Perspective: 2014 AIChE Alan P. Colburn Award

Engineering Red-Blood-Cell-Membrane–Coated Nanoparticles for Broad Biomedical Applications

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DOI 10.1002/aic.14735
Published online January 27, 2015 in Wiley Online Library (wileyonlinelibrary.com)

Keywords: biomimicry, cell membrane, nanomedicines, nanoparticles, red blood cells

Introduction

Red blood cells (RBCs) are nature's long-circulating delivery vehicles. They possess various remarkable properties and continue to inspire the design and engineering of man-made delivery systems.[1] Inherently suited for intravascular delivery, RBCs are intrinsically biocompatible, biodegradable, and nonimmunogenic. They form natural compartments capable of protecting encapsulated cargos, and this allows them to circulate in the bloodstream for a long period of time (up to 120 days). In addition, their semipermeable membrane affords sustained release to small-molecule drugs, yet they are ideal for retaining large proteins while providing them access to the substrates. Delivery vehicles possessing one or several of these properties have long been desired for efficacious therapeutics.[2]

A long-sought strategy for RBC-mimicking delivery vehicles is to load natural RBCs with therapeutic agents without compromising the structural integrity and biological functions of the RBCs.[3] Various loading techniques, including automated loading devices, have been developed to enable the encapsulation of payloads with molecular weights of over 180 kDa into RBCs with maintenance of the carriers' biological competence.[4,5] In addition to the interior of RBCs, their exterior surface has also been coupled with therapeutic molecules, either covalently or physically, for various delivery applications. These RBC-based delivery vehicles, namely carrier RBCs, have been developed for the delivery of numerous therapeutic agents, including proteins, nucleic acids, and small-molecule drugs. Several of them have entered clinical tests to treat various diseases, including cancers and enzyme deficiencies.[6,7]

Meanwhile, advances in molecular biology have provided unprecedented understanding of the connections between the physicochemical characteristics of RBCs and their biological functions.[8,9] This understanding, in turn, has inspired researchers to model drug carriers after RBCs. Designs that mimic the physicochemical characteristics and biological functions of RBCs, particularly those that enable their passage through narrow constrictions while maintaining a long *in vivo* survival, have been integrated into the engineering of drug carriers and have resulted in novel delivery systems with improved drug tolerability, circulation lifetime, and efficacy.[10]

Recently, the pursuit of RBC-mimicking nanoparticles led us to develop an intriguing approach for functionalizing synthetic nanoparticles with natural RBC membranes.[11] In this approach, we first collected intact cellular membranes and then coated them onto polymeric nanoparticle cores, such as those made from poly(lactic-*co*-glycolic acid) (PLGA); this resulted in red-blood-cell-membrane–coated nanoparticles (RBC-NPs; Figure 1). Our aim was to fabricate cell-mimicking nanoparticles through a top-down approach that bypassed the labor-intensive processes of protein identification, purification, and conjugation. The natural membranes also provide a bilayered medium for transmembrane protein anchorage while preventing common chemical modifications that could compromise the integrity and functionalities of these proteins. The independent preparation of cellular membranes and particle cores before coating offers a new level of engineering flexibility toward highly functional biomimetic nanoparticles.

Since their initial development, RBC-NPs have provided an unprecedented capability for harnessing the natural functionalities of native cells that would otherwise be difficult to replicate. They have since inspired us to develop novel nanotherapeutics for better disease intervention. In this Perspective, we review our recent progress in developing RBC-NPs for three distinct biomedical applications: long-circulating nanocarriers for drug delivery, biomimetic nanosponges for detoxification, and nanotoxoids for safe and effective toxin vaccination.

Long-Circulating Carriers for Drug Delivery

Long-circulating nanocarriers have significant clinical impact as they promise sustained systemic delivery and better targeting through both passive and active

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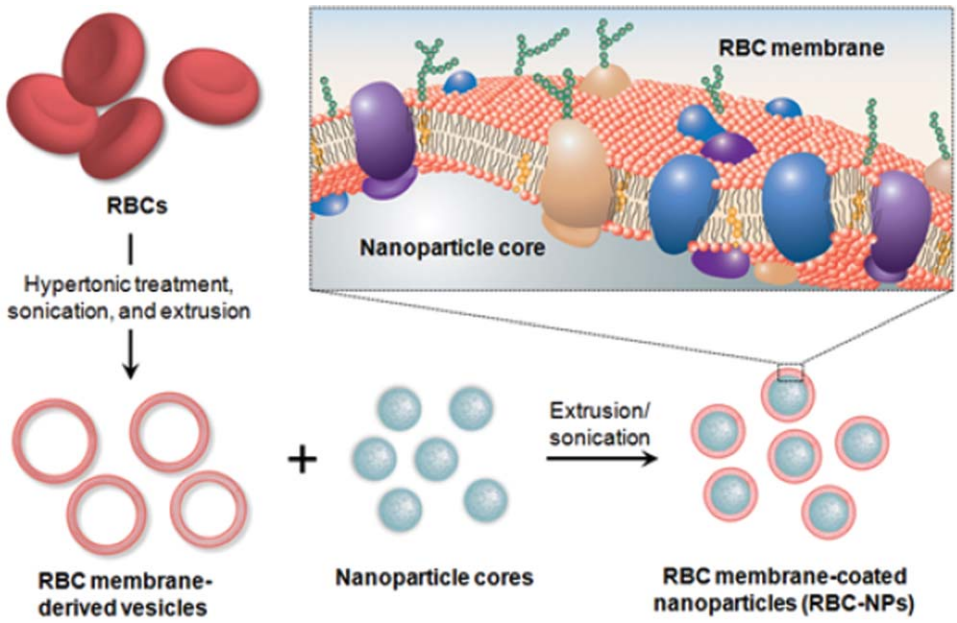

Figure 1. Schematic illustration of the preparation process of the RBC-NPs.

The process can be divided into two steps: the derivation of membrane vesicles from RBCs and the fusing of the vesicles onto the surfaces of the nanoparticle cores.

mechanisms.[12–14] Numerous approaches, mostly through the modification of the nanoparticle size, surface chemistry, geometry, and flexibility, have been exploited to extend the particle residence time *in vivo*.[15–17] In particular, surface coatings that can help minimize the clearance of nanoparticles by the reticuloendothelial system, including liver, spleen, bone marrow, and lymph nodes, are essential for increasing the nanoparticle's blood circulation time. The current gold standard for nanoparticle stealth coating is poly (ethylene glycol) (PEG), which improves the circulation time by stabilizing and protecting nanoparticles from opsonization, a plasma protein deposition process that signals Kupffer cells in the liver to remove the carriers from circulation.[13] The success of PEG has led to several clinical products.[14] However, the desire to further improve nanoparticle circulation, together with the recent observation of anti-PEG immunological response, has triggered a continuous search for new synthetic materials and natural immunosuppressive proteins to modify nanoparticles for prolonged circulation.[18]

Our strategy in this thrust is to replicate the protein makeup on the RBC surfaces responsible for long circulation onto synthetic nanoparticles. Apparently, conventional chemical conjugation approaches would be impractical for achieving this goal given the abundance and complexity of proteins associated with the RBC membranes. Instead, we collected entire cellular membranes and developed an effective coating technique to wrap polymeric nanoparticles of various sizes with unilamellar, bilayered RBC membranes [Figure 2(A)].[11,19] We demonstrated that the antigen density on the RBC-NPs was comparable to that of the native RBCs; this indicated that the entire RBC membranes were translocated to the nanoparticle surfaces. Using immunostaining, we further verified that the membranes presented in a right-side-out orientation on the nanoparticle surfaces; this ensured proper expression of critical surface antigens for molecular interactions. When added to macrophage cells, RBC membrane coatings resulted in a 64% reduction in particle internalization under the experimental conditions; this confirmed the translocation of immune-evasive functionality from the RBCs to the nanoparticles.[20] When injected into mice, these nanoparticles showed a longer elimination half-life compared to PEG-coated counterpart nanoparticles [Figure 2(B)].[11] Together, this cell-membrane-coating strategy provides synthetic nanoparticles superior retardation for *in vivo* clearance. Therapeutic potentials underlying such nanoparticle design strategies are emerging. Meanwhile, advances in polymer engineering offer flexibility to mimic other properties of RBCs critical for long circulation, such as their shape and elasticity. Such flexibility will also allow the nanoparticle circulation time to be tailored for specific diseases.

RBC-NPs confer the combined advantages of both a long circulation lifetime and controlled drug release. To gain a better understanding of the drug loading, drug-release kinetics, and cell-based efficacy of these nanoparticles, we used two common loading strategies to encapsulate doxorubicin into the RBC-NPs: physical encapsulation by the coprecipitation of doxorubicin with PLGA and chemical conjugation by covalent linking of doxorubicin molecules to the terminal of PLGA chains.[21] We found that the chemical conjugation approach resulted in a more sustained drug-release profile, and the coated membrane provided a barrier, which retarded the outward diffusion of encapsulated drug molecules. Regardless of the drug-loading methods, in comparison to free doxorubicin, RBC-NPs exhibit approximately a 10-fold increase in LC50 (50% of lethal concentration) against Kasumi-1, an acute myeloid leukemia cell line.

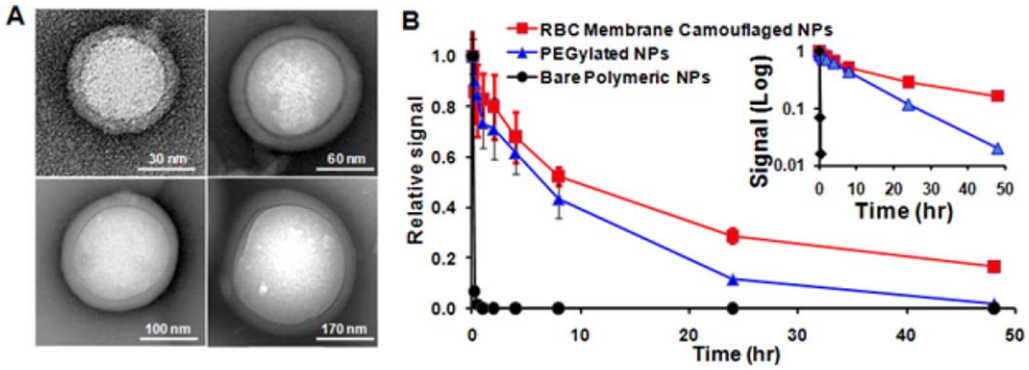

Figure 2. (A) Representative transmission electron microscopy (TEM) images of RBC-NPs with differently sized polymeric cores. The nanoparticles were negatively stained with uranyl acetate and subsequently visualized with TEM. (B) *In vivo* circulation time of RBC-NPs made from PLGA cores with a diameter of 75 nm. Fluorescence-labeled RBC-NPs were injected intravenously through the tail vein of mice. At various time points, blood was drawn intraorbitally and measured for fluorescence intensity to evaluate the systemic circulation lifetime of the nanoparticles ($n = 6$ per group). Reproduced with permission from the National Academy of Sciences[11] and the Royal Society of Chemistry.[19]

The compelling structural characteristics and functionalities of the RBC-NP platform further raised our curiosity over the interfacial interactions between natural cellular membranes and polymeric nanoparticle substrates. We then investigated several key interfacial properties of the RBC-NPs, with a particular interest in gaining an understanding of how the physicochemical properties of the nanoparticles, such as the particle size, ζ potential, and surface chemistry, would affect the membrane-coating process.[19,20] We found that the RBC membranes completely covered negatively charged polymeric nanoparticles in a right-side-out manner and enhanced the particles' colloidal stability. The membrane-coating process was applicable to particle substrates with a diameter ranging from 65 to 340 nm. Our studies showed that the interplay between the cell membrane moieties, such as RBC glycans, and the substrate surface characteristics strongly influenced the affinity between the RBC membranes and the synthetic polymer cores. Hence, this drove and directed the membrane-particle coating process. These findings shed light on the dynamics between the cellular membrane and nanoscale substrates and provided valuable information toward future development and characterization of cellular-membrane–coated nanodevices.[22]

We recently extended the membrane-coating technique from polymeric nanoparticles to inorganic gold nanoparticles (AuNPs).[23] AuNPs have found widespread applications as imaging agents and drug carriers in biology and medicine.[24] Their modification with the entirety of a cell membrane provided improved functions and advanced biomimetic features. Facilitated by external mechanical forces, RBC membranes spontaneously fused onto solid AuNPs to form red-blood-cell-membrane–coated gold nanoparticles (RBC-AuNPs). The resulting RBC-AuNPs possessed right-side-out RBC membranes and the associated membrane proteins, which provided the AuNPs with immunosuppressive functionalities for evading macrophage uptake. In addition, the membrane coating effectively shielded the particles from interacting with thiolated compounds. When synthetic AuNPs were integrated with natural cellular membranes, the particles were bestowed with a wide range of functionalities responsible for the cells' diverse antigenic, transport, and mechanical characteristics. The RBC-AuNPs embody a new materials design strategy and present an intriguing class of advanced materials for a broad range of biomedical applications.

Biomimetic Nanosponges for Detoxification

In addition to immune evasion, RBC-NPs also inspired us to harness other functionalities of natural RBCs for novel therapeutics. We were particularly interested in detoxification treatments to cleanse the body of virulence factors, such as those caused by bacterial infections, venomous injuries, and biological weaponry.[25] Among various toxins, our focus was on pore-forming toxins (PFTs), one of the most common protein toxins found in nature.[26] These toxins disrupt cells by forming pores in cellular membranes and altering their permeability for bioactivity. The inhibition of PFTs is beneficial in the fight against various bacterial infections and venomous attacks by animals, such as sea anemones, scorpions, and snakes.[27] To neutralize PFTs, numerous detoxification platforms, including antisera,[28] monoclonal antibodies,[29] small-molecule inhibitors,[30] and molecularly imprinted polymers,[31] have been developed. However, these platforms act by targeting the specific molecular structures of toxins and require customized treatments for different diseases. With over 80 families of PFTs so far having been identified, existing detoxification strategies are challenged by an overwhelming number of distinctive molecular structures and epitopic targets of PFTs.[32]

To address this challenge, we took the advantage of the functional similarity among PFTs in the perforation of cellular membranes and developed RBC-NPs as toxin nanosponges for PFT neutralization [Figure 3(A,B)].[33] The RBC membrane shell, according to the coating process, provides a substrate mimicking RBCs to absorb a wide range of PFTs, regardless of their molecular structures. Meanwhile, the inner polymeric core stabilizes the RBC membrane shell to enable the prolonged systemic circulation essential for absorbing

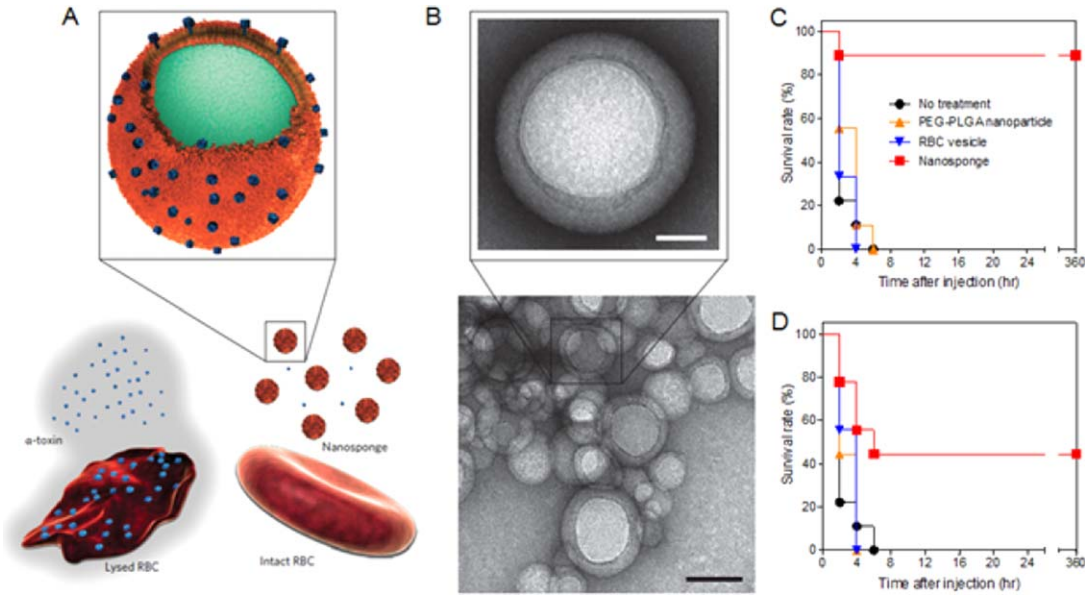

Figure 3. (A) Schematic structure of the toxin nanosponges and their mechanism of neutralizing PFTs. The nanosponges consisted of substrate-supported RBC bilayer membranes, into which PFTs could be incorporated. After being absorbed and arrested by the nanosponges, the PFTs were diverted away from their cellular targets, thereby avoiding target cells and preventing toxin-mediated hemolysis. (B) TEM visualization of nanosponges mixed with α-toxin (scale bar = 80 nm) and the zoomed-in view of a single toxin-absorbed nanosponge (scale bar = 20 nm). The sample was negatively stained with uranyl acetate before TEM imaging. (C,D) Survival rates of mice over 15 days after an intravenous injection of α-toxin (75 mg/kg). Nanosponges (80 mg/kg), RBC vesicles, or PEG-PLGA nanoparticles were administered intravenously 2 min either (C) before or (D) after toxin injection. All injections were performed via the tail vein ($n = 9$). Reproduced with permission from Nature Publishing Group.[33]

toxins in the bloodstream. The unique core-shell structure also locks in the absorbed toxins from further bioactivity. Such nanosponges act as decoys to divert the toxin away from their cellular targets. In a mouse model, the nanosponges markedly reduced the toxicity of staphylococcal α-hemolysin and improved the survival rate of toxin-challenged mice [Figure 3(C,D)]. Such action-mechanism–targeted detoxification by nanosponges can be distinguished from the current paradigm of detoxification treatments, where toxin antagonists rely primarily on structure-specific epitopic binding. By targeting a common mechanism shared by a broad range of toxins, the nanosponges introduce a unique strategy to use injectable nanocarriers for biodetoxification.

In addition to absorbing PFTs, RBC-NPs also hold great promise to neutralize other chemical and biological molecules that target RBCs. One particular example is type II immune hypersensitivities driven by pathological antibodies that target self-antigens, either naturally occurring or due to exposure to an exogenous substance present on the cellular exterior or extracellular matrix.[34] This disease type makes up many of the most prevalent autoimmune diseases, including pernicious anemia, Graves' disease, and myasthenia gravis, and autoimmune hemolytic anemia (AIHA) and immune thrombocytopenia.[35,36] In addition, these diseases may occur after the administration of a new drug or after certain infections. Currently, therapies for these immune-mediated diseases remain relatively nonspecific via broad immune suppression and, thus, present significant iatrogenic risk.[37,38]

In this regard, we recently explored the capability of RBC-NPs as an alternative target for pathological antibodies in an antibody-induced anemia disease model (Figure 4).[39] In our study, we demonstrated that these nanoparticles bound and neutralized anti-RBC polyclonal IgG effectively and, thus, preserved circulating RBCs. As a result, they abrogated the effect of pathological antibodies and minimized the disease burden without the need for drug-based immune suppression. Similar to nanosponges for PFT detoxification, these biomimetic nanoparticles enabled the indiscriminate absorption of pathological antibodies, regardless of their epitope specificities. Unlike conventional immune therapies, these biomimetic nanoparticles have no drug payload to suppress normal lymphocytes or immune effector cells. Additionally, unlike blood transfusions, which serve as a replacement therapy, RBC-NPs serve to deplete circulating antibody levels without contributing further toxic metabolites due to the hemolysis of transfused cells. Moreover, it has been demonstrated in animal models of autoimmune diseases that the primary target antigens can vary and shift over the course of the diseases. The exploitation of target cell membranes in their entirety overcomes the various antigen specificities and presents a previously unidentified approach in intercepting the autoreactive antibody mechanism of type II immune hypersensitivity reactions.

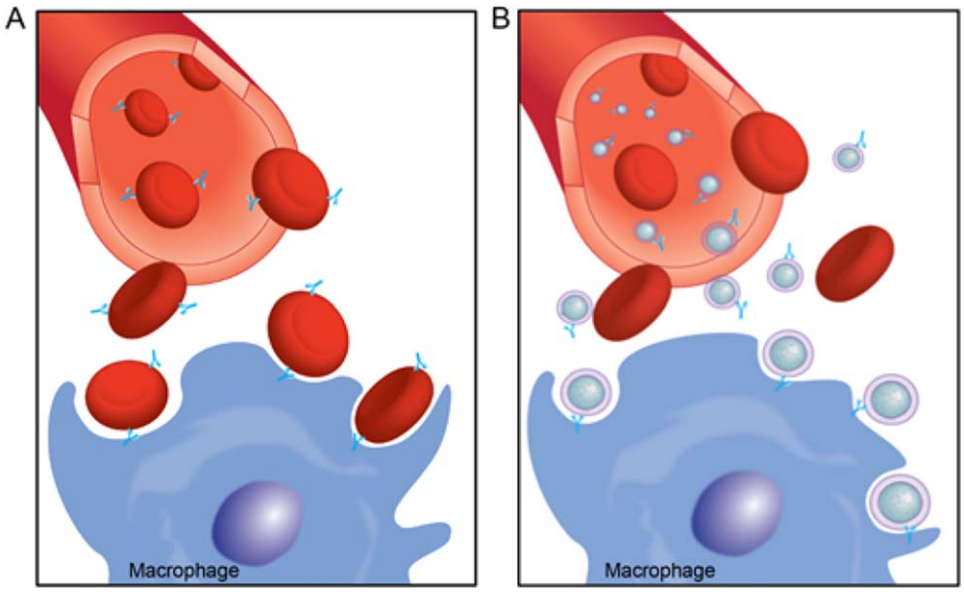

Figure 4. Schematic representation of RBC-NPs neutralizing anti-RBC antibodies (anti-RBCs). (A) Anti-RBCs opsonization of RBCs for extravascular hemolysis via phagocytosis, as observed in AIHA. (B) RBC-NPs absorption and neutralization of anti-RBCs and, thus, protection of RBCs from phagocytosis. For illustration purposes, antibodies were drawn exclusively on the nanoparticles. Reproduced with permission from the National Academy of Sciences.[39]

Nanotoxoids for Safe and Effective Toxin Vaccination

Bacterial toxins alter the normal metabolism of host cells, and many protein toxins have been identified as the primary causative factors in infectious diseases. The role of toxins in infections has prompted the development of toxoid vaccines, which are inactivated forms of toxins that can be administered to mount an antitoxin immune response. Conventional toxoid preparation methods involve protein denaturation through heat or chemical treatment for toxin neutralization, but these disruptive techniques compromise the antigenic information in the toxin proteins and, thereby, inevitably causing a tradeoff between toxoid safety and efficacy. The shortfalls of denaturation-based toxoid preparation are evidenced in the decades-long effort in the development of α-hemolysin toxoid against *Staphylococcal aureus* infections, as early development of denaturation-based α-hemolysin toxoid vaccines have been marred by either residual toxicity or inadequate potency.[40] More recent efforts have focused on the development of nontoxic but structurally conserved toxin mutants with advanced biomolecular techniques. In particular, site-directed mutagenesis has been applied to produce toxin mutants with minimal antigenic alterations from the target toxins; this minimizes the tradeoff between safety and efficacy.[41]

To address this challenge, we see the potential of RBC-NPs as a safe and effective vaccination platform as they offer a unique approach to inactivating PFTs without protein denaturation. With this platform, nanoparticle vectors are applied to intercept toxins' virulence mechanism, thereby enabling unaltered toxins to be administered for immune processing (Figure 5).[42] With staphylococcal α-hemolysin as a model toxin and through its mixture with preformed RBC-NPs, we demonstrated the facile preparation of toxin-loaded nanotoxoids.[43] On one hand, this platform takes advantage of particle-stabilized biomembranes to absorb and lock in PFTs within the membranes; this precludes the toxins from initiating their normal virulence mechanisms. On the other hand, as detained toxins retain their protein structure, they elicit superior immune responses. We showed that mice vaccinated with particle-detained α-hemolysin generated significantly higher antitoxin immune responses as compared to those vaccinated with heat-denatured toxins. Most impressively, mice receiving three weekly doses of a particle-detained α-hemolysin vaccine became completely immune to the toxin. High doses of α-hemolysin, which can cause serious tissue damage in unvaccinated animals, did not inflict any observable effect on the vaccinated mice upon subcutaneous injection.

The biocompatible nature of RBC membranes and PLGA polymers allow the immune system to selectively process the toxin cargoes while ignoring the rest of the nanoparticle vectors. No antinanoparticle immune response was observed, despite the high antitoxin responses generated by the nanotoxoid. Notably, an RBC-NP-based vaccine system also allows for the detainment of other membrane-active protein toxins. For example, we demonstrated the successful neutralization of two other types of PFTs, an oligomerizing streptolysin-O from *Streptococcus* bacteria and a small peptide from bee venom.[43] Given the broad presence of membrane-damaging virulence factors in pathogenic microbes such as *Escherichia coli*, *Helicobacter pylori*, *Clostridium perfringens*, and *Bacillus anthracis*,[44] RBC-NPs offer

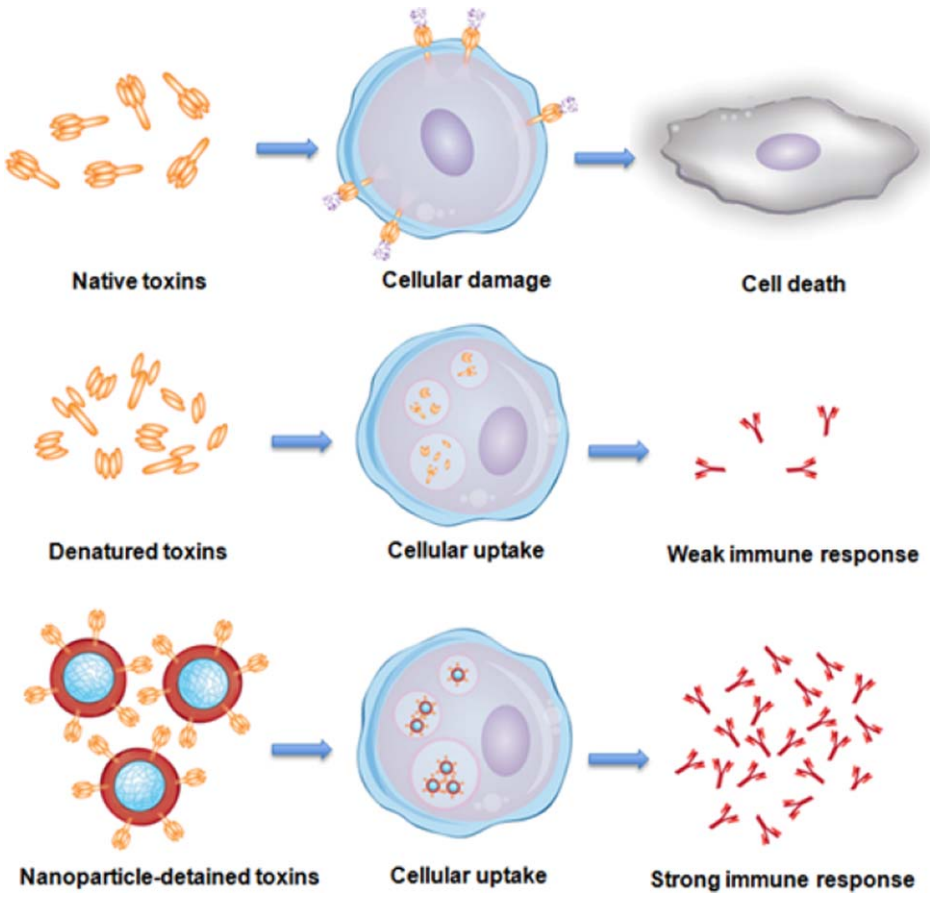

Figure 5. Schematics demonstrating the benefit of toxin detainment by nanoparticle carriers. Native toxins are cytotoxic and are unsafe to be administered for vaccination (top row). Conventional toxoid preparation disrupts toxin antigens and compromises their immunogenicity (middle row). Nanoparticle detainment neutralizes toxins by interfering with their virulence mechanism. Structurally intact toxin antigens can thus be safely administered to mount a strong antitoxin immune response (bottom row). Reproduced with permission from Elsevier.[42]

a versatile platform for vaccine development against many infectious diseases. In addition to the membrane-coated exterior that serves to sequester PFTs, the nanoparticles possess other characteristic features that can facilitate the immune processing of the toxin antigens. For instance, the nanoparticles' high stability and small sizes facilitate antigen delivery to lymphatic organs, such as the spleen and lymph nodes.[43] The nanoparticle/toxin complexes also possess a particulate morphology that is more prone to cellular ingestion compared to free proteins. This property allows toxin antigens to be efficiently taken up and metabolized by antigen-presenting cells for immune processing. Along with the antigenically preserved toxin antigens, these other factors likely contribute to the enhanced antibody responses as well.

The ability to neutralize toxins via the detainment strategy also highlights the intricate biomolecular machineries behind the virulence mechanisms of protein toxins.[45] For instance, PFTs such as α-hemolysin and streptolysin-O require membrane interactions and oligomerizing actions with other toxin monomers for channel formation and cellular disruption. Nanoparticle detainment immobilizes the toxin molecules, alters their cellular distribution, and thus restrict their interactions with targeted cellular substrates. We envision that this detainment concept may be extended to other toxin categories that require interactions with specific substrates and receptors to take effect. For example, toxins that interact with membrane receptors (ie, neurotoxins) or cytosolic substrates (ie, Shiga toxin) can likewise be detained by nanoparticulates to preclude their virulence activities and to facilitate their cellular digestion and immune processing. Toward future development, however, rigorous safety characterizations of particle-detained formulations are warranted, as sequestered toxins can be potentially bioactive. Methods that help secure the toxin detainment, enhance particle stability, and accelerate particle cellular uptake are expected to benefit the overall vaccine system as they minimize the risks of premature toxin release. Given the engineering flexibility of nanomaterials, numerous toxin association and immune modulation approaches are possible.[46] Meanwhile, as antibiotic resistance rises at an alarming rate, the urgent need for emerging antimicrobial measures can benefit from creative engineering in nanotechnology. As the nanoparticle-mediated

toxin detainment approach promises vaccine formulations with a higher potency, the RBC-NP platform is expected to become a new generation of nanotoxoid vaccines that can improve the management of infectious diseases.[47,48] By promoting antivirulence immunity against the pathogenic factors of bacteria, the vaccination approach could reduce the occurrence of microbial infections without the reliance on antibiotics.

Summary and Outlook

The RBC-NP platform uses a novel top-down approach to transfer the entire RBC exterior, including both lipids and membrane-associated proteins, onto synthetic nanoparticles. Such biomimetic nanoparticles are disguised as RBCs to evade the immune system, although the synthetic cores are able to carry chemotherapy drugs. The RBC-NPs have demonstrated tremendous therapeutic implications for systemic drug delivery, biodetoxification, and toxin vaccination. From a drug-delivery perspective, the RBC-NPs not only enable extended systemic circulation but also allow for the implementation of synthetic biomaterials, including biocompatible polymers with established clinical uses for drug delivery. From a detoxification perspective, the RBC-NPs function as a toxin nanosponge to absorb and neutralize a wide spectrum of membrane-damaging toxins, regardless of their molecular structures. By targeting a common mechanism shared by various types of toxins, the RBC-NPs introduce a unique strategy for the use of injectable nanocarriers for biodetoxification. From a toxoid vaccine perspective, the RBC-NPs provide a novel toxin-detainment strategy to safely deliver nondisrupted toxins for immune processing. The resulting nanotoxoids yield stronger immunogenicity and superior efficacy compared with commonly used protein-denaturation–based toxin vaccination approaches.

The further development of RBC-NPs for clinical use needs to address blood-supply issues. For *in vivo* animal testing of the RBC-NPs, RBCs collected from syngeneic animal models can be used. This can prevent the immune responses associated with the use of animals with different blood types. For future translation to humans, RBCs can be collected from a blood bank. As a result, we expect the resulting RBC-NPs need to match patients on the basis of their blood types (A, B, AB, or O type) and Rh compatibility (Rh+ or Rh−) according to a cross-match test, as in the case of a blood transfusion. Note that RBC-NPs made of type O, Rh− RBCs will be applicable to the entire human population. This would simplify the clinical translation of RBC-NPs by allowing the preparation of a singular formulation.

The RBC membrane-coating technique has recently been extended to other nanostructures, including gold nanocages,[49] silica,[50] and gelatin nanoparticles,[51] for more versatile applications. The RBC-NP platform has also inspired the development of novel molecular probes to better understand the interactions between the cell membrane and synthetic nanoparticles.[52] Moreover, the success of RBC membrane coating has also motivated the application of other cellular membranes as coating materials for advanced functionalities. For example, we successfully collected cancer cell membranes from mouse melanoma cells and subsequently coated them onto PLGA nanoparticles.[53] When coupled with immunological adjuvants, these nanoparticles promote a tumor-specific immune response for use in vaccine applications.

The use of cellular membranes to coat nanoparticles has emerged as a robust and versatile approach for integrating natural and synthetic biomaterials to form functional nanostructures. Such nanoparticle functionalization represents as a feasible method for developing novel, nature-inspired nanotherapeutics with complex antigenic information and surface properties. In the future, cell-membrane-coated nanoparticles will continue to inspire us and other researchers to develop new nanotherapeutics for effective disease intervention. They are expected to lead to a new paradigm of thinking on design and applications in nanomedicine.

Literature Cited

1. Hu CMJ, Fang RH, Zhang LF. Erythrocyte-inspired delivery systems. *Adv Healthc Mater*. 2012;1(5):537–547.
2. Muzykantov VR. Drug delivery by red blood cells: vascular carriers designed by mother nature. *Expert Opin Drug Deliv*. 2010;7(4):403–427.
3. Ihler GM, Glew RH, Schnure FW. Enzyme loading of erythrocytes. *Proc Natl Acad Sci U S A*. 1973;70(9):2663–2666.
4. Bax BE, Bain MD, Fairbanks LD, et al. A 9-yr evaluation of carrier erythrocyte encapsulated adenosine deaminase (ADA) therapy in a patient with adult-type ADA deficiency. *Eur J Haematol*. 2007;79(4):338–348.
5. Hamidi M, Zarrin A, Foroozesh M, Mohammadi-Samani S. Applications of carrier erythrocytes in delivery of biopharmaceuticals. *J Control Release*. 2007;118(2):145–160.
6. Godfrin Y, Horand F, Franco R, et al. International seminar on the red blood cells as vehicles for drugs. *Expert Opin Biol Ther*. 2012;12(1):127–133.
7. Magnani M. Erythrocytes as carriers for drugs: the transition from the laboratory to the clinic is approaching. *Expert Opin Biol Ther*. 2012;12(2):137–138.
8. Mohandas N, Gallagher PG. Red cell membrane: past, present, and future. *Blood*. 2008;112(10):3939–3948.
9. Wan JD, Forsyth AM, Stone HA. Red blood cell dynamics: from cell deformation to ATP release. *Integr Biol*. 2011;3(10):972–981.
10. Doshi N, Zahr AS, Bhaskar S, Lahann J, Mitragotri S. Red blood cell-mimicking synthetic biomaterial particles. *Proc Natl Acad Sci U S A*. 2009;106(51):21495–21499.
11. Hu CMJ, Zhang L, Aryal S, Cheung C, Fang RH, Zhang LF. Erythrocyte membrane-camouflaged polymeric nanoparticles as a biomimetic delivery platform. *Proc Natl Acad Sci U S A*. 2011;108(27):10980–10985.
12. Moghimi SM, Hunter AC, Murray JC. Long-circulating and target-specific nanoparticles: theory to practice. *Pharmacol Rev*. 2001;53(2):283–318.
13. Peer D, Karp JM, Hong S, FaroKhzad OC, Margalit R, Langer R. Nanocarriers as an emerging platform for cancer therapy. *Nat Nanotechnol*. 2007;2(12):751–760.
14. Davis ME, Chen Z, Shin DM. Nanoparticle therapeutics: an emerging treatment modality for cancer. *Nat Rev Drug Discov*. 2008;7(9):771–782.

15. Geng Y, Dalhaimer P, Cai SS, et al. Shape effects of filaments versus spherical particles in flow and drug delivery. *Nat Nanotechnol*. 2007;2(4):249–255.
16. Alexis F, Pridgen E, Molnar LK, Farokhzad OC. Factors affecting the clearance and biodistribution of polymeric nanoparticles. *Mol Pharm*. 2008;5(4):505–515.
17. Yoo JW, Chambers E, Mitragotri S. Factors that control the circulation time of nanoparticles in blood: challenges, solutions and future prospects. *Curr Pharm Des*. 2010; 16(21):2298–2307.
18. Hu CMJ, Fang RH, Luk BT, Zhang LF. Polymeric nanotherapeutics: clinical development and advances in stealth functionalization strategies. *Nanoscale*. 2014;6(1): 65–75.
19. Luk BT, Hu CMJ, Fang RH, et al. Interfacial interactions between natural RBC membranes and synthetic polymeric nanoparticles. *Nanoscale*. 2014;6(5):2730–2737.
20. Hu CMJ, Fang RH, Luk BT, et al. "Marker-of-self" functionalization of nanoscale particles through a top-down cellular membrane coating approach. *Nanoscale*. 2013;5(7):2664–2668.
21. Aryal S, Hu CMJ, Fang RH, et al. Erythrocyte membrane-cloaked polymeric nanoparticles for controlled drug loading and release. *Nanomedicine*. 2013; 8(8):1271–1280.
22. Wu Z, Li T, Li J, et al. Turning erythrocytes into functional micromotors. *ACS Nano*. 2014;8(12):12041–12048.
23. Gao W, Hu CMJ, Fang RH, Luk BT, Su J, Zhang L. Surface functionalization of gold nanoparticles with red blood cell membranes. *Adv Mater*. 2013;25(26):3549–3553.
24. Giljohann DA, Seferos DS, Daniel WL, Massich MD, Patel PC, Mirkin CA. Gold nanoparticles for biology and medicine. *Angew Chem Int Ed Engl*. 2010;49(19):3280–3294.
25. Rasko DA, Sperandio V. Anti-virulence strategies to combat bacteria-mediated disease. *Nat Rev Drug Discov*. 2010;9(2):117–128.
26. Gilbert RJC. Pore-forming toxins. *Cell Mol Life Sci*. 2002;59(5):832–844.
27. Andreeva-Kovalevskaya ZI, Solonin AS, Sineva EV, Ternovsky VI. Pore-forming proteins and adaptation of living organisms to environmental conditions. *Biochemistry (Mosc)*. 2008;73(13):1473–1492.
28. Beghini DG, Hernandez-Oliveira S, Rodrigues-Simioni L, Novello JC, Hyslop S, Marangoni S. Anti-sera raised in rabbits against crotoxin and phospholipase A2 from *Crotalus durissus cascavella* venom neutralize the neurotoxicity of the venom and crotoxin. *Toxicon*. 2004;44(2): 141–148.
29. Chen ZC, Moayeri M, Zhao HY, Crown D, Leppla SH, Purcell RH. Potent neutralization of anthrax edema toxin by a humanized monoclonal antibody that competes with calmodulin for edema factor binding. *Proc Natl Acad Sci U S A*. 2009;106(32):13487–13492.
30. McCormick CC, Caballero AR, Balzli CL, Tang AH, O'Callaghan RJ. Chemical inhibition of alpha-toxin, a key corneal virulence factor of *Staphylococcus aureus*. *Invest Ophthalmol Vis Sci*. 2009;50(6):2848–2854.
31. Hoshino Y, Koide H, Furuya K, et al. The rational design of a synthetic polymer nanoparticle that neutralizes a toxic peptide *in vivo*. *Proc Natl Acad Sci U S A*. 2012;109(1):33–38.
32. Bayley H. Membrane-protein structure piercing insights. *Nature*. 2009;459(7247):651–652.
33. Hu CMJ, Fang RH, Copp J, Luk BT, Zhang LF. A biomimetic nanosponge that absorbs pore-forming toxins. *Nat Nanotechnol*. 2013;8(5):336–340.
34. Jacobson DL, Gange SJ, Rose NR, Graham NMH. Epidemiology and estimated population burden of selected autoimmune diseases in the united states. *Clin Immunol Immunopathol*. 1997;84(3):223–243.
35. Weetman AP. Medical progress: Graves' disease. *N Engl J Med*. 2000;343(17):1236–1248.
36. Hudson BG, Tryggvason K, Sundaramoorthy M, Neilson EG. Alport's syndrome, Goodpasture's syndrome, and type IV collagen. *N Engl J Med*. 2003;348(25):2543–2556.
37. Hansel TT, Kropshofer H, Singer T, Mitchell JA, George AJT. The safety and side effects of monoclonal antibodies. *Nat Rev Drug Discov*. 2010;9(4):325–338.
38. Tabas I, Glass CK. Anti-inflammatory therapy in chronic disease: challenges and opportunities. *Science*. 2013; 339(6116):166–172.
39. Copp JA, Fang RH, Luk BT, et al. Clearance of pathological antibodies using biomimetic nanoparticles. *Proc Natl Acad Sci U S A*. 2014;111(37):13481–13486.
40. Kernodle DS. Expectations regarding vaccines and immune therapies directed against *Staphylococcus aureus* alpha-hemolysin. *J Infect Dis*. 2011;203(11):1692–1693.
41. Kennedy AD, Wardenburg JB, Gardner DJ, et al. Targeting of alpha-hemolysin by active or passive immunization decreases severity of USA300 skin infection in a mouse model. *J Infect Dis*. 2010;202(7):1050–1058.
42. Hu CMJ, Zhang LF. Nanotoxoid vaccines. *Nano Today*. 2014;9(4):401–404.
43. Hu CMJ, Fang RH, Luk BT, Zhang LF. Nanoparticle-detained toxins for safe and effective vaccination. *Nat Nanotechnol*. 2013;8(12):933–938.
44. Los FCO, Randis TM, Aroian RV, Ratner AJ. Role of pore-forming toxins in bacterial infectious diseases. *Microbiol Mol Biol Rev*. 2013;77(2):173–207.
45. Ivarsson ME, Leroux JC, Castagner B. Targeting bacterial toxins. *Angew Chem Int Ed Engl*. 2012;51(17):4024–4045.
46. Hoshino Y, Kodama T, Okahata Y, Shea KJ. Peptide imprinted polymer nanoparticles: a plastic antibody. *J Am Chem Soc*. 2008;130(46):15242–15243.
47. Zhang L, Pornpattananangkul D, Hu CMJ, Huang CM. Development of nanoparticles for antimicrobial drug delivery. *Curr Med Chem*. 2010;17(6):585–594.
48. Gao W, Thamphiwatana S, Angsantikul P, Zhang L. Nanoparticle approaches against bacterial infections. *WIREs Nanomed Nanobiotechnol*. 2014;6(6):532–547.
49. Piao J, Wang L, Gao F, You Y, Xiong Y, Yang L. Erythrocyte membrane is an alternative coating to polyethylene glycol for prolonging the circulation lifetime of gold nanocages for photothermal therapy. *ACS Nano*. 2014;8(10):10414–10425.

50. Parodi A, Quattrocchi N, van de Ven AL, et al. Synthetic nanoparticles functionalized with biomimetic leukocyte membranes possess cell-like functions. *Nat Nanotechnol*. 2013;8(1):61–68.
51. Li LL, Xu JH, Qi GB, Zhao XZ, Yu FQ, Wang H. Core-shell supramolecular gelatin nanoparticles for adaptive and "on-demand" antibiotic delivery. *ACS Nano*. 2014; 8(5):4975–4983.
52. Fan Z, Zhou H, Li P, Speer J, Cheng H. Structural elucidation of cell membrane-derived nanoparticles using molecular probes. *J Mater Chem B*. 2014;2(46):8231–8238.
53. Fang RH, Hu CMJ, Luk BT, et al. Cancer cell membrane-coated nanoparticles for anticancer vaccination and drug delivery. *Nano Lett*. 2014;14(4):2181–2188.