelet membranes, allowing for targeted drug delivery with minimal immune responses. (A color version of this figure is available in the online journal.)

at injury sites along vascular vessel walls.[3–5] Upon activation, platelets undergo a shape change, deform extensively and "spread" along the vascular wall at injury sites.[3] The thrombogenic properties of endothelial collagen and the hemostatic behavior of natural platelets interact to form the basis for the hemostatic activity and clot contraction that occurs at the vascular wall.[3,6]

Hemostasis occurs in two phases, known as primary and secondary hemostasis. During primary hemostasis, platelets target and aggregate at the injury site to form a "plug" of sorts.[7] Platelets bind to exposed sub-endothelial collagen at the injury site via the von Willebrand factor (VWF), which is released from the Weibel–Palade bodies of injured vessel walls.[8] The A1 domain of the secreted VWF interacts with GPIbα, a ligand-binding protein within the glycoprotein GP-Ib-V-IX complex on the platelet's surface, allowing the platelets to adhere to the damaged vascular wall.[9–11] GPIbα also allows platelets to activate under high-shear conditions, which facilitates platelet aggregation at wound sites.[10] The activated platelets then spread along the wall and adhere to each other, forming a platelet plug to stem initial blood loss. Secondary hemostasis is characterized by the activation of the blood coagulation cascade and results in the formation

of a fibrin clot.[7] These events occur alongside primary hemostasis, but rather than facilitating platelet aggregation, they induce the formation of an insoluble fibrin clot through the binding of the pro-coagulant tissue factor (TF) molecule to coagulation factor fVII, forming a TF/fVII complex that can cleave factorX into factorXa, a molecule that generates thrombin. Thrombin activates the adherent platelet aggregate, allowing several other pro-coagulant factors to bind to the surface of the aggregate. Thrombin also activates fibrinogen, which in turn produces a cross-linked fibrin mesh. Platelet activation also results in an increase of GPIIb-IIIa, a platelet surface integrin that serves as a receptor for fibrinogen and carries out mediated fibrinogen binding during the fibrin-platelet crosslinking process.[10,12] The fibrin mesh and platelet aggregate form a complete clot that can prevent further blood loss.[7,13]

Following the cessation of bleeding, cytoplasmic motility proteins, including actin and myosin, facilitate platelet clot contraction, resulting in expulsion of serum from the clot.[14] The contractile forces generated by the platelet-rich plasma within the clot increases with time, forming a seal at the clot site to fortify the hemostatic capabilities of the clot. Local fibrin concentration and clot stiffness increase as clot contraction occurs over time; therefore, it is likely that platelets actively increase their contractile forces in response to the changing fibrin dynamics over time. Indeed, atomic force microscopy experiments with single platelets attached to fibrinogen-coated surfaces demonstrate that force generation by individual platelets increases in response to increased cantilever stiffness.[15] Additionally, platelets become increasingly activated on increasingly stiff fibrinogen-coated surfaces.[16] Clot contraction promotes wound healing by decreasing the clot surface area, which allows greater blood flow to the healing tissue.[14] Clot contraction is supplemented by fibrinolysis, the process by which clots are degraded, following contraction in order to prevent thrombosis. Interestingly, clot retraction plays a role in the dynamics of fibrinolysis, by increasing clot stability and decreasing clot susceptibility to fibrinolysis.[17,18]

## Platelets and the immune system

The unique structure and composition of platelets allow them to interface with the immune system after injury by targeting wound sites, facilitating healing, and preventing infection or contamination from occurring at the site (Figure 1). Platelets are involved in several of the mechanisms comprising the first lines of defense against pathogenic substances in the body; circulating platelets have scavenger receptors, including CD36, on their surfaces that constantly scan the surrounding area for the presence of potentially dangerous molecules or molecular patterns that could be indicative of pathogenic activity.[19] As part of the innate immune system, platelets have a significant role in the inflammatory response at sites of injury and/or disease through activation of and close interaction with leukocytes (regulated by cathepsin G), secretion of chemokines and cytokines that attract other immune cells, and through additional mechanisms that influence the body's adaptive immunity.[10,20–23] Tissue inflammation and healing are further modulated by protease-activated receptors (PARs) found on platelet surfaces; these PARs stimulate the release of alpha granules, which contain various growth factors and angiogenic factors that aid in tissue repair.[24,25] Many glycoproteins found on the platelet surface membrane, such as CD55 and CD59, are a part of the complement system, which eliminates particulate invaders by providing recognition mechanisms for phagocytes that can then clear invaders from the body via phagocytosis.[4] Other glycoprotein receptors on the platelet surface membrane, such as (GP)IIb-IIIa, GPIbα, FcγRIIa, and toll-like receptors (TLRs), are triggered when bacteria bind to the platelet surface; these receptors, in turn, induce the secretion of antimicrobial peptides known as platelet microbicidal proteins, which mediate chemotaxis of phagocytes, and cause the platelet to shift from a quiescent to an activated state.[10,26,27] Additionally, TLRs are critical to thrombosis, activation of the immune system cells, and determination of both non-specific and specific immune responses to various pathogens.[28,29] Platelets also express Fc-receptors for antibodies, which, together with the complement receptors, allow them to bind virus-antibody complexes.[30]

Interactions between pathogens and platelet surface receptors can trigger platelet activation and thrombus formation. Bacterial binding can occur via direct or indirect methods, including binding directly to a receptor on the platelet's surface, binding of secreted bacterial toxins to platelet surface receptors, or binding to a plasma protein that functions as a ligand for a platelet membrane receptor.[10] Platelets can also interface with viral particles as part of the body's immune response; however, in the case of certain viral infections such as HIV, dengue virus, and hepatitis C, this interaction can result in the destruction of platelets or platelet function and can lead to potentially fatal levels of thrombocytopenia and bleeding.[30,31]

Despite the variety of capabilities that platelets possess to fight pathogenic agents within the body, interactions between platelets and pathogens can also create severe problems for the immune system. For example, the thrombotic response of platelets in the presence of bacteria can lead to events such as strokes or pulmonary embolisms if the thrombus forms on a heart valve. Even microthrombi can present large problems if they result in blockade of the body's microvasculature.[10] Additionally, in cancer patients, the innate behavior of platelets that causes them to adhere to pathogens and injured vasculature results in the aggregation of platelets around tumor cells. This can have the unintended effect of promoting tumor survival in the bloodstream without creating a noticeable immune response, allowing the tumor cells to metastasize and extravasate to tissues or organs other than their tissue of origin.[32]

## The need for platelet-mimetic materials

As discussed in previous sections, several conditions, such as traumatic injury, viral or bacterial infection, and chemotherapy, result in an insufficient number of natural platelets to maintain hemostasis and innate immunity within the body. The decrease in platelet levels within the body

during viral infections can lead to severe and potentially fatal bleeding, demonstrating the need for platelet-mimetic materials for the treatment of diseases such as HIV and Hepatitis C.[30] In cases of traumatic injury, patients often bleed out in the pre-hospital phase; therefore, platelet-mimetic materials could provide sufficient stabilization in traumatic injury victims to facilitate survival until hospital care is received.[33] In cancer patients, chemotherapy significantly decreases platelet counts and often results in significant bleeding problems, requiring multiple platelet transfusions; these immunocompromised patients would benefit greatly from synthetic platelet strategies.[34,35]

Platelet-mimetic materials could also improve targeted drug delivery, since current targeting technologies are hampered by nonspecific binding and insufficient margination and circulation and thus cannot target specific molecules or structures effectively.[36] Since platelets have the ability to marginate to the vascular wall, target injury sites, and can effectively circulate throughout the body, the use of platelet-mimetic technologies that mimic these features could allow researchers and clinicians to more effectively deliver drugs to specified locations within the body for improved therapeutic action. These agents could be used to treat bleeding and hemorrhages, as well as thrombosis, atherosclerosis, and cancer.

There are a wide variety of applications for platelet-mimetic materials, and current strategies typically focus on replicating various biochemical or mechanical properties of platelets to replicate specific desired functions of natural platelets, such as stabilization and augmentation of clot formation (Figure 1).[36–38] Current research in the field of platelet-mimetic technologies utilizes a wide variety of strategies including both naturally derived platelet derivatives and synthetic platelet strategies. In subsequent sections, we review various strategies that encompass a large range of approaches to achieve various goals such as wound targeting, drug delivery, and immune system evasion through platelet membrane cloaking.

## Platelet-mimetic strategies and technologies

### Natural platelet derived approaches

Allogeneic platelet transfusion is the most commonly used platelet therapy in the clinic. In clinical cases involving low platelet counts, platelet transfusions are often used in order to supplement or replace the activity of the body's own natural platelets.[39] Platelet transfusion has the advantage of utilizing natural platelets that innately possess the ability to biologically, biochemically, and mechanically interface with the immune system, endothelial cells, and other platelets to the required degree to supplemental lost platelet function, especially platelet functions required for hemostasis. However, platelet transfusion is limited by the relative lack of donors, storage cost, and short shelf life of donated platelets, and the fact that most platelet therapy is done prophylactically rather than therapeutically.[40,41] The short shelf life of stored platelets makes the development of new therapeutic natural platelet-based technologies difficult.[42] Even though recent technologies have improved the shelf life of natural platelets to up to seven days, this shelf life does not allow for a "bank" of platelets to be generated, which may be necessary for therapeutic applications requiring large volumes of platelets; furthermore, stored platelets are placed at an increasing risk of bacterial contamination that could result in a severe immunologic response if used in a transfusion.[43,44] Additionally, studies by Ponschab et al.[44] demonstrated that platelets increasingly lose their ability to aggregate over the short period of time in which they are kept in storage, further complicating the use of natural platelets as a viable means of treatment for vascular injury or disease. Platelet transfusion also has the inherent risk of transmission of blood-borne diseases; polymorphism of human platelet antigen (HPA) and human leukocyte antigen (HLA) after transfusion can also lead to complications such as transfusion refractoriness, which is the failure to achieve the desired level of platelets following transfusion, and von Willebrand's disease.[22,43,45] Platelet transfusion can also be potentially pro-inflammatory or highly immunogenic; recipients of donated platelets run the risk of alloimmunization, exposure to the transmission of infections, graft-vs-host disease, or transfusion-related immunosuppression, which could increase their vulnerability to a host of other harmful pathogens.[20,46]

Therefore, the short shelf life of natural platelets poses a considerable restriction on the practicality of platelet transfusion therapy. This shortcoming is being combated through the use of lyophilized platelets, which can be dehydrated and freeze-dried in order to improve the length of their shelf life.[46] Freeze-dried platelets can be prepared by paraformaldehyde-treating platelets, washing in citrated saline prior to freezing, and rehydrating in citrated saline upon thawing. This freezing and thawing process allows the lyophilized platelets to retain their hemostatic functionality, unlike previously investigated cryopreserved lyophilized platelets washed in standard saline that were unable to form the characteristic primary hemostatic platelet plug after reconstitution.[47] However, in vivo studies in animal models indicate that these freeze-dried lyophilized platelets, although capable of forming a hemostatic plug, have a short duration of circulation, lasting only 9.5 min prior to being cleared from the bloodstream.[48] These lyophilized platelets have also been shown to have the potential to increase thrombogenicity and antigenicity, and aggregate in the spleen.[46] Therefore, the use of lyophilized platelets lacks the reliability that is required for large scale clinical application of naturally derived platelet replacements.

All platelet-substitute technologies utilizing donated natural platelets, including transfusion and lyophilized approaches, have a major drawback – introducing allogeneic platelets into the body can result in an immune response that causes the recipient's body to reject the donated platelets, preventing them from addressing the injury or infection in question and further weakening the body as it attempts to heal from an injury or fight harmful pathogens. The risks of recipients developing adverse immune responses can be minimized through blood-matching; however, blood-matching complicates this therapeutic strategy by requiring clinicians to search through already limited stores of platelets in an attempt to find a matched source, making naturally derived platelet substitutes inefficient

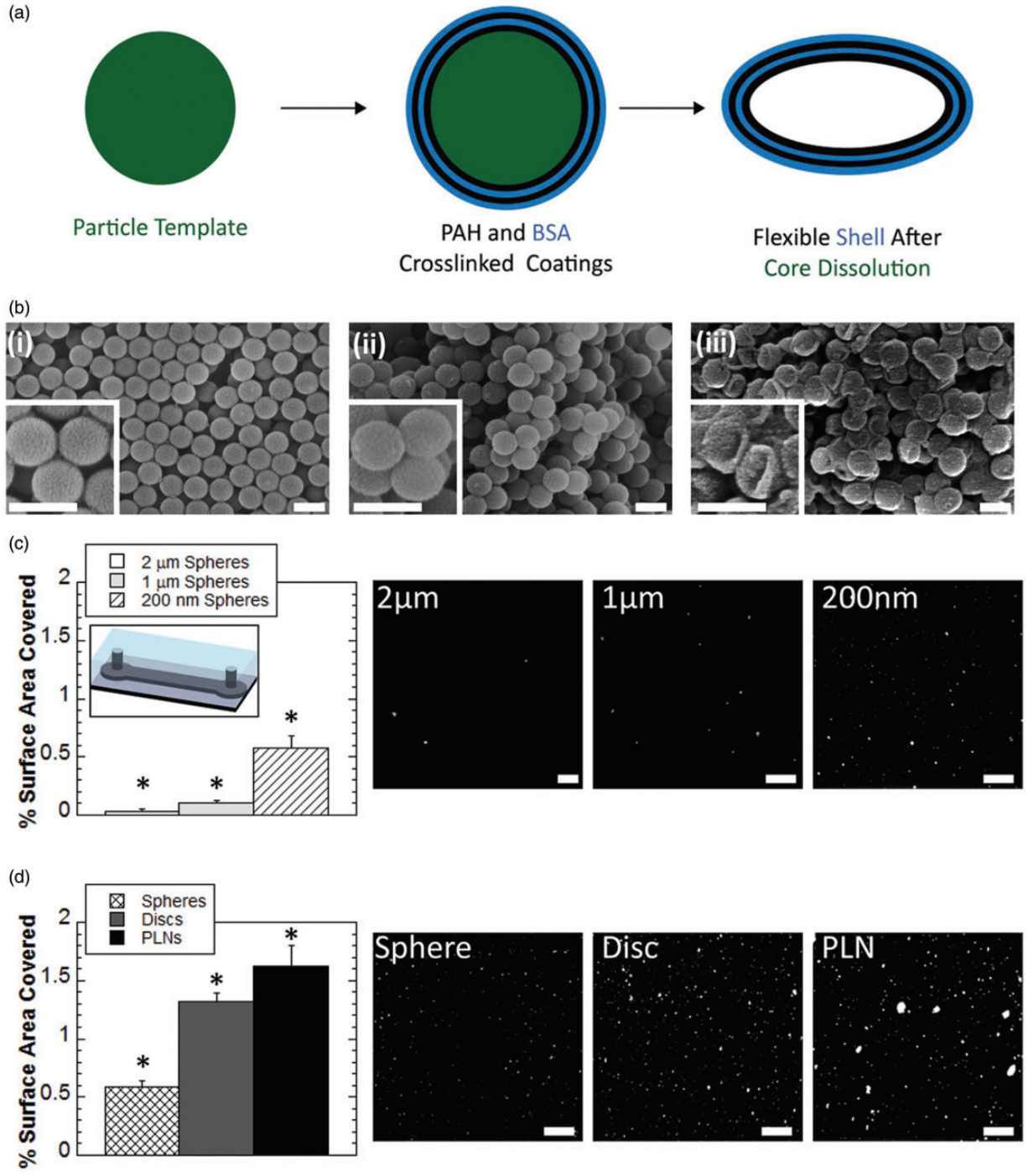


**Figure 2** Synthesis and analysis of platelet-like nanoparticles developed by Anselmo et al.[36] (a) schematic and (b) scanning electron microscope images of layer-by-layer synthesis of nanoparticles using alternating layers of bovine serum albumin and poly(allylamine hydrochloride) (PAH) and a degraded core to achieve deformable particles with platelet-like discoid morphology. Scale bars = 200 nm. (c) Percentage of surface area coverage of ovalbumin-coated spherical particles of various sizes; (d) Percentage of surface area bound by platelet-like nanoparticles in comparison with ovalbumin-coated particles bound by larger spherical or discoid particles. Scale bars = 20 nm. This is an unofficial adaptation of an article that appeared in an ACS publication. ACS has not endorsed the content of this adaptation or the context of its use. Reproduced from (Anselmo AC, Modery-Pawlowski CL, Menegatti S, Kumar S, Vogus DR, Tian LL, Chen M, Squires TM, Sen Gupta A, Mitragotri S. Platelet-like nanoparticles: mimicking shape, flexibility, and surface biology of platelets to target vascular injuries. *ACS Nano* 2014 **25**;8:11243–53.) (A color version of this figure is available in the online journal.)

and unreliable for use in emergency trauma-related treatments.[45] As an alternative approach, studies performed by Nguyen et al.[24] demonstrated that the body's existing platelets can be differentially stimulated using the protease activated receptor agonists PAR-1-agonist and PAR-4-agonist to selectively secrete VEGF, to promote angiogenesis, or endostatin, an anti-angiogenic factor. While the conclusions they drew from this study need to be tested under more applicable physiological conditions, such as in the presence of microbial agents, this method may present a useful

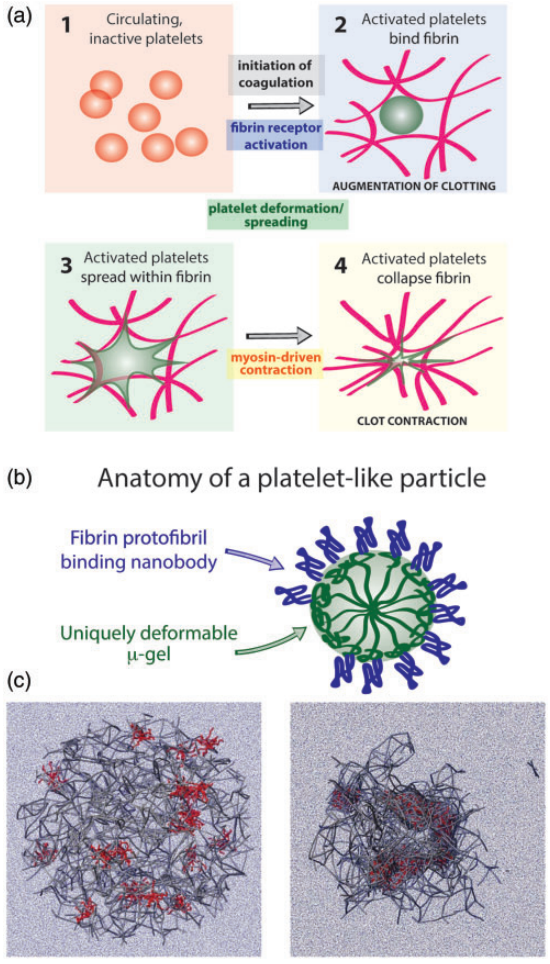


**Figure 3** Structure and behavior of platelet-like particles developed by Brown et al. (a) Schematic of natural platelet behavior mimicked by platelet-like particles during clot formation and collapse. (b) Schematic of the structure of the platelet-like particles; ultra-low cross-linked pNIPAM-based microgels are coupled to H6 sdFvs to confer fibrin specificity. (c) In silico demonstration of clot collapse induced by platelet-like particles. Reprinted by permission from Macmillan Publishers Ltd: [Nature Materials] (Brown AC, Stabenfeldt SE, Ahn B, Hannan RT, Dhada KS, Herman ES, Stefanelli V, Guzzetta N, Alexeev A, Lam WA, Lyon LA, Barker TH. Ultrasoft microgels displaying emergent platelet-like behaviours. *Nat Mater* 2014;**13**:1108–14.), copyright (2014). (A color version of this figure is available in the online journal.)

future method of utilizing natural platelets and their existing immunological and healing mechanisms on an amplified scale without the need for platelet transfusions.

## Artificial platelets

Artificial platelet-mimetic technologies address the immunogenicity issues raised by natural platelet-based treatments, because artificial materials do not need to be blood-matched prior to therapeutic use, but must still be evaluated for biocompatibility and other potential adverse effects, such as thrombotic responses.[49] The use of artificial platelet-mimetic materials prevents adverse immune responses from developing within the body as well as decreases clearance of targeted therapeutic nanoparticles from the bloodstream. Clearance of nanoparticle-based technologies is low because they are generally not recognized or marked by the immune system, and clearance can be further modified through nanoparticle size or material parameters.[36,50] Synthetic materials have further advantages over natural platelets due to their longer shelf life, scale-up potential, and ease of manufacturing and reproducibility.[3] The material properties of artificial platelet-mimetic devices can also be tuned to reflect the mechanical properties of natural platelets in both their resting and active conformations, giving researchers a greater level of control over obtaining specific desirable characteristics, such as clearance time, for various particular clinical applications.

A multitude of design strategies have been utilized to create artificial platelets and typically entail the coupling of a particle or polymer platform with a binding motif that facilitates interactions at a wound site (Figure 1). Particle platforms previously utilized for artificial platelets have included liposomes, albumin microparticles, latex particles, and even erythrocytes.[3] Wound targeting strategies vary widely and having included targeting elements which bind to the subendothelial matrix, platelets, both the subendothelial matrix and platelets, and fibrin. Examples include coupling of full-length fibrinogen, fibrinogen-derived RGD-peptides and other fibrinogen-mimetic peptides, platelet surface glycoproteins, and fibrin-specific single domain variable fragment antibodies (sdFvs). Past and state-of-the art strategies to create artificial platelets have been reviewed in detail by others recently.[3,51,52] Several recent attempts to develop artificial platelet technologies have focused on designing particles capable of mimicking the shape and mechanical properties of natural platelets; we primarily focus this review on such attempts.

Platelets are generally found in the bloodstream in their inactive, or quiescent, state, and only shift to their active, spread conformation in the presence of an injury or pathogen.[36] Attempts to mimic natural platelet shape and mechanics could potentially lead to particles that are capable of replicating the shape change of natural platelets following activation. Efforts in this area include the creation of particles capable of mimicking the mechanical properties of active platelets, then coupling with various binding agents to confer specificity for molecules present in active wound sites.[11,36,38] Doshi et al.[11] formed synthetic platelet-like particles by stretching polymeric particles into a discoid shape reminiscent of the true shape of natural platelets, crosslinking alternating layers of bovine serum albumin and polyelectrolytes over the stretched discoid particles, then degrading the polymeric core to leave a flexible discoid capable of imitating the mechanical stretching of natural platelets.[11] Anselmo et al.[36] also made use of a layer-by-layer synthesis method with a degraded core to imitate innate platelet deformability while synthesizing their own platelet-like nanoparticles (Figure 2).[36] In an alternative approach, Brown et al.[38,53] used soft pNIPAM-based microgels synthesized via precipitation polymerization to yield ultra-low cross-linked particles with a high degree of flexibility that allow for extensive spreading within fibrin clots.

Following structural design development, platelet-like particles with appropriate mechanical properties can be functionalized with various molecules to confer targeting

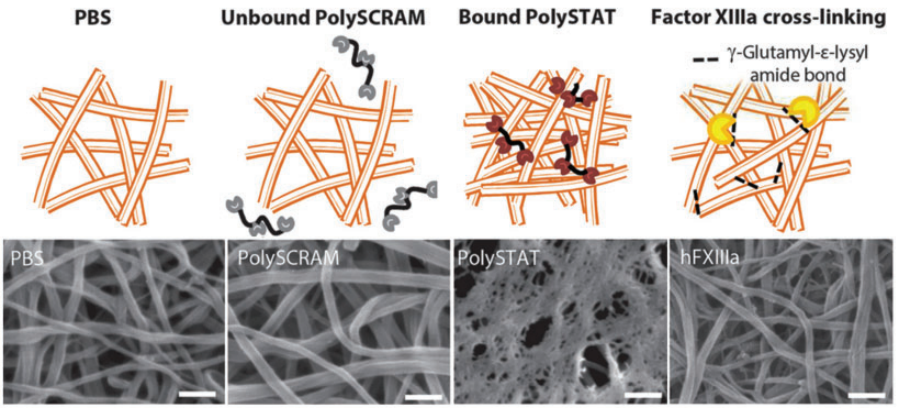


**Figure 4** Schematics and scanning electron microscope images of in vitro PolySTAT-induced fibrin clots. Fibrin clots showed binding specificity for PolySTAT over PBS and scrambled nonbinding polymer PolySCRAM. PolySTAT induced clot formation through fibrin crosslinking in a manner reminiscent of natural factor XIIIa-induced crosslinking. Scale bars = 250 nm. From (Chan LW, Wang X, Wei H, Pozzo LD, White NJ, Pun SH. A synthetic fibrin cross-linking polymer for modulating clot properties and inducing hemostasis. *Sci Transl Med* 2015;**7**:277ra29.). Reprinted with permission from AAAS. (A color version of this figure is available in the online journal.)

capabilities onto the particle surface. This can be done via a variety of targeting methods, including peptides, antibodies, and growth factors. Doshi et al.[11] conferred targeting abilities to their particles via the VWF-A1 domain or GPIbαN (an amino terminal domain of GPIbα), which resulted in particles that adhere to injured endothelial vascular walls under high shear stress, and to each other to form an aggregate at the injury site. Particles synthesized by Anselmo et al.[36] were heteromultivalently surface-decorated with peptides to give them binding specificity for exposed collagen and VWF in the vascular injury site and GPIIb-IIIa on activated natural platelets. This method allows for simultaneous targeting of the wound site and aggregation of platelets to each other, increasing the efficacy of the particles in targeting wound sites and decreasing bleeding time. A study performed by Ravikumar et al.[54,55] also involved particles surface-decorated with VWF and collagen-binding ligands, with the purpose of promoting binding to VWF under shear flow and binding to collagen independent of shear flow in order to imitate the dual-binding adhesion and aggregation capability of natural platelets. The platelet-like particles developed by Brown et al.[38] use sdFvs, identified through phage display techniques, to obtain a high specificity for fibrin, allowing the particles to target fibrin clots, and therefore secondary hemostasis (Figure 3). These platelet-like particles were found to spread within the fibrin network, and induce collapse of the fibrin network, without binding fibrinogen present in the blood stream or in areas without injured vasculature. The particle-mediated clot collapse was found to be dependent on the deformability of the particles coupled with high affinity for fibrin, conferred by the sdFv.[38] Fibrin monomers cleaved from fibrinogen by thrombin enzymes also possess the ability to self-polymerize and assemble into a three-dimensional scaffold. Transglutaminase factor XIIIa supplements the scaffold by increasing the fiber density and helps to stabilize the clot by creating crosslinks between the fibers.[56] Pun and her coworkers developed a synthetic hemostatic polymer known as PolySTAT that mimics the crosslinking behavior of factor XIIIa to stabilize clots and inhibit fibrinolysis via incorporation of polymers that are resistant to plasmin enzyme degradation (Figure 4). PolySTAT was synthesized from poly(HEMA) copolymerized with NHSMA monomers to create a polymer capable of a high degree of peptide grafting. Once inserted into the body, PolySTAT bound noncovalently to fibrin monomers to strengthen clots. Haji-Valizadeh et al.[8] developed an alternative strategy for creating artificial platelets, involving the use of self-assembled peptide-lipid nanoconstructs that can adhere to injury sites and facilitate aggregation of active platelets. The nanoconstructs were coated in a factor FVIII-VWF binding peptide that was able to promote the injury-site adhesion component of the design, while additional fibrinogen-mimetic peptides on the nanoconstruct surface functioned to promote platelet aggregation. The use of the factor FVIII-VWF binding peptide allowed the nanovehicles to target and bind to injury sites without obstructing the binding mechanisms of natural platelets, which resulted in a greater degree of overall aggregation than was possible with the synthetic or natural platelets alone.[8] Overall, these recent approaches have resulted in platelet-mimetics that more closely recapitulate natural platelet mechanics and shape.

## Cloaking and active targeting mechanisms for drug delivery

Platelet technology also has potential applications in the field of targeted drug delivery. The concept of ''pairing'' a drug delivery vehicle with native cells can be illustrated within the natural interactions of the immune system, as platelets' endothelial cell adhesion molecules often bind to antibodies to increase the efficacy of both components in binding to their targets in the injured vascular endothelium.[57] The discoidal shape of platelets also makes them a prime candidate for providing cloaking mechanisms to drug delivery particles, since more discoidal particles tend to marginate to vessel walls under shear flow, allowing specific targeting of injured vascular sites.[58] Platelet

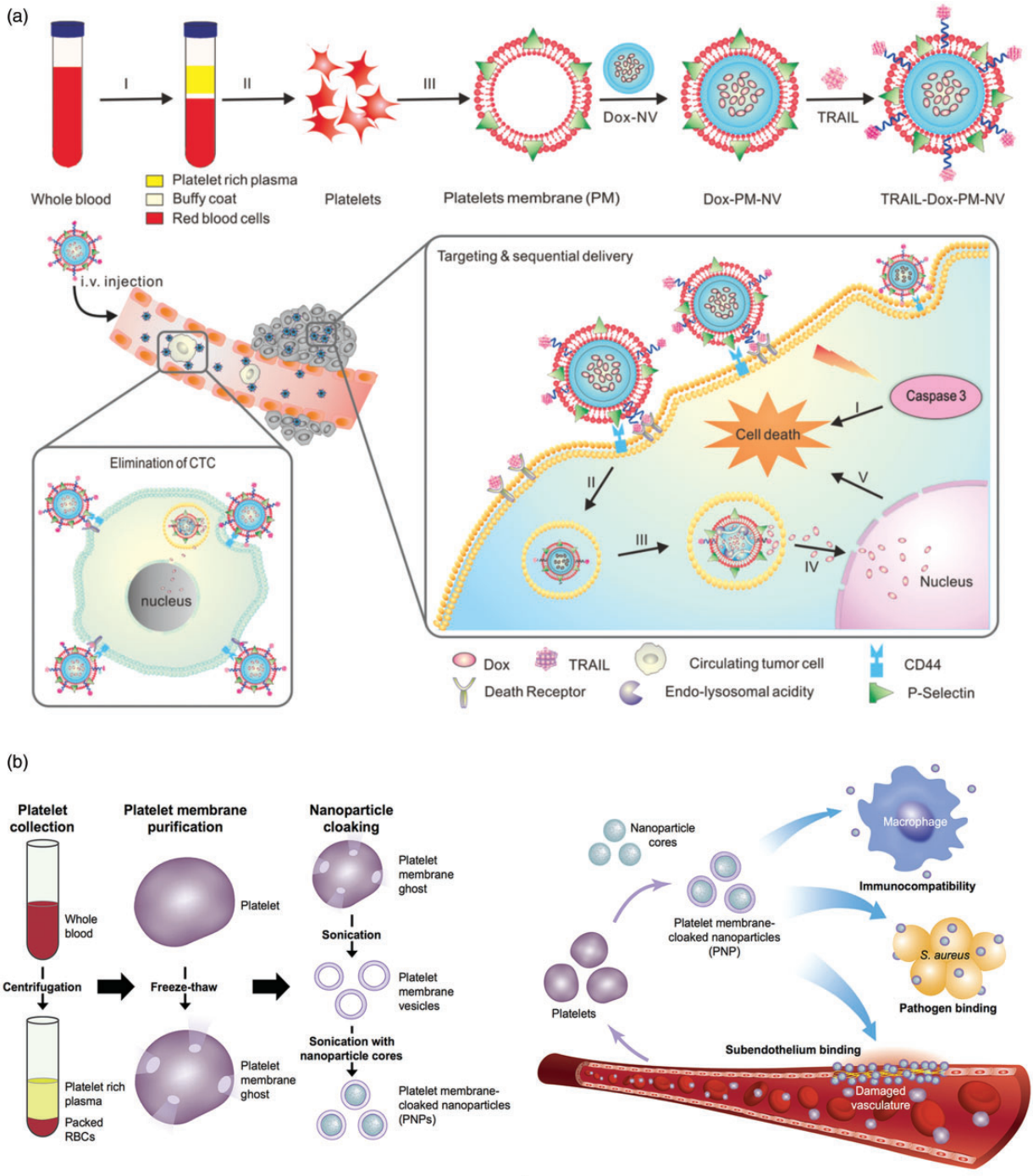


**Figure 5** (a) Schematic of platelet membrane-enclosed nanovehicle drug delivery mechanism developed by Hu et al.[59] Platelet membranes are isolated via centrifugation and then used to enclose the doxorubicin-nanovehicle particle. The membrane-doxorubicin-nanovehicle particle is loaded with TNF-related apoptosis ligand (TRAIL) and inserted into the bloodstream, where it can target and deliver doxorubicin and TRAIL to circulating tumor cells. Reprinted by permission from Wiley (Hu Q, Sun W, Qian C, Wang C, Bomba HN, Gu Z. Anticancer platelet-mimicking nanovehicles. *Adv Mater* 2015 September). (b) Drug delivery vehicle developed by Hu et al.,[32] in which PLGA nanoparticles are enclosed in platelet membranes obtained via centrifugation to confer immunocompatibility and targeting specificity for injured vascular endothelium to the PLGA nanoparticles. Reprinted by permission from MacMillan Publishers Ltd: [Nature] (Hu C-MJ, Fang RH, Wang K-C, Luk BT, Thamphiwatana S, Dehaini D, Nguyen P, Angsantikul P, Wen C, Kroll A, Carpenter C, Ramesh M, Qu V, Patel S, Zhu J, Shi W, Hofman F, Chen C, Gao W, Zhang K, Chien S, Zhang L. Nanoparticle biointerfacing by platelet membrane cloaking. *Nature* 2015 September 16;**526**:118–21.), copyright (2015). (A color version of this figure is available in the online journal.)

membrane-cloaked nanoparticles can be used in a similar manner to deliver drugs to specific structures, such as wound sites or thrombi, in the body without causing a severe immune response, which could prevent the drug capsules from being delivered effectively. This membrane-encapsulated strategy allows for greater circulation time, since high clearance rates are often a hindrance to effectively targeted drug delivery, decreased immunogenicity, and improved specific targeting.[59] Cloaked drug delivery can also suppress the complement system, increasing the

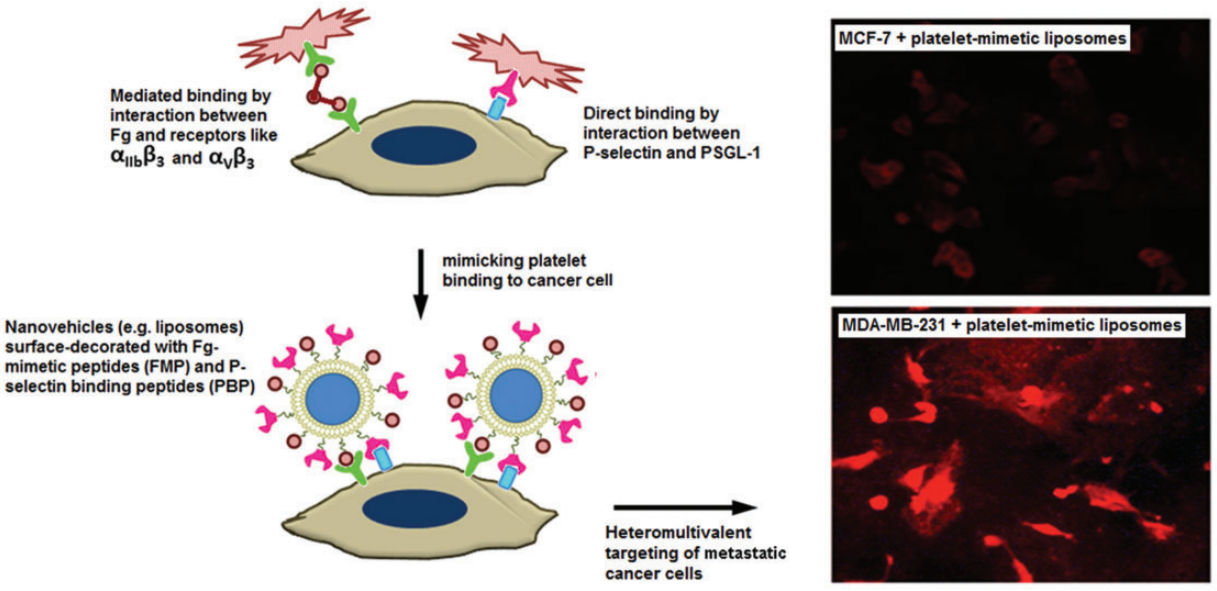


**Figure 6** Drug delivery nanovehicles are surface-decorated with peptides to target and bind P-selectin and FMP. Once bound, these constructs can target and deliver anticancer drugs to metastatic cancer cells. Reprinted (adapted) with permission from (Modery-Pawlowski CL, Master AM, Pan V, Howard GP, Sen Gupta A. A platelet-mimetic paradigm for metastasis-targeted nanomedicine platforms. *Biomacromolecules.* 2013;**14**:910–9). Copyright (2014) American Chemical Society. (A color version of this figure is available in the online journal.)

antimicrobial efficacy of the system, and has several potential applications in the field of cancer nanomedicine due to the natural aggregation of platelets around metastatic tumors.[32,59,60]

The Gu research group has developed a platform for delivering anticancer drugs within a platelet membrane-enclosed nanovehicle (Figure 5(a)). The outer platelet membrane shell of the particle is coated with proteins such as P-selectin, which allow the particle to bind CD44 receptors on cancer cells, and the inner nanogel is loaded with doxorubicin, a small-molecule anticancer drug that can be released upon internalization and subsequent digestion of the particle by cancer cells.[59] In vivo testing of the particles in MDA-MB-231 tumor-bearing mice revealed that the particles exhibit significant antitumor effects with minimal immunogenicity.[59] Hu et al.[32] developed a nanoparticle drug delivery system cloaked in platelet plasma membranes to reduce clearance of the nanovehicles by macrophages in the immune system (Figure 5(b)).[32] In their design, polymeric nanoparticles were loaded with drugs and enclosed in platelet plasma membranes that had previously been treated to remove thrombotic molecules, thus decreasing the chance of an immune response when inserted into the body.[32]

Alternative methods of active targeting involve nanoconstructs that have been surface-decorated with various peptides or ligands that can bind to areas on active platelets, injured vessel walls, or cancer cells. Modery et al.[61] developed a platform for targeting injury sites using surface-decorated nanoconstructs. The nanoconstructs are coated with peptides that have a high affinity for GPIIb-IIIa and P-selectin, which bind active platelets in sites of vascular disease or injury. These peptides allow the nanoconstructs to target specific wound sites with a high level of specificity and release thrombolytic or anti-proliferative therapeutic drugs at sites of injury, thrombosis, or vascular disease such as atherosclerosis.[61] Similar heteromultivalent ligand-receptor pathway surface-decorated nanoconstructs were later used by this group to target and deliver anticancer drugs to metastatic MDA-MB-231 and MCF-7 cancer cells (Figure 6).[37]

## Conclusion

Platelets have a multitude of functions in addition to their vital role in coagulation and hemostasis, including modulating immune functions through interactions with pathogens and cytokine and growth factor release, immune cloaking, and modulation of cancer metastasis. The design of platelet mimetic technologies has shown promise for augmentation of hemostasis following traumatic injury, targeted delivery of cancer therapeutics, and improved immunocompatibility of nanoparticle platforms. As researchers continue to develop more sophisticated strategies for mimicking platelet features and functions, and as we learn more about platelet biology, we can likely expect to continue to see additional application of artificial platelet technologies. Thus, while artificial platelet technologies emerged in an effort to merely supplement the hemostatic function of platelets, the potential application of artificial platelet technologies clearly extends into numerous additional areas including targeted drug delivery, cancer therapeutics and immune modulation.

**Author Contribution:** SN and ACB wrote the manuscript and prepared figures.

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**DECLARATION OF CONFLICTING INTERESTS**

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