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Circulatory-cell-mediated nanotherapeutic approaches in disease targeting

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Circulating blood cells, and cell-derived microvesicles, are emerging as pragmatic delivery systems that can smartly complement the already existing nanotherapeutic platforms evaluated to treat or diagnose diseases. The valuable distinctive features of circulatory cells over synthetic nanocarriers encompass their biological origin which confers immune transparence, known biodegradability, high drug loading, relatively long half-life and a targeting capacity associated with their physiological surface functionality. Absence of nuclei in red blood cells and platelets provides further rationale for their use as cargo vehicles for nucleotoxic agents. Ongoing developments in cell-based and cell-inspired nanotherapies can move drug delivery into reachable frontiers and exhibit high potentiality for translatability into clinical use.

Introduction

Q3 Many therapeutic compounds have suboptimal pharmacokinetics and biodistribution, and their toxic effects impede patient management. Engineering drug formulations and conceiving delivery platforms that could provide improved bioavailability, stealth function, circulation half-life and minimized side-effects (through altered tissue and organ distribution or via altered route of excretion), enhancing targeting of disease sites, are needed. The most advanced approaches considered so far, relying on synthetic nanocarrier conjugation with blood protein carriers, or functionalization [1], have partially failed to deliver optimal drug targeting and safety. Using circulatory cells as drug delivery systems (DDS), in a strategy of drug camouflage, can help circumvent artificial nanocarrier limitations [2]. Translational applications of cell-based carriers are pursued actively, encouraged by already successful clinical applications [3–6]. This review provides a recent background on the properties, practical advantages and limits of blood cells as drug carriers. We also discuss the development of novel synthetic nanocarriers that are designed to resemble circulatory cells by mimicking their membranes as a means to enhance the safety and biocompatibility level and decrease reactions from the immune system.

Advantages of circulatory cells as nanotherapeutics

Circulatory cell DDS exhibit immune transparence and biocompatibility if *ex vivo* processing for drug encapsulation maintains their native physiological properties. Most cells exhibit long residence times in the circulation and are naturally decorated with receptors and ligands providing targeting capacity toward pathogenic sites (e.g., damaged vasculature or tumoral tissues). Blood cells are obvious DDS candidates based on the decades of transfusion medicine experience (Table 1). The technologies and procedures for the collection of blood and the transfusion of therapeutic cellular blood products (in particular packed red blood cell concentrates and platelet concentrates) are well established, making red blood cells (RBC) and platelets a pragmatic primary choice in the establishment of cell-based DDS platforms (Fig. 1). The methodologies used to ensure Q4

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TABLE 1

Translational features to consider when using transfusion-grade blood cells as source materials for nanotherapeutic drug delivery approaches.

	Red blood cell concentrate	Platelet concentrate
Donor source	Allogeneic or autologous	Allogeneic or autologous
Collection entity	Blood establishments	Blood establishments
Collection procedure	Whole blood or apheresis	Whole blood or apheresis
Volume (ml)/collection procedure	50–200	30–300
Mean cell number/μl	5×10^6	1×10^6
Quality and safety testing procedures	Established at national level	Established at national level
Viral testing procedures	HIV, HBV, HCV	HIV, HBV, HCV
Pathogen inactivation procedures	Under development	Two photoinactivation procedures licensed[a]
Immunohematology compatibility testing (allogeneic source)	In place using licensed reagents and kits	In place using licensed reagents and kits
Regulatory situation	Licensed procedures for transfusion	Licensed procedures for transfusion
Typical shelf-life	Up to 42 days at 2–6 °C, liquid	5–7 days at 20–24 °C, liquid
Size	6–8 μm (high loading capacity)	2–4 μm
Drug entrapment	Tonicity change; incubation; electroporation; cell-penetrating peptide	Short-term incubation
In vivo half-life (days)	>100	7–10
Disease-targeting capacity	Yes	Yes

[a] In European Union.

efficient encapsulation or conjugation methods of drugs or nanoparticles (NPs) to circulatory cells are highly dependent upon the type of cells considered, the biochemical specificity of their membrane (in particular the expression of markers) and the clinical applications. Procedures used include passive cellular uptake, endocytosis or phagocytosis, active reversible physicochemical modifications of membranes to increase diffusion capacity, loading by chemical modifications (covalent conjugation, biotinylation) or gene modification and delivery [7].

Red blood cells

Properties

RBC, the most abundant blood cells ($4.2–6.1 \times 10^6$/μl), have a discoid shape and are 6–8 μm in size (Fig. 1). Membrane components (carbohydrates, phospholipids, cholesterol and proteins) reinforce the cellular stability, providing flexibility and deformability to infiltrate into small vascular structures and interact within the cell microenvironment. Membrane decoration by antigens and adhesive proteins facilitates targeting to vascular tissues [5]. RBC cytoplasm is relatively simple, mostly containing hemoglobin. This is the result of erythroblast enucleation, an asymmetric cytokinesis-like process in the last step of erythropoiesis that ejects nuclei out of mature RBC and maximizes loading capacity for hemoglobin. Senescent RBC are degraded by macrophages of the reticuloendothelial system (RES) in the spleen, bone marrow and liver [8].

The advantages offered by RBC as DDS encompass: (i) a large size, which confers a potentially high capacity for loading drugs; (ii) a lack of nucleus, which makes them suitable as a carrier of nucleotoxic agents; and (iii) a residence time of over 100 days in the blood circulation, which can prolong the release kinetics of drugs to maximize bioefficacy and decrease systemic toxicity [5,6,9,10]. The RBC membrane withstands controlled transient reversible deformability, important for drug loading, and can be conjugated with NPs and proteins for enhanced targeting [11]. Artificial *ex vivo* aging of the RBC membrane enhances recognition by tissue macrophages supporting immunological or immunomodulatory actions [11]. As such, nanotherapeutic-based RBC have reached clinical stages.

Ex vivo *processing for drug entrapment*

Strategies using RBC as DDS, bioreactors or imaging tools involve *ex vivo* drug cellular entrapment, or coupling of drugs or NPs to the membrane surface. Drug entrapment is achieved by incubating RBC in a hypotonic solution allowing controllable intracellular drug diffusion, whereas subsequent hypertonicity transfer reseals the pore membrane for long-term and stable drug entrapment. Two methodologies (pre-swell and hypotonic dialysis), amenable to 50–300 ml of RBC suspension, respectively, have been developed into clinically compliant procedures [10]. Alternative methods include: (i) static incubation without tonicity change; (ii) electroporation; and (iii) cell-penetrating peptide methods (Fig. 2) [8,10]. Physical or chemical stresses to the membrane should be limited to avoid fast uptake by the RES. By contrast, improved targeting of macrophages and enhanced erythrophagocytosis through controlled alterations of the RBC membrane by oxidation, heat or coating with antibody, ovalbumin or a Toll-like receptor 3 agonist [12], or by triggering phosphatidylserine exposure by calcium ionophore treatment, can serve some clinical applications [11].

Therapeutic targets

Molecules up to 500 kDa, such as replacement therapy biomolecules [e.g., thymidine phosphatase, coagulation Factor IX, adenosine deaminase (ADA), acid alpha-glucosidase (GAA) or glucocerebrosidase], metabolic processes (e.g., L-asparaginase), chemotherapeutic agents (e.g., doxorubicin) or antigens (e.g., tyrosinase-related protein 2 self-tumor antigen, coagulation Factor VIII) to modulate an immune response are candidates for RBC entrapment (Fig. 3a) [5,6,10]. Enzymes with short residence times in the blood circulation, or toxic when infused in a nonencapsulated form, can be entrapped in RBC to prolong half-life, decrease posology and improve tolerance [13].

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