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Review Article

Red Blood Cells as Drug Delivery and Imaging Agents: An Update

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Abstract

Red blood cells are essential physiologic cellular components that are produced constantly at abundant levels throughout life, making them an easily obtainable vehicle for both targeted cellular delivery of exogenous drugs as well as non-invasive in vivo imaging of the vasculature. As red blood cells are readily acquired in sufficient quantities and minimally immunogenic by nature, red blood cell-based agents may allow delivery of pharmaceuticals into the body under conditions of both reduced toxicity and increased physiologic half-life, when compared to the non-carrier-based drug equivalent. Research efforts in this area have recently seen major advances that are likely to accelerate the translation of red blood cell-based carrier methods into the clinical setting in the near future. This includes the coupling of therapeutic agents to the external surface of red blood cells, as well as more refined methods for internal encapsulation of drugs within red blood cells or cell-based derivatives. Here we review some of the more recent advances in the field regarding the loading of various therapeutic agents onto red blood cells as well as the use of red blood cell based imaging agents for in vivo vascularity imaging.

Keywords: Red blood cells; Erythrocytes; Nanoparticles; Drug delivery; Imaging markers

Abbreviations

ALL: Acute Lymphocytic Leukemia; R: Arginine; ASO: Antisense Oligonucleotide; N: Asparagine; D: Aspartic Acid; COPD: Chronic Obstructive Pulmonary Disease; CD: Cluster Of Differentiation; CT: Computed Tomography; DNA: Deoxyribonucleic Acid; DSP: Dexamethasone Sodium Phosphate; G: Glycine; ICG: Indocyanine Green; IR: Infrared; MRI: Magnetic Resonance Imaging; miR: MicroRna; MNGIE: Mitochondrial Neurogastrointestinal Encephalomyopathy; nm: Nanometer; PEG: Polyethylene Glycol; PGLA: Poly(lactic-co-glycolic acid); PET: Positron Emission Tomography; PD-L1: Programmed Death Ligand 1; RNA: Ribonucleic Acid; SPIO: Superparamagnetic Iron Oxide; TPZ: Tirapazamine; FDG: [18F]-Fluorodeoxyglucose

Introduction

Over the years, considerable research efforts have been expended towards the development of drug delivery systems capable of overcoming significant toxicities and/or improving suboptimal clinical efficacies of otherwise promising pharmaceutical compounds. Many of these delivery approaches are based on so called "carrier" systems where the agent of interest is complexed with chemical and/or biologic entities that perform a number of in vivo functions, such as protection of drug from premature serum degradation and inactivation, avoidance of solicitation of undesirable immunologic reactions, and reduction of drug metabolism by off-target organs and other tissues. This includes the development of synthesized nanoparticle platforms where desired biophysical properties are engineered to enhance the delivery and uptake of the transported agent to the tissues of interest. These nanoparticle platforms have been largely organized into three classes of nanoparticles: lipid-derived nanoparticles, polymeric nanoparticles, and inorganic nanoparticles [1]. Although many of these nanoparticle platforms demonstrate promising potential, the development of many has also been severely hampered by either feared or poorly characterized toxicities, or significant nanoparticle accumulation in off-target tissues including the liver and reticulo-endothelial systems [2-4].

Advantages of Red Blood Cells as Drug Delivery Systems

A number of significant limitations that impede the development of exogenously-derived nanoparticle systems can be readily circumvented by the adoption of endogenous red blood cells as the underlying drug carrier system. Unlike exploratory nanoparticle systems, red blood cells have well characterized in vivo properties, with an average life span of 120 days, and ultimate clearance by the spleen, liver, and reticulo-endothelial system [5,6]. The inherent phospholipid and cytoskeletal structure of red blood cell membranes as well as the expression of immune-modulating surface markers such as CD47 mask the transported agent from otherwise rapid clearance by immunologic mechanisms (opsonization) or biliary or renal excretion, allowing for an improved pharmacokinetic profile otherwise not possible for some pharmaceuticals [6]. Other non cell based nanoparticles ultimately require surface coating with immune-evading polymers such as Polyethylene Glycol (PEG) in order to achieve a somewhat similar bioavailability profile [7]. The immune-evading properties of red blood cells have also inspired the use of cellular membrane derived particles to encapsulate a number of compounds, including iron oxide particles, gold nanorods, and chemotherapeutics [8-11]. The encapsulation of agents within red blood cells also allows for more gradual drug release into the

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circulation, depending on the specific drug diffusion properties or cell membrane transport potential. Red blood cells are also routinely collected for transfusion purposes within a short time period under safe and unburdensome conditions and can be obtained in relatively large volumes. In addition, red blood cells possess a myriad of cytoplasmic enzymes and proteins that can potentially be appropriated for therapeutic outcomes. More recently, successful targeted agent release has been demonstrated in preclinical models by coupling of drug to the external membrane surface of red blood cells, representing an exciting novel application of a red blood cell based drug delivery system [12]. Some of the more promising red blood cell delivery approaches shall be discussed in greater depth below.

Methods of Drug Encapsulation into Red Blood Cells

Whole red blood cell based systems: Several approaches have been developed to facilitate drug entry into red blood cells or their derivatives (hemoglobin deficient erythrocyte ghosts and membrane derived vesicles) that have been described in the literature. Two major methods have been broadly adopted by many investigators due to relative efficient agent loading, straightforward and scalable methodology, and acceptable toxicities. One major method that has been successfully used is based on exposure of red blood cells to an electric field, resulting in the formation of multiple transient pores in the cell membrane, a technique known as electroporation. The electric field-induced transient pore formation allows intracellular uptake of the desired agent. In addition, fine tuning of electroporation conditions can modulate pore size, thus improving agent uptake efficiency and specificity [13-15]. Another commonly utilized approach to intracellular agent incorporation involves transient exposure of red blood cells to a hypotonic solution that leads to osmotic shock-induced pore formation. As with electroporation, pore formation results in intracellular agent influx. Intracellular agent entrapment is then achieved by subsequent cell immersion into a hypertonic solution resulting in rapid membrane resealing [16]. These methods have been successfully used to encapsulate proteins, RNA, DNA, imaging agents, and other small pharmaceuticals.

Red blood cell membrane-based nanosystems: Red blood cells can also be fragmented to generate smaller sized drug carriers on the scale of nanometers capable of encapsulating therapeutics or even other drug loaded nanoparticles, providing much of the same benefits that whole cell derived carriers possess. Two major methods for the creation of smaller red blood cell-based carriers use either sonication or mechanical extrusion [17]. Sonication involves physical membrane disruption and fragmentation of red blood cells using sound energy, creating spherical particles that are nanometers in size [18]. Extrusion is another process for creating smaller membrane-based carriers in which red blood cells are forced through a layer of nanoporous material resulting in membrane disruption and fragmentation with reformation of smaller sized vesicular structures [19].

Red blood cell "hitchhiking": More recently, Brenner et al demonstrated that drug containing nanoparticles could be directly absorbed onto the surface of red blood cells. Such nanoparticles could then be transferred with high efficiency to the first organ downstream of the intravascular injection site; a process Brenner termed "red blood cell-hitchhiking" [20]. Using this approach, Brenner showed that this hitchhiking approach could increase liposomal nanoparticle uptake into the first downstream organ such as the lung by up to 40-fold, when compared to free nanoparticles. Brenner showed that intra-carotid injection of hitchhiked red blood cells delivered greater than 10% of the injected nanoparticle dose to the brain, significantly higher than what could typically be accomplished with other targeted therapies, including antibody-based strategies. The putative mechanism of nanoparticle transfer to the target organ is believed to result from direct mechanical transfer of nanoparticles from the surface of red blood cells to the capillary wall, as the cells undergo spatial deformation while being squeezed through narrow capillary lumens during cell transit. In addition, the group also found nanoparticle uptake by leukocytes residing within the vessel wall. Both the nanoparticle affinity to red blood cells as well as nanoparticle transfer efficiency to the target organ is dependent on the physical composition of the nanoparticles, with some nanoparticle constructs showing higher target organ-to-liver and target organ-to-blood ratios than other constructs, thus leading to the possibility that nanoparticle composition design can be tailored for specific organ uptake [12].

Red Blood Cell Based Therapeutics

Incorporation of enzymes into red blood cells: The incorporation of enzymes into red blood cells has been investigated as a novel enzyme replacement technology in patients suffering from particular enzyme deficiencies. Intracellular enzymatic entrapment is postulated to help protect susceptible enzymes from premature degradation and metabolism and thus increase overall enzymatic half-life, prolonging drug efficacy over a longer temporal window. While multiple different enzyme targets have been studied in preclinical animal models, there have been a few instances where enzyme incorporation into red blood cells has entered clinical trials. For example, the incorporation of the enzyme L-asparaginase into red blood cells has been explored in clinical trials involving patients suffering from Acute Lymphocytic Leukemia (ALL) [21]. The proposed mechanism of action is based on the premise that certain malignancies such as ALL are relatively deficient in the amino acid asparagine and that further asparagine depletion in tumor cells stimulates tumor cell death. As L-asparaginase converts L-asparagine into aspartic acid and ammonia, the enzyme is postulated to accelerate asparagine depletion in tumor cells, whereas normal cells are somewhat resistant to the agent, due to intact intracellular asparagine biosynthesis [22]. Asparaginase encapsulated red blood cells are also being investigated in clinical trials in patients with pancreatic cancer and breast cancer as well (U.S. clinical trials NCT03665441 and NCT03674242).

In an exploratory therapy approach to drug addiction, Rossi et al encapsulated the recombinant bacterial enzyme cocaine esterase into red blood cells in vitro. Rossi found that enzyme stability could be maintained for at least 4 hours, and that the enzyme-loaded cells could efficiently degrade cocaine in a time-dependent and concentration-dependent manner [23]. In addition, it was also shown that red blood cell encapsulation of cocaine esterase resulted in a longer in vivo half life compared to the unencapsulated enzyme, suggesting that cell-encapsulated cocaine esterase may represent a novel approach to drug detoxification.

With regards to genetic diseases related to particular enzyme deficiencies, red blood cells loaded with the enzyme thymidine phosphorylase have been tested in patients suffering from the

autosomal recessive disorder Mitochondrial Neurogastrointestinal Encephalomyopathy (MNGIE). These patients suffer from a lack of thymidine phosphorylase, leading to excessive accumulation of deoxyribonucleosides thymidine and deoxyuridine, that ultimately results in mitochondrial dysfunction. Thymidine phosphorylase encapsulated red blood cells have been shown to decrease concentrations of these deoxyriboncleosides in MNGIE patients [24,25]. Similar enzyme replacement strategies have been pursued in preclinical models of several other metabolic disorders and malignancies, including red blood entrapment of methionine gamma-lyase for cancer, glutamate dehydrogenase for hyperammonemia, and phenylalanine ammonia-lyase for Phenylketonuria [23, 26-28].

Red blood cell coated nanoparticles have even been proposed as a method for detoxification from microbial infection. Ben-Akiva et al encapsulated biodegradable Poly(Lactic-Co-Glycolic Acid) (PGLA) nanoparticles with red blood cell membranes [29]. The PGLA particles had been engineered with anisotropic morphologies ranging from prolate ellipsoidal to oblate ellipsoidal shapes, allowing for increased surface area-to-volume particle ratios compared to spherical forms. Ben-Akiva hypothesized that particle anisotropy could synergize with red blood cell membrane coating, leading to improved pharmacokinetic and therapeutic nanoparticle properties. The group found that the prolate ellipsoidal-shaped nanoparticles coated with red blood cell membranes demonstrated a longer circulatory half-life (171 minutes) when compared to either the oblate ellipsoidal form (82 minutes) or the spherical form (64 minutes). These cellular membrane coated nanoparticles were then tested in a mouse model for their toxin absorbing capacities. Mice were injected with a lethal dose of the bacterial alpha toxin and the survival of mice was then tracked over time. While mice in the control group had a median survival time of 2.75 hours, 50% of mice injected with the prolate ellipsoidal nanoparticles and 33% of mice injected with oblate ellipsoidal nanoparticles remained alive one week after nanoparticle injection. Such findings support further investigation of these nanoparticles as novel treatment approaches to sepsis from known toxicities.

Non-Enzymatic Drug Incorporation into Red Blood Cells

Corticosteroid encapsulated red blood cells: Red blood cell incorporation of a number of non-enzymatic drugs has been characterized in preclinical and clinical studies. In 2018, Coker et al demonstrated in a clinical trial of healthy volunteers that encapsulation of Dexamethasone Sodium Phosphate (DSP) into red blood cells allowed for a sustained release of DSP that could be detected up until 35 days after a single infusion of a loading dose of 16.9 mg of drug [30]. DSP-loaded red blood cells have been investigated in clinical trials for a number of inflammatory diseases. These include irritable bowel disease, inflammatory bowel disease, cystic fibrosis, and Chronic Obstructive Pulmonary Disease (COPD) [31-36].

Chemotherapeutic encapsulated red blood cells: Development of red blood cell carriers of chemotherapeutics has also been an area of active investigation [37]. Malhotra et al developed red blood cell membrane-derived vesicles containing complexes of a chemotherapeutic camptothecin with an amphiphilic fluorophore imaging agent. It was shown that the vesicle derived carriers were nonphagocytic with minimal stimulation of cytokine release from macrophages in vitro. The carriers could also be internalized by lung carcinoma cells. Intravenous injection of the camptothecin loaded carriers into mice showed no significant accumulation in the kidneys or heart or other vital organs [38]. Chen et al demonstrated that doxorubicin could be internalized into Prussian blue nanoparticles coated with red blood cell membranes with relatively high loading capacity (up to 130% by weight), and that red blood cell membrane coating of the nanoparticles resulted in significantly improved doxorubicin retention [11]. Zhang et al encapsulated the chemotherapeutic paclitaxel with an infrared dye IR780 into a hyaluronic acid-modified red blood cell membrane-based carrier, for the purposes of combined photochemotherapy. The presence of red blood cell membranes decreased macrophage mediated phagocytosis in vitro, when compared to nanoparticles not shielded by cellular membranes [39]. Shi et al developed a multilayered nanoparticle in which the chemotherapeutic doxorubicin and phototherapy agent Purpurin 18 were contained within a dextran-polymer derived matrix that was then further surrounded by red blood cell membrane components modified with the tripeptide NGR (asparagine-glycine-arginine). Shi showed that the carriers were capable of significantly impeding growth of HepG2 hepatocellular carcinoma cells using the combination of sonodynamic therapy and chemotherapy with minimal side effects [40].

Nucleic acid encapsulated red blood cells: Red blood cell carriers have also been utilized to deliver nucleic acids to target tissues. For example, Usman et al created extracellular vesicles from type O red blood cells that were then loaded with antisense oligonucleotides (ASOs) [13]. These antisense oligonucleotides antagonize miR-125b, an oncogenic mRNA found in leukemia, prostate cancer, and breast cancer. miR-125b loaded vesicles were then injected directly into mice bearing CA1a breast cancer tumors. In comparison to tumors injected with vesicle carriers lacking ASOs or with unencapsulated ASOs, tumors injected with miR-125b vesicles underwent overall tumor shrinkage. Using a leukemia MOLM13 cell line modified to express the imaging markers luciferase and green fluorescent protein, Usman created a leukemic xenograft mouse model that was then systemically injected with miR-125b vesicles. Usman showed that injection of miR-125b vesicles resulted in a suppression of tumor cell proliferation, as measured by total tumor derived bioluminescence expression.

Site-directed red blood cell therapeutics: Site directed therapies have also been explored with red blood cell based carrier systems. Jiang et al encapsulated natural melanin derived from cuttlefish into red blood cells to create melanin-loaded nanoparticles. Natural melanin has potent photothermal properties under near infrared light exposure. Jiang found that melanin loaded red blood cells could accumulate in A549 tumor bearing mice and demonstrated high photothermal efficacy against the tumor upon excitation with an 808 nm laser [41]. Other groups have modified red blood cell membranes to directly target tissues of interest with cargo drugs. For example, Wen et al modified red blood cell membranes to contain the tumor targeting tripeptide arginylglycylaspartic acid (RGD) and then encapsulated albumin nanoparticles containing the chemotherapeutic gefitinib. This construct was shown to target A549 tumor cells in a mouse model resulting in a decrease in tumor size and a prolonged mouse survival time [42]. Targeted delivery

of tumor antigens to the spleen by engineered red blood cell-based nanoparticles for immunotherapy purposes was demonstrated by Han et al in 2019 [43]. Han fused red blood cells with tumor cells to create so called "Nano-Ag@erythrosomes." Han's group subjected red blood cells and tumor cells to both sonication and extrusion to create nanoparticles containing fused membrane components from both cell types. They found that these nanoparticles were quickly cleared from the blood by the spleen in mice. Splenic dendritic cells and macrophages exposed to the nanoparticles upregulated expression of multiple cytokines, including pro-inflammatory type I interferons. Han tested these nanoparticles in mouse models bearing tumors derived from B16F10 and 4T1 tumor cell lines and showed tumor growth inhibition in these mice after combining nanoparticle injection with Programmed Death Ligand 1 (PD-L1) blockade mediated by an anti-PD-L1 antibody. Using a similar tactic, Chen et al created red blood cell-based vesicles by fusion of membrane fragments of both red blood cells and cells from the head and neck squamous carcinoma cell line WSU-HN6 [44]. The red blood cell membrane components of the nanoparticles had been modified by membrane attachment of oligopeptides composed of eight aspartic acids which preferentially bind bone resorption sites. These vesicle constructs were used to encapsulate branched polymer nanoparticles containing the photosensitizer agent IR780 and the hypoxia-activated prodrug tirapazamine (TPZ). Exposure of IR780 to laser irradiation generated significant thermal energy as well as singlet oxygen species, exacerbating tumor hypoxia. The subsequent hypoxia then stimulated conversion of TPZ into cytotoxic radicals in the tumor microenvironment. After intravenous injection of the vesicle constructs into mice bearing WSU-HN6 mandibular tumors, Chen found significant preferential vesicle accumulation at the sites of mandibular bone invasion by tumor. Photothermal ablation of tumor sites after vesicle accumulation resulted in a relatively significant inhibition in tumor growth.

Red blood cell based imaging agents: Multiple investigators have explored the use of red blood cell-derived constructs as in vivo imaging markers of the vasculature or other target organs. For example, red blood cell based carriers of Super Paramagnetic Iron Oxide (SPIO) nanoparticles have been used to image the vasculature with Magnetic Resonance Imaging (MRI) [45-49]. While SPIOs have historically been used as extracellular MRI contrast agents in the clinic, the encapsulation of SPIOs into red blood cells significantly extends their half-life in vivo. Using hypotonic shock to load SPIOs into mouse red blood cells, Antonelli et al injected SPIO-loaded red blood cells into mice, showing that the red blood cell coating of SPIOs prolonged SPIO half-life in the bloodstream up to 12 days [47].

Aryal et al used a membrane fusion technique to complex gold nanoparticles to the surface of red blood cells [50]. These gold complexed red blood cell constructs demonstrated a blood pool circulation time of about 100 days before undergoing splenic sequestration, allowing their potential use as a long-lived blood pool imaging agent with Computed Tomographic (CT) imaging.

Burns et al created near infrared imaging nanoparticle probes by encapsulating the dye Indocyanine Green (ICG) into red blood cell nanoparticles created by sequential membrane extrusion. The nanoparticles were subsequently injected into a mouse model of intraperitoneal ovarian cancer. Using a spatially-modulated illumination system for fluorescence imaging, Burns showed preferential nanoparticle accumulation in vivo and ex vivo within intraperitoneal tumors relative to major organs [51].

Red blood cells have also been studied as cell-based vehicles for imaging of the vasculature using Positron Emission Tomography (PET). As red blood cells demonstrate inherently high surface expression of the glucose transporter GLUT1 [52], a number of investigators have shown that red blood cells are capable of significant intracellular incorporation of the PET imaging agent, [18F]-Fluorodeoxyglucose (FDG). After FDG-labeled red blood cells were injected intravenously in small animal models, the total body vasculature could be imaged with PET imaging [53-55]. Matsusaka et al showed that heat-damaged red blood cells labeled with FDG could be used to image the splenic tissue in a rat model with PET [56]. Wang et al showed that FDG labeled red blood cells could be used to indirectly detect changes in myocardial and cerebrovascular perfusion in normal rats with PET imaging after exposure to pharmacologic vasodilators [57]. In addition, Wang showed that changes in myocardial perfusion in rat models of myocardial infarction and diabetic cardiomyopathy could also be detected by PET imaging with FDG-labeled red blood cells.

Conclusions

Advances in cellular engineering have led to the development of multiple promising red blood cell based drug delivery systems that offer distinct advantages over both unmodified therapeutics or other non-cellular nanoparticle carrier platforms. These red blood cell based vehicles are either currently being investigated in patients or demonstrate significant promise in preclinical animal models. At least some of these technologies are likely to be pursued into the clinic as novel alternative treatments for diverse disorders, including genetic disorders, metabolic abnormalities, and cancer. In addition, red blood cells have recently been shown to function as in vivo imaging agents with multiple imaging modalities in animal models, some of which have high potential for direct translation into the clinical realm.

References

1. Mitchell MJ, Billingsley MM, Haley RM, Wechsler ME, Peppas NA, Langer R. Engineering precision nanoparticles for drug delivery. Nature reviews Drug discovery. 2021; 20: 101-24.
2. Najahi-Missaoui W, Arnold RD, Cummings BS. Safe Nanoparticles: Are We There Yet?. Int J Mol Sci. 2020; 22: 385.
3. De Jong WH, Borm PJ. Drug delivery and nanoparticles:applications and hazards. Int J Nanomedicine. 2008; 3: 133-49.
4. Yao Y, Zang Y, Qu J, Tang M, Zhang T. The Toxicity Of Metallic Nanoparticles On Liver: The Subcellular Damages, Mechanisms, And Outcomes. Int J Nanomedicine. 2019; 14: 8787-804.
5. Mohandas N, Gallagher PG. Red cell membrane: past, present, and future. Blood. 2008; 112: 3939-48.
6. Neri S, Swinkels DW, Matlung HL, van Bruggen R. Novel concepts in red blood cell clearance. Curr Opin Hematol. 2021; 28: 438-44.
7. Suk JS, Xu Q, Kim N, Hanes J, Ensign LM. PEGylation as a strategy for improving nanoparticle-based drug and gene delivery. Adv Drug Deliv Rev. 2016; 99: 28-51.
8. Ren X, Zheng R, Fang X, Wang X, Zhang X, Yang W, et al. Red blood cell membrane camouflaged magnetic nanoclusters for imaging-guided photothermal therapy. Biomaterials. 2016; 92: 13-24.
9. Li J, Yao H, Wei Z, Li X, Mu X, Wu L, et al. Seedless synthesis of gold

nanorods with (+)-catechin-assisted and red blood cell membranes coating as a biomimetic photothermal agents. Materials Technology. 2018; 33: 825-34.

10. Ye S, Wang F, Fan Z, Zhu Q, Tian H, Zhang Y, et al. Light/pH-Triggered Biomimetic Red Blood Cell Membranes Camouflaged Small Molecular Drug Assemblies for Imaging-Guided Combinational Chemo-Photothermal Therapy. ACS Appl Mater Interfaces. 2019; 11: 15262-75.

11. Chen W, Zeng K, Liu H, Ouyang J, Wang L, Liu Y, et al. Cell Membrane Camouflaged Hollow Prussian Blue Nanoparticles for Synergistic Photothermal-/Chemotherapy of Cancer. Advanced Functional Materials. 2017; 27: 1605795.

12. Brenner JS, Pan DC, Myerson JW, Marcos-Contreras OA, Villa CH, Patel P, et al. Red blood cell-hitchhiking boosts delivery of nanocarriers to chosen organs by orders of magnitude. Nat Commun. 2018; 9: 2684.

13. Usman WM, Pham TC, Kwok YY, Vu LT, Ma V, Peng B, et al. Efficient RNA drug delivery using red blood cell extracellular vesicles. Nat Commun. 2018; 9: 2359.

14. Hasenkamp S, Russell KT, Horrocks P. Comparison of the absolute and relative efficiencies of electroporation-based transfection protocols for Plasmodium falciparum. Malar J. 2012; 11: 210.

15. Saulis G, Saulė R. Size of the pores created by an electric pulse: Microsecond vs millisecond pulses. Biochimica et Biophysica Acta (BBA) - Biomembranes. 2012; 1818: 3032-9.

16. Bustamante López SC, Meissner KE. Characterization of carrier erythrocytes for biosensing applications. J Biomed Opt. 2017; 22: 91510.

17. Hanley T, Vankayala R, Lee CH, Tang JC, Burns JM, Anvari B. Phototheranostics Using Erythrocyte-Based Particles. Biomolecules. 2021; 11.

18. Kuo YC, Wu HC, Hoang D, Bentley WE, D'Souza WD, Raghavan SR. Colloidal Properties of Nanoerythrosomes Derived from Bovine Red Blood Cells. Langmuir. 2016; 32: 171-9.

19. Guo P, Huang J, Zhao Y, Martin CR, Zare RN, Moses MA. Nanomaterial Preparation by Extrusion through Nanoporous Membranes. Small. 2018; 14: e1703493.

20. Brenner JS, Mitragotri S, Muzykantov VR. Red Blood Cell Hitchhiking: A Novel Approach for Vascular Delivery of Nanocarriers. Annu Rev Biomed Eng. 2021; 23: 225-48.

21. Hunault-Berger M, Leguay T, Huguet F, Leprêtre S, Deconinck E, Ojeda-Uribe M, et al. A Phase 2 study of L-asparaginase encapsulated in erythrocytes in elderly patients with Philadelphia chromosome negative acute lymphoblastic leukemia: The GRASPALL/GRAALL-SA2-2008 study. Am J Hematol. 2015; 90: 811-8.

22. Batool T, Makky EA, Jalal M, Yusoff MM. A Comprehensive Review on L-Asparaginase and Its Applications. Appl Biochem Biotechnol. 2016; 178: 900-23.

23. Rossi L, Pierigè F, Aliano MP, Magnani M. Ongoing Developments and Clinical Progress in Drug-Loaded Red Blood Cell Technologies. Bio Drugs. 2020; 34: 265-72.

24. Moran NF, Bain MD, Muqit MM, Bax BE. Carrier erythrocyte entrapped thymidine phosphorylase therapy for MNGIE. Neurology. 2008; 71: 686-8.

25. Bax BE, Bain MD, Scarpelli M, Filosto M, Tonin P, Moran N. Clinical and biochemical improvements in a patient with MNGIE following enzyme replacement. Neurology. 2013; 81: 1269-71.

26. Gay F, Aguera K, Sénéchal K, Tainturier A, Berlier W, Maucort-Boulch D, et al. Methionine tumor starvation by erythrocyte-encapsulated methionine gamma-lyase activity controlled with per os vitamin B6. Cancer Med. 2017; 6: 1437-52.

27. Rossi L, Pierigè F, Carducci C, Gabucci C, Pascucci T, Canonico B, et al. Erythrocyte-mediated delivery of phenylalanine ammonia lyase for the treatment of phenylketonuria in BTBR-Pah(enu2) mice. J Control Release. 2014; 194: 37-44.

28. Sanz S, Lizano C, Luque J, Pinilla M. In vitro and in vivo study of glutamate dehydrogenase encapsulated into mouse erythrocytes by a hypotonic dialysis procedure. Life Sci. 1999; 65: 2781-9.

29. Ben-Akiva E, Meyer RA, Yu H, Smith JT, Pardoll DM, Green JJ. Biomimetic anisotropic polymeric nanoparticles coated with red blood cell membranes for enhanced circulation and toxin removal. Sci Adv. 2020; 6: eaay9035.

30. Coker SA, Szczepiorkowski ZM, Siegel AH, Ferrari A, Mambrini G, Anand R, et al. A Study of the Pharmacokinetic Properties and the In Vivo Kinetics of Erythrocytes Loaded With Dexamethasone Sodium Phosphate in Healthy Volunteers. Transfus Med Rev. 2018; 32: 102-10.

31. Bossa F, Latiano A, Rossi L, Magnani M, Palmieri O, Dallapiccola B, et al. Erythrocyte-mediated delivery of dexamethasone in patients with mild-to-moderate ulcerative colitis, refractory to mesalamine: a randomized, controlled study. Am J Gastroenterol. 2008; 103: 2509-16.

32. Castro M, Knafelz D, Rossi L, Ambrosini MI, Papadatou B, Mambrini G, et al. Periodic treatment with autologous erythrocytes loaded with dexamethasone 21-phosphate for fistulizing pediatric Crohn's disease: case report. J Pediatr Gastroenterol Nutr. 2006; 42: 313-5.

33. Castro M, Rossi L, Papadatou B, Bracci F, Knafelz D, Ambrosini MI, et al. Long-term treatment with autologous red blood cells loaded with dexamethasone 21-phosphate in pediatric patients affected by steroid-dependent Crohn disease. J Pediatr Gastroenterol Nutr. 2007; 44: 423-6.

34. Annese V, Latiano A, Rossi L, Lombardi G, Dallapiccola B, Serafini S, et al. Erythrocytes-mediated delivery of dexamethasone in steroid-dependent IBD patients-a pilot uncontrolled study. Am J Gastroenterol. 2005; 100: 1370-5.

35. Rossi L, Serafini S, Cenerini L, Picardi F, Bigi L, Panzani I, et al. Erythrocyte-mediated delivery of dexamethasone in patients with chronic obstructive pulmonary disease. Biotechnol Appl Biochem. 2001; 33: 85-9.

36. Rossi L, Castro M, D'Orio F, Damonte G, Serafini S, Bigi L, et al. Low doses of dexamethasone constantly delivered by autologous erythrocytes slow the progression of lung disease in cystic fibrosis patients. Blood Cells Mol Dis. 2004; 33: 57-63.

37. Vincy A, Mazumder S, Amrita, Banerjee I, Hwang KC, Vankayala R. Recent Progress in Red Blood Cells-Derived Particles as Novel Bioinspired Drug Delivery Systems: Challenges and Strategies for Clinical Translation. Front Chem. 2022; 10: 905256.

38. Malhotra S, Dumoga S, Sirohi P, Singh N. Red Blood Cells-Derived Vesicles for Delivery of Lipophilic Drug Camptothecin. ACS Appl Mater Interfaces. 2019; 11: 22141-51.

39. Zhang Y, Xia Q, Wu T, He Z, Li Y, Li Z, et al. A novel multi-functionalized multicellular nanodelivery system for non-small cell lung cancer photochemotherapy. J Nanobiotechnology. 2021; 19: 245.

40. Shi X, Zhang Y, Tian Y, Xu S, Ren E, Bai S, et al. Multi-Responsive Bottlebrush-Like Unimolecules Self-Assembled Nano-Riceball for Synergistic Sono-Chemotherapy. Small Methods. 2021; 5: e2000416.

41. Jiang Q, Luo Z, Men Y, Yang P, Peng H, Guo R, et al. Red blood cell membrane-camouflaged melanin nanoparticles for enhanced photothermal therapy. Biomaterials. 2017; 143: 29-45.

42. Wen Q, Zhang Y, Muluh TA, Xiong K, Wang B, Lu Y, et al. Erythrocyte membrane-camouflaged gefitinib/albumin nanoparticles for tumor imaging and targeted therapy against lung cancer. Int J Biol Macromol. 2021; 193: 228-37.

43. Han X, Shen S, Fan Q, Chen G, Archibong E, Dotti G, et al. Red blood cell-derived nanoerythrosome for antigen delivery with enhanced cancer immunotherapy. Sci Adv. 2019; 5: eaaw6870.

44. Chen H, Deng J, Yao X, He Y, Li H, Jian Z, et al. Bone-targeted erythrocyte-cancer hybrid membrane-camouflaged nanoparticles for enhancing photothermal and hypoxia-activated chemotherapy of bone invasion by OSCC. J Nanobiotechnology. 2021; 19: 342.

45. Markov DE, Boeve H, Gleich B, Borgert J, Antonelli A, Sfara C, et al. Human erythrocytes as nanoparticle carriers for magnetic particle imaging. Physics in medicine and biology. 2010; 55: 6461-73.

46. Boni A, Ceratti D, Antonelli A, Sfara C, Magnani M, Manuali E, et al. USPIO-loaded red blood cells as a biomimetic MR contrast agent: a relaxometric study. Contrast media & molecular imaging. 2014; 9: 229-36.

47. Antonelli A, Sfara C, Rahmer J, Gleich B, Borgert J, Magnani M. Red blood cells as carriers in magnetic particle imaging. Biomed Tech (Berl). 2013; 58: 517-25.

48. Rahmer J, Antonelli A, Sfara C, Tiemann B, Gleich B, Magnani M, et al. Nanoparticle encapsulation in red blood cells enables blood-pool magnetic particle imaging hours after injection. Physics in medicine and biology. 2013; 58: 3965-77.

49. Antonelli A, Pacifico S, Sfara C, Tamma M, Magnani M. Ferucarbotran-loaded red blood cells as long circulating MRI contrast agents: first in vivo results in mice. Nanomedicine (Lond). 2018; 13: 675-87.

50. Aryal S, Nguyen TDT, Pitchaimani A, Shrestha TB, Biller D, Troyer D. Membrane Fusion-Mediated Gold Nanoplating of Red Blood Cell: A Bioengineered CT-Contrast Agent. ACS Biomater Sci Eng. 2017; 3: 36-41.

51. Burns JM, Shafer E, Vankayala R, Kundra V, Anvari B. Near Infrared Fluorescence Imaging of Intraperitoneal Ovarian Tumors in Mice Using Erythrocyte-Derived Optical Nanoparticles and Spatially-Modulated Illumination. Cancers (Basel). 2021; 13: 2544.

52. Montel-Hagen A, Blanc L, Boyer-Clavel M, Jacquet C, Vidal M, Sitbon M, et al. The Glut1 and Glut4 glucose transporters are differentially expressed during perinatal and postnatal erythropoiesis. Blood. 2008; 112: 4729-38.

53. Matsusaka Y, Nakahara T, Takahashi K, Iwabuchi Y, Nishime C, Kajimura M, et al. (18)F-FDG-labeled red blood cell PET for blood-pool imaging: preclinical evaluation in rats. EJNMMI Res. 2017; 7: 19.

54. Choi JW, Budzevich M, Wang S, Gage K, Estrella V, Gillies RJ. In vivo positron emission tomographic blood pool imaging in an immunodeficient mouse model using 18F-fluorodeoxyglucose labeled human erythrocytes. PloS one. 2019; 14: e0211012.

55. Yin WY, Yuan J, Zhang ZM, Mei C, Xu W, Tang YX, et al. (18) F-fluorodeoxyglucose positron emission tomography-computed tomography for assessing organ distribution of stressed red blood cells in mice. Scientific reports. 2021; 11: 2505.

56. Matsusaka Y, Nakahara T, Takahashi K, Iwabuchi Y, Ogata Y, Nishime C, et al. Preclinical evaluation of heat-denatured [(18)F]FDG-labeled red blood cells for detecting splenic tissues with PET in rats. Nucl Med Biol. 2018; 56: 26-30.

57. Wang S, Budzevich M, Abdalah MA, Balagurunathan Y, Choi JW. In Vivo Imaging of Rat Vascularity with FDG-Labeled Erythrocytes. Pharmaceuticals (Basel). 2022; 15.