

# Recent advancements in erythrocytes, platelets, and albumin as delivery systems

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**Abstract:** In the past few years, nanomaterial-based drug delivery systems have been applied to enhance the efficacy of therapeutics and to alleviate negative effects through the controlled delivery of targeting and releasing agents. However, few drug carriers can achieve high targeting efficacy, even when targeting modalities and surface markers are introduced. Immunological problems have also limited their wide applications. Biological drug delivery systems, such as erythrocytes, platelets, and albumin, have been extensively investigated because of their unique properties. In this review, erythrocytes, platelets, and albumin are described as efficient drug delivery systems. Their properties, applications, advantages, and limitations in disease treatment are explained. This review confirms that these systems can be used to facilitate a specific, biocompatible, and smart drug delivery.

**Keywords:** albumin, biological drug delivery systems, erythrocyte, platelet

## Introduction

Drug delivery systems aim to improve the pharmacological properties of drugs and to achieve maximal therapeutic efficacy and minimal side effects by directing therapeutic cargos to target cells and tissues.<sup>1,2</sup> Novel drug delivery systems have been developed because conventional and new drugs provide intrinsic disadvantages, such as poor stability, poor solubility, gastrointestinal reactions, and unwanted toxicity.

With remarkable advancements in nanotechnology, nanomaterials and nanostructures have been widely applied. Nanotechnology- and nanoscience-related studies have been extensively conducted over the past decades. Nanomaterial-based drug delivery systems have also been rapidly developed.<sup>3,4</sup> Various nanomaterials, including nanoparticles,<sup>5</sup> polymeric micelles,<sup>6</sup> liposomes,<sup>7</sup> and nanotubes<sup>8</sup> have been utilized as drug carriers. Nanocarrier-delivered drugs provide several advantages over free drugs; for instance, nanocarriers can prolong circulation time, slow down metabolism, induce controlled drug release, and improve patient convenience and compliance.<sup>9</sup> When used for chemotherapy, nanocarriers can enhance drug targeting to tumors because of their selective accumulation in tumors through enhanced permeability and retention (EPR) effects. EPR effects or passive targeting effects that often occur in solid tumors involve nanocarriers or macromolecules that preferentially accumulate in tumors rather than in normal tissues, because of leaky vasculature and poor lymphatic drainage in tumor cells and tissues.<sup>10</sup> However, some hypoxic regions of solid tumors may fail to elicit EPR effects because of poor angiogenesis.<sup>11</sup> Thus, active targeting strategies, including coupling with homing peptides,<sup>12</sup> antibodies,<sup>13,14</sup> and growth factors,<sup>15</sup> have been employed to overcome the limitations of passive targeting. Although major advancements have been observed in this field, neither passive targeting nor active targeting can achieve

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specific nonrandom targeting that remains chance dependent. Human bodies are equipped with innate immune defenses, such as the reticuloendothelial system (RES), which rapidly recognizes and destroys foreign objects. As such, nanocarriers may be engulfed by macrophages of the mononuclear phagocyte system before these carriers reach target sites.<sup>16</sup> As the immune system catches up in the game of hide and seek, biological drug carriers have been developed to bypass immune surveillance.<sup>16,17</sup>

Biological carriers, such as erythrocytes, platelets, and albumin, can bypass immune surveillance and provide several advantages, including long circulation time, good biodegradability, abundant surface ligands, and flexible morphology. Therefore, these carriers are optimum biological delivery systems and potential solutions to overcome the limitations of nanomaterial-based drug carriers.<sup>18,19</sup> This review describes erythrocytes, platelets, and albumin as promising biological drug carriers and their applications in various diseases.

## Erythrocytes as drug delivery systems

### Properties of erythrocytes

Erythrocytes or red blood cells (RBCs) are enucleated biconcave disk-shaped cells in humans. RBCs are characterized by a diameter of  $\sim 7\ \mu\text{m}$ , a thickness of  $\sim 2\ \mu\text{m}$ , and a plasma membrane surface area of  $\sim 160\ \mu\text{m}^2$ . Erythrocytes are the most abundant blood components, and  $1\ \mu\text{L}$  of human blood contains  $\sim 4\text{--}5$  million RBCs. The half-life of human erythrocytes ranges from 100 days to 120 days. Various receptors, proteins, and functional groups on the membrane of erythrocytes provide binding sites for antibodies, specific ligands, and drug.<sup>20–22</sup>

The complex and unique membranes of RBCs are involved in the delivery of oxygen from lungs to different tissues.<sup>23</sup> RBCs travel  $\sim 250\ \text{km}$  through the cardiovascular system containing capillary networks, which can be as narrow as one-third of a diameter of cells.<sup>24</sup> The cytoskeleton, especially hexagonal actin–spectrin lattice underlying the plasmalemma, supports the membrane of RBCs, and the integrity of the cytoskeleton is based on a series of “horizontal” and “vertical” linkages between membrane protein complexes within the fluid phospholipid membrane bilayer. These structures ensure that the erythrocyte membrane simultaneously undergoes reversible changes between expanded and tight networks to maintain integrity and to avoid intravascular hemolysis.<sup>25</sup> These damaged and aged RBCs are eliminated by tissue macrophages in the liver and spleen,

and RBCs continuously circulate within the bloodstream but pass through the interstitium in splenic follicles and hepatic sinusoid. Thus, RBCs are suitable for intravascular delivery to treat blood, or endothelium-related diseases.<sup>1,26</sup> Erythrocytes can be supplied as ideal drug delivery systems because of these important features; since the discovery of RBCs as potential carrier systems, numerous substances have been bound to these blood cells or used to encapsulate them for disease treatment.<sup>27–32</sup>

## Preparation approaches for RBCs as drug delivery systems

### Encapsulation

Several methods have been used to encapsulate cargos in RBCs. Osmosis-based methods developed in the 1970s have been considered standard methods, because erythrocytes can act as an osmometer. In a hypotonic solution, erythrocytes reversibly swell to  $\sim 25\%$  of their original volume, and pores ranging from  $200\ \text{\AA}$  to  $500\ \text{\AA}$  are temporarily formed in the membrane. Thus, substances can enter erythrocytes through these pores. In an isotonic solution, the shape of RBCs is regained, and their pores close; substances are then encapsulated within erythrocytes.<sup>33</sup> This mechanism exhibits some variations, including hypotonic hemolysis, hypotonic pre-swelling, and hypotonic dilution. Considered as the most simple and rapid method of encapsulation, hypotonic dilution is the first process in which therapeutic moieties are loaded into erythrocytes.<sup>34–36</sup>

Another encapsulation method is electroporation or electro-insertion; in this process, erythrocytes are exposed to a strong external electrical field that causes irreversible changes in the RBC membrane.<sup>37,38</sup> However, Kinoshita and Tsong<sup>39</sup> suggested that desirable membrane permeability can be produced through transient electrolysis. Kitao et al<sup>40</sup> pioneered the successful application of chemical perturbation in human and mouse RBCs in 1980. However, this method has been rarely employed because it induces irreversible destructive changes in the membrane. Alternative approaches, such as lipid fusion and endocytosis, have also been applied to encapsulate agents into erythrocytes.<sup>41,42</sup>

### Membrane coupling or binding

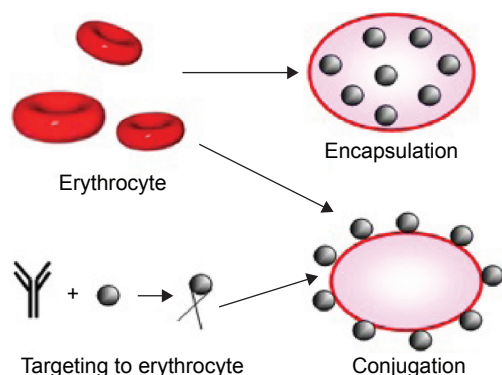
Among conventional strategies, reversible or irreversible coupling of a therapeutic moiety to RBCs is the most extensively utilized strategy. Nonspecific chemical cross-linkers have been used to bind molecules to the surface of erythrocytes.<sup>43,44</sup> However, implementation of these strategies has been impeded by specific cross-links, such as biotin–avidin pair,

which allow biotinylated molecules, including nucleic acids, to couple to sulfhydryl groups, amino acids, and other specific groups expressed on the membrane of RBCs. Through these methods, various agents, such as viral antigens and immunoglobulins, have been coupled to the RBC surface.<sup>21,45</sup> In addition to biotin–avidin, various chemical linkers have been utilized to attach different molecules, including small therapeutic molecules, such as daunorubicin,<sup>46</sup> and large molecules, such as hyperbranched chemical linkers.<sup>47</sup> Furthermore, single or combined molecules can conjugate with the surface of RBCs. In another approach, site-specific drug binding occurs as follows.<sup>48</sup> Antibodies, peptides, or other affinity ligands are initially anchored to the surface proteins of erythrocytes; therapeutic agents bind to and conjugate with these ligands.<sup>48</sup> Agents can also be attached to RBC surfaces through physical interactions; for instance, polystyrene particles can adhere to the surface of erythrocytes via nonspecific van der Waals and hydrogen bonding, which work through electrostatic and hydrophobic forces, respectively, between particles and erythrocytes.<sup>49</sup>

Figure 1 illustrates the two main preparation strategies for RBCs as a drug delivery system.

## Application of erythrocytes as a drug delivery system

Erythrocytes encapsulate and protect L-asparaginase (L-ASNase) from degradation; the encapsulated L-ASNase exhibits a longer half-life and lower incidence and severity of side effects, such as allergic reactions than native L-ASNase. Encapsulated L-ASNase is a promising product with a good safety profile to treat children and adults with refractory or relapsing acute lymphoblastic leukemia.<sup>50,51</sup> For instance, the toxic effect of erythrocyte-delivered etoposide on



**Figure 1** Two main strategies of preparing erythrocytes as drug delivery systems. **Notes:** In the method of encapsulation, therapeutic agents are inserted within erythrocytes, while in the method of conjugation, they are attached to the erythrocyte membrane.

macrophages is greater than that of free chemotherapeutic drugs; this phenomenon indicates the usefulness of RBCs in the delivery of this cytotoxic agent to target macrophages.<sup>52</sup> Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE), an autosomal recessive disease, is caused by mutations in the nuclear gene *ECGF1* coding for thymidine phosphorylase (TP); thus, high deoxythymidine and deoxyuridine levels accumulate in the body and induce gastrointestinal motility disorders, such as stomachache, progressive external ophthalmoplegia, hearing loss, and peripheral sensorimotor polyneuropathy. Moran et al<sup>53</sup> found that deoxythymidine and deoxyuridine levels decrease significantly, and symptoms have been ameliorated in patients with MNGIE when TP is carried by RBCs. Levene et al concluded that serious toxicities likely preclude a clinical trial of TP carried by RBCs in patients with MNGIE.<sup>54</sup> Harisa et al<sup>32</sup> demonstrated that human erythrocytes can be successfully loaded with pravastatin, and relatively high drug loading and encapsulation efficiency can be obtained. Moreover, no significant loading parameters and morphological changes in erythrocytes have been observed in entrapping pravastatin; this finding indicates that erythrocytes are potential carriers for pravastatin. Methotrexate (MTX), an antimetabolite and antifolate agent used in solid tumors and hematological diseases, can be encapsulated by erythrocytes, and the average survival time of rat hepatoma cells is enhanced with MTX-loaded erythrocyte treatment compared with that of cells treated with native MTX.<sup>55</sup> Biagiotti et al<sup>56</sup> confirmed that immunosuppressants can be encapsulated into erythrocytes in the presence of corresponding target proteins, and RBCs can serve as a promising delivery system for immunosuppressive agents.<sup>56</sup> The use of RBCs as a drug delivery system for chemotherapeutic agents, especially in vitro and in vivo use of antineoplastic agents, has been widely investigated. Other therapeutic drugs delivered by RBCs include gentamicin for bacterial infection,<sup>57</sup>  $\delta$ -aminolevulinic acid dehydratase for lead poisoning,<sup>58</sup>  $\beta$ -glucocerebrosidase for enzyme replacement therapy in Gaucher's disease,<sup>59</sup> adenosine deaminase for adenosine deaminase deficiencies,<sup>60</sup> enalaprilat for hypertension management and congestive heart failure,<sup>61</sup> and heparin for thromboembolism<sup>62</sup> and carrier for thrombolytic agents.<sup>63</sup> Table 1 provides some examples of therapeutic moieties loaded by erythrocytes.<sup>28,46,64–75</sup>

## Advantages

Erythrocytes applied as drug delivery systems have been extensively investigated because of several factors. For instance, erythrocyte sources are abundant, and the structure

**Table 1** Examples of therapeutic moieties loaded by erythrocytes

Therapeutic moieties	Application	Approaches	Study types	References
Dexamethasone	Crohn's disease; ulcerative colitis	Encapsulation	Both in vitro and in vivo	64–66
Dexamethasone	Chronic obstructive pulmonary disease	Encapsulation	In vivo	67
Dexamethasone	Cystic fibrosis	Encapsulation	In vivo	68
Dexamethasone	Ataxiateleangiectasia	Encapsulation	In vivo	69
Daunorubicin	Acute leukemia	Binding	Both in vitro and in vivo	46
Doxorubicin	Lymphoma	Binding	In vivo	70
5-Fluorouracil	Malignant ascites	Encapsulation	In vivo	28
Phenylalanine ammonia lyase	Phenylketonuria	Encapsulation	In vivo	71
IFN- $\alpha$ and Ribavirin	Hepatitis C	Encapsulation	In vitro	72
Glutamine synthetase	Hyperammonemia	Encapsulation	In vivo	73
Factor IX	Hemophilia B	Encapsulation	In vitro	74
Inositol hexaphosphate	Sickle cell disease	Encapsulation	In vitro	75

**Abbreviation:** IFN- $\alpha$ , interferon- $\alpha$ .

and properties of erythrocytes are well understood. RBCs also possess good biocompatibility and biodegradability without inducing immunological reactions and producing toxic by-products. The membrane properties of RBCs permit relatively high drug loading and slow molecular release. Furthermore, their circulation time in the bloodstream is prolonged. RBCs are phagocytosed by macrophages in the liver and spleen. Thus, RBCs introduce cargos into the RES of cells, thus they can be beneficial for the treatment of macrophage-related hepatic diseases<sup>22,23,48,76</sup> (Figure 2).

## Drawbacks

Similar to other drug delivery systems, carrier erythrocytes are limited by various factors. For example, encapsulation may cause an osmotic stress-induced damage to the RBC membrane. Coupling therapeutic moieties to RBCs can lead to the loss of the mechanical stability and plasticity of erythrocytes. These morphological and physiological

changes in erythrocytes may trigger an unwanted removal of RBCs by RES; as a consequence, their circulation time in the bloodstream is decreased. Molecules encapsulated in or coupled with RBCs may induce erythrocyte leakage and thus elicit toxic effects. Furthermore, preparation strategies for erythrocyte carriers have yet to be standardized; these carriers are also more varied than synthetic carrier systems. The storage of erythrocyte carriers and risk of blood contamination before, during, and after drug loading should also be highly considered.<sup>27,77</sup>

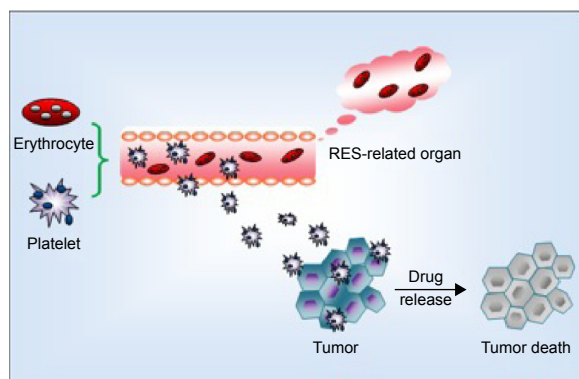
## Advancements

Drug selection and RBC–drug relationship have been improved because of various limitations. Prodrugs, such as corticosteroid prodrugs for prolonged circulation and nucleoside/nucleotide prodrugs for macrophage targeting, can improve the performance of RBC-based drug carriers. If an encapsulated or coupled molecule is inactive, erythrocytes can transform and release prodrugs in their active forms. This strategy can reduce off-target effects and prolong the half-life of drugs. Kwon et al<sup>78</sup> introduced a new strategy of protein loading by using membrane-translocating low molecular weight protamine, which causes negligible changes in RBC membrane. Other novel engineered substances, such as nanoparticles and polymeric multilayered microcapsules, combined with RBCs are also currently used.<sup>22,79,80</sup>

## Platelets as drug delivery systems

### Properties of platelets

Platelets, with an average diameter of 2–3  $\mu\text{m}$ , are discoid fragments from megakaryocytes of the bone marrow and the smallest components of the blood. Approximately  $10^8$  cells can be produced by megakaryocytes daily. Thus,  $2 \times 10^{11}$  to  $5 \times 10^{11}$  platelets can be generated; when demands are high,



**Figure 2** Illustration of the mechanism of erythrocytes and platelets as drug delivery systems.

**Note:** Erythrocytes are targeting to RES-related organs and platelets are targeting to tumor.

**Abbreviation:** RES, reticuloendothelial system.



this number increases tenfold. The platelet concentration in the circulating blood is  $\sim 150 \times 10^9/L$  to  $350 \times 10^9/L$  with an average life span of 7–10 days; after this period, platelets are removed by reticuloendothelial cells from the liver and spleen.<sup>81,82</sup>

Platelets participate in several physiological and pathological processes, including hemostasis, wound healing, thrombosis, inflammation, and atherosclerosis.<sup>83</sup> In hemostasis, damaged small blood vessels and capillaries promote vasoconstriction to prevent blood loss. In addition, platelets adhere to the site of injury by interacting with von Willebrand factor and collagen; some mediators work with various potent functional molecules, including adenosine diphosphate and thromboxane A2. Thus, platelets act as a storage “depot” and trafficking “vehicle” in blood vessels.<sup>84</sup>

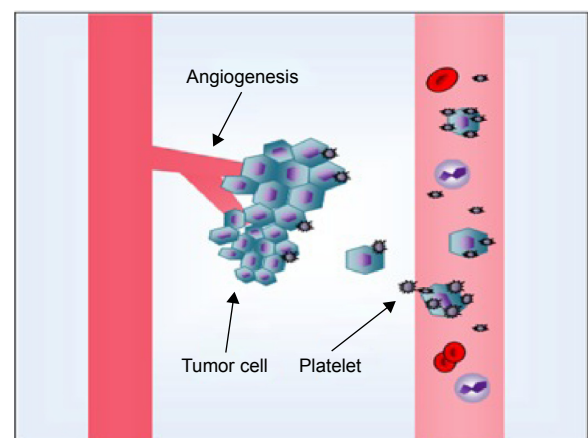
Pathological conditions occur because of nonspecific activity of platelets, stimulation of agonist secretion, and platelet aggregation in atheromatous plaques; these conditions decrease the number of platelets in circulation and cause ischemia.<sup>85</sup> The mechanism by which platelets engulf small molecules is different from conventional phagocytosis, in which an engulfed substance is metabolized. Compared with other carriers, encapsulated substances in the platelet remain intact, and are thus referred to as coverocytes.<sup>86</sup>

The relationship between platelets and malignancy was first discovered in 1865 by Armand Trousseau, a French clinician who reported that migratory thrombophlebitis can be an indicator of occult malignancies, including pancreatic carcinoma. Named after this clinician, Trousseau syndrome is characterized by a hypercoagulable state with advanced malignancy. Seven years later, Riess<sup>87</sup> described a “massive increase of platelets” in patients with carcinoma. In 1878,<sup>88</sup> Billroth found that platelets not only participate in tumor growth but also contribute to metastatic spread. A century later, researchers found that thrombocytopenia alleviates metastasis in mice. Tumor cells induce platelet aggregation, and active platelets protect or cloak circulatory tumor cells from physical factors such as shear stress within the vasculature, and help them escape from immune surveillance. Subsequently, platelets cause tumor cells to transfer to secondary sites on the vascular wall; platelets also secrete proteases and cytokines that promote the formation of new blood vessels, which are necessary for tumor-associated angiogenesis and growth. Platelet count is increased by tumor cells. Therefore, the correlation between platelets and tumors can be described as a vicious cycle: tumor increases platelet count; the increased platelet count promotes tumor growth and metastasis; as a consequence, platelet count is

further increased (Figure 3).<sup>89,90</sup> The phenomenon by which tumor cells cause platelet aggregation was first discovered in 1968 and referred to as tumor-induced platelet aggregation (TCIPA).<sup>91</sup> TCIPA is considered the fundamental to utilize platelets as drug delivery systems for malignant tumor treatment. The mechanism of TCIPA is illustrated in Figure 2.

## Application of platelets as a drug delivery system

Sarkar et al<sup>92</sup> showed that platelets can take up drugs at a relatively high drug loading concentration, and the efficiency of inducing cytotoxicity is significantly increased when platelets are used as drug delivery carriers in vitro and in vivo. They concluded that platelets can be potential drug carriers when a low drug concentration is used in a targeted mode. Anti-thrombotic agents are commonly utilized to manage patients at a high risk of thrombosis and hemorrhage. Platelets have also been effectively exploited as carriers for antithrombotic agents, especially for arterial clots.<sup>93,94</sup> Davies et al<sup>95</sup> constructed a strategy to deliver europium luminescent complex and gold nanoparticles (AuNPs) into human platelets by using a low-pH insertion peptide. The radiolabeling of platelets to assess survival and recovery has been developed for more than 4 decades. However, this technique has been banned for use in clinical studies. By contrast, nonradioactive labeling of platelets has been used frequently in clinical studies, including evaluation of the survival of transfused platelets and analysis of the pathogenesis of stroke.<sup>96</sup> In our study,



**Figure 3** The relationship between platelets and malignancy.

**Notes:** Tumor cells induce platelet aggregation, and then active platelets protect or cloak circulatory tumor cells from physical factors, as well as help them escape from immune surveillance. Subsequently, platelets facilitate tumor cells to transfer to the secondary sites on the vascular wall and secrete proteases and cytokines that promote the formation of new blood vessels. On the other hand, tumor cells increase the platelet count.

**Table 2** Examples of therapeutic moieties loaded by platelets

Therapeutic moieties	Application	Study types	References
Factor VIII	Hemophilia A	In vivo	101
Factor IX	Hemophilia B	In vivo	84
Doxorubicin	Lung adenocarcinoma; ehrlich ascites carcinoma	Both in vitro and in vivo	92
Ferucarbotran superparamagnetic nanoparticles	Transfusion medicine and cardiovascular medicine	In vitro	96

doxorubicin is loaded into platelets, and high drug loading and encapsulation efficacy are detected. Doxorubicin is also released by a pH-based trigger. Inhibition and apoptosis rates are higher in the group that received drug-loaded platelets than in the untreated group.

Nanoparticles mimicking platelets have been proposed for drug and gene delivery to treat various diseases. Kona et al<sup>97</sup> formulated drug-loaded poly(lactic-co-glycolic acid) nanoparticles and conjugated the external fraction of platelet glycoprotein Ib (GPIb) via carbodiimide chemistry. They confirmed that the GPIb-conjugated poly(lactic-co-glycolic acid) nanoparticles can act as a controlled and targeted drug carrier under flow conditions at vascular injury sites.<sup>97</sup> Lin et al obtained similar results.<sup>98</sup> Modery-Pawłowski et al<sup>99</sup> found that platelet mimetics is a promising method in the treatment of metastasized tumor. Researchers prepared polymeric nanoparticles enclosed in the plasma membrane of human platelet; they verified that platelet membrane-cloaked nanoparticles exhibit less uptake by macrophage-like cells than uncoated nanoparticles do, and particle-induced complement activation is reduced. Furthermore, therapeutic efficacy is enhanced when vancomycin and docetaxel are applied to an experimental mouse model of systemic bacterial infection and a rat model of coronary restenosis via platelet-mimetic nanoparticles.<sup>100</sup>

Other applications of platelets as drug carriers are summarized in Table 2.<sup>92,101</sup>

## Advantages

Biocompatibility, which is defined as “expression of the benignity of the relation between a material and its biological environment”, is considered a fundamental property of a carrier. The biocompatibility of platelets is superior to that of other drug delivery systems because platelets are components of the human body. In theory, ~500 molecules can be loaded on  $1 \times 10^{-15}$ /L nanoparticles; in reality, the amount is much lower. The loading efficacy of platelets is relatively high, with an average of 50,000–70,000 molecules encapsulated by the same number of platelets.<sup>86,102</sup> Another property of platelets is that targets are well defined: these targets are mostly sites with high density of proliferating cells or injured sites. Thus, side effects caused by nonspecific targeting are decreased because

of the inherent targeting capacity of platelets. Furthermore, platelet-encapsulated drugs are protected from physical stress and immunological reactions; as such, the systemic clearance of the encapsulated drugs is similar to that of single platelet, and circulation time is prolonged. Controlled drug delivery is quite important, and platelets can facilitate controlled drug release by artificially exposing drugs to agonists. According to Sarkar et al, lethal effects are exacerbated by enhanced apoptosis when cytotoxic drugs are carried by platelets; this phenomenon indicates the synergetic effect of platelets and drugs. As such, low amounts of drugs can be used to achieve the same treatment efficacy, and few side effects are induced. Another advantage of platelets over other carriers is that the method can be individualized because a patient's own platelets are the ones used to deliver drugs.<sup>92</sup> The method is also appropriate for patients who need transfusion.

## Drawbacks

The main drawbacks of platelets are similar to those of RBCs. These limitations include proper storage and contamination. In genetic therapy, whether the platelet-specific expression of transgenes affects the expression of endogenous proteins remains unknown. Furthermore, whether the usage of platelets as drug carriers causes thrombogenesis should be further investigated.

## Albumin as a drug delivery system

### Properties of albumin and its preparation

Albumin, which is the most abundant plasma protein, acts as a molecular taxi for various small insoluble substances, such as hormones, nutrients, and toxins. The albumin concentration in an adult ranges from 35 g/L to 50 g/L.<sup>103</sup> Albumin, with an average half-life of 19 days, is synthesized in the liver and its molecular weight is 66.5 kDa.<sup>104</sup>

Babson and Winnick<sup>105</sup> demonstrated for the first time that tumor cells can trap plasma proteins and utilize their degradation products for proliferation. Since then, other researchers have revealed the ability of albumin to accumulate in malignant tissues via the EPR effect. The leakiness of the defective blood vessels of tumor tissues enables macromolecules to pass the endothelial barrier, but normal

tissues are permeable only to small molecules.<sup>71,104</sup> As such, the proper size of macromolecules that can extravasate into tumor tissues but not into healthy tissues ranges from 2 nm to 10 nm, and the diameter of serum albumin is 7.2 nm. In addition, Stehle et al proposed that albumin is a major nutrient and energy source for tumor cell growth.<sup>106</sup> In 2003, Wunder et al<sup>107</sup> showed that albumin accumulates in the arthritic paws of mice. Therefore, albumin is a potential carrier that can be used to deliver drugs to the inflamed joints of patients with rheumatoid arthritis.<sup>104</sup>

Albumin as a drug delivery system is prepared via two primary ways: 1) encapsulation of drugs into albumin nanoparticles, which are dependent on physical interaction between drugs and albumin, and 2) coupling of drugs to endogenous or exogenous albumin and conjugation with bioactive proteins.

## Application of albumin as a drug delivery system

The MTX bound to human serum albumin (MTX-HSA) is the first albumin conjugate evaluated in Phase I/II clinical studies. In the assessment of antitumor activity in preclinical *in vivo* models, complete remission/cure is achieved in soft-tissue sarcoma SXF 1301 by using MTX-HSA; free MTX lasts for a short period. In the prostate cancer model PRXF PC3M, MTX-HSA induces a greater growth inhibition than native MTX dose. These results demonstrate that antitumor effectiveness can be enhanced when MTX is conjugated with albumin.<sup>108</sup> MTX-HSA can be combined with cisplatin, and the toxicity of the antitumor activity against urothelial carcinomas is tolerable.<sup>109</sup> One of the successful applications of albumin as a drug delivery system is demonstrated by the albumin–paclitaxel (PAC) nanoparticle Abraxane, which was first approved in 2005 by the US Food and Drug Administration to treat metastatic breast cancer; this drug was also approved in 41 countries in the same year. Teneriello et al<sup>110</sup> verified that nanoparticle albumin-bound PAC is active in patients with recurrent ovarian, peritoneal, or fallopian tube cancer, and toxicity is manageable, as revealed by Phase II evaluation. HSA has been proposed as a carrier for renal targeting drugs, but glomeruli are not entirely permeable to HSA because of the molecular weight of albumin. Therefore, Yuan et al<sup>111</sup> used three peptide fragments (PFs), namely PF-A1-123, PF-A124-298, and PF-A299-585, obtained through the cyanogen bromide degradation of HSA to carry agents and selectively accumulate in kidneys; they verified that PFs can be applied as potential drug delivery systems for renal targeting, and PF-A299-585 may be an optimal

carrier. Cationic bovine serum albumin as a gene delivery system has also been demonstrated as a promising strategy to treat lung and metastatic cancers.<sup>112</sup> Asmatulu et al<sup>113</sup> fabricated albumin-based drug-carrying microcomposite spheres loaded with cyclophosphamide and 5-fluorouracil; they subsequently investigated the potential of these spheres for breast cancer treatment *in vitro* by using fibroblast cells (3T3) and breast cancer cells (MDA-486). Nude mice have also been used for *in vivo* evaluation. The results showed that chemotherapeutic efficacy is enhanced when the mice are exposed to other drug delivery systems possibly because of the albumin-induced increase in uptake of microspheres by malignant cells. Peralta et al<sup>114</sup> revealed that gold nanorods combined with the chemotherapeutic drug PAC are successfully encapsulated into HSA nanoparticles (HSAPs) to form PAC–AuNP–HSAPs. They verified that PAC–AuNP–HSAPs are more efficient than free PAC in inhibiting the proliferation of 4T1 mouse breast cancer cells and inducing the apoptosis of these cancer cells. Zhang et al<sup>115</sup> demonstrated that cytotoxicity is enhanced by two to five times in tumor cells when ruthenium-based anticancer complexes  $[\text{RuCl}_5(\text{ind})]^{2-}$  bind to HSA; no side effects are detected in normal cells *in vitro*. Compared with the use of single drugs, the use of the HSA– $[\text{RuCl}_5(\text{ind})]^{2-}$  complex promotes tumor cell apoptosis. Albumin drug nanoparticles combined with phospholipid perfosine or the antibodies trastuzumab (Herceptin) and bevacizumab (Avastin) can effectively treat patients with head, neck, or breast cancer.<sup>116</sup> Thus, albumin is a potential drug delivery system.<sup>117</sup> Other uses of albumin as a drug carrier are listed in Table 3.<sup>112,118–127</sup>

## Advantages

Albumin is an optimal candidate for drug delivery because of several advantages, including abundant sources, nontoxicity, biodegradability, nonimmunogenicity, and preferential uptake in tumor and inflamed tissues. Albumin is also stable at various temperatures (it can be heated at 60°C for up to 10 hours without deleterious effects), pH (it is stable at pH 4–9), and in various organic solvents.<sup>117</sup> Therefore, albumin can enhance the solubility of poorly water-soluble molecules, prolong the circulation time of drugs, and minimize side effects by improving targeting. Furthermore, albumin provides a depot for various drug binding sites and high binding capacity for different drugs.<sup>117,128</sup>

## Drawbacks

In preparing albumin–drug conjugates with or without additional targeting ligands, the structure of albumin may

**Table 3** Examples of therapeutic moieties loaded by albumin

Therapeutic moieties	Application	Approaches	Study types	References
Deferasirox	Neurodegenerative disorders	Conjugation	In vitro	118
Noscapine	Breast cancer	Coacervation	In vitro	119
Tacrolimus	Rheumatoid arthritis tissues	Conjugation	Both in vitro and in vivo	120
Azidothymidine	AIDS	Conjugation	In vitro	121, 122
Exendin-4	Type 2 antidiabetic	Conjugation	In vivo	123
Aspirin	Intra-articular therapy	Coacervation	In vitro	124
Obidoxime	Poisoning by organophosphorus compounds	Conjugation	In vitro	125, 126
siRNA	Lung metastatic cancer	Encapsulation	Both in vitro and in vivo	112
Doxorubicin	Breast carcinoma; ovarian carcinoma; small cell lung cancer; orthotopic pancreas carcinoma	Conjugation	Both in vitro and in vivo	127

**Abbreviation:** siRNA, small interfering RNA.

be disrupted; as a result, these conjugates may accumulate in nontarget tissues and may be eradicated by the mononuclear phagocyte system.<sup>128</sup> Furthermore, the performances of nanomaterials and albumin in vitro differ from the interaction of albumin and nanomaterials in vivo; undesirable results, such as the risk of capillary blockage because of increased size and potential toxicity caused by conformational changes in albumin, may also be obtained.<sup>9</sup>

## Others

In addition to erythrocytes, platelets, and albumin as drug delivery systems, apoferritin has been used to encapsulate cisplatin and carboplatin.<sup>129</sup> Ivy nanoparticles also exhibit great potential for the delivery of chemotherapeutic drugs in cancer therapy.<sup>130</sup> Wang et al<sup>131</sup> suggested that tea nanoparticles can be useful to prevent multidrug resistance in tumor cells and to enhance the chemotherapeutic efficacy in tumor treatment. Yi et al<sup>132</sup> also confirmed that tea nanoparticles can function as a multifunctional nanocarrier for cancer therapy. Fungal nanoparticles have also been proposed as nanocarriers for cancer treatment.<sup>133</sup> Low-density lipoprotein,<sup>134</sup> nonpathogenic bacteria,<sup>135,136</sup> and dendritic and eukaryotic cells<sup>137</sup> have also been used to deliver drugs, enzymes, and genes to specific sites.

## Conclusion

Biological agents have been investigated as drug delivery systems in various research and clinical applications, especially for antineoplastic agent delivery, because of their excellent properties. The advantages of biological delivery carriers have been verified by numerous studies. Nevertheless, further research should be conducted to clarify the following questions: 1) Can thrombus be formed through the reduplicative application of platelets as drug carriers? 2) Can carrier erythrocytes deliver oxygen from lungs to different tissues? 3) Can carrier platelets still participate in

physiological and pathological processes; if so, what is their role in human bodies?

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## Disclosure

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