Journal of Controlled Release 328 (2020) 313–324

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

Cytopharmaceuticals: An emerging paradigm for drug delivery

Weishuo Li, Zhigui Su, Meixi Hao, Caoyun Ju*, Can Zhang*

State Key Laboratory of Natural Medicines and Jiangsu Key Laboratory of Drug Discovery for Metabolic Diseases, Center of Advanced Pharmaceuticals and Biomaterials, China Pharmaceutical University, Nanjing 210009, PR China

ARTICLE INFO

Keywords:
Cytopharmaceuticals
Targeted drug delivery
Carrier cell
Nanomedicine

ABSTRACT

Cytopharmaceuticals, in which drugs/nanomedicines are loaded into/onto autologous patient- or allogeneic donor-derived living cells *ex vivo*, have displayed great promise for targeted drug delivery in terms of improved biocompatibility, superior targeting, and prolonged circulation. Despite certain impressive therapeutic benefits in preclinical studies, several obstacles retard their clinical application, such as the lack of facile and convenient methods of carrier cell acquisition, technologies for preparing cytopharmaceuticals at scale with undisturbed carrier cell viability, and modalities for monitoring the *in vivo* fate of cytopharmaceuticals. To comprehensively understand cytopharmaceuticals and thereby accelerate their clinical translation, this review covers the main sources of various cytopharmaceuticals, technologies for preparing cytopharmaceuticals, the *in vivo* fate of cytopharmaceuticals including carrier cells and loaded drugs/nanomedicines, and the application prospects of cytopharmaceuticals. It is our hope that this review will elucidate the bottlenecks associated with cytopharmaceutical preparation, leading to the acceleration of future industrialization of cell-based formulations.

1. Introduction

Conventional targeted drug delivery systems (TDDSs) can be categorized into passive and active types. Passive TDDSs are generally dependent on the enhanced permeability and retention (EPR) effect, which is based on the longevity of TDDSs in blood and their accumulation at pathological sites (*e.g.* tumor or inflammation site) with leaky vasculature [1]. While active TDDSs depend on the attachment of specific ligands of TDDSs to recognize and bind surface markers on pathological cells or in their surrounding microenvironment [2]. Although conventional TDDSs have increased the therapeutic efficacy and decreased the undesired side effect because of the improved biodistribution and pharmacokinetics of the delivered drugs to certain degree, these systems are still limited by their unsatisfactory targeting efficiency [3]. For example, a recent study found that in preclinical tumor models, only 0.7% of intravenously administered drugs accumulated at the diseased site [4]. There could be two main reasons for the poor targeting efficiency of conventional TDDSs. First, conventional TDDSs are usually identified as foreign agents that are easily cleared by the reticuloendothelial system (RES), resulting in a short circulation time as well as poor passive targeting capacity [5]. Second, even if the active targeting strategy based on ligand–receptor interactions is applied, targeting efficiency is not greatly improved. This is largely owing to the heterogeneous expression of membrane receptors among individual patients and different tumors as well as during different tumor stages within a single tumor [6]. Additionally, immune responses against the administrated foreign TDDSs also dampen their benefits [5]. Novel TDDSs with enhanced targeting efficiency and therapeutic efficacy are thus greatly needed.

Cytopharmaceuticals represent a new cell-based formulation in which drugs/nanomedicines are loaded into/onto autologous patient- or allogeneic donor-derived living cells *ex vivo*. Since the unique characteristics of living cells as carrier cells of cytopharmaceuticals, such as a long circulation time, high motility, flexible morphology, active tropism towards certain tissues, as well as low immunogenicity, cytopharmaceuticals have emerged as attractive modalities for addressing the aforementioned challenges of conventional TDDSs [7–9]. When cytopharmaceuticals are infused into patients, their *in vivo* behavior is completely inherited from the corresponding carrier cells. That is, cytopharmaceuticals can overcome the multiple *in vivo* physiological/pathological barriers faced by conventional TDDSs, effectively deliver the cargos (*i.e.* drugs or nanomedicines) to pathological sites with improved targeting efficiency, and ultimately maximize treatment efficacy and minimize side effects. For example, neutrophil cytopharmaceuticals of liposomal paclitaxel (PTX) were linked to 1162- and 86-fold higher PTX concentrations in the brain than Taxol and liposomal PTX, respectively, slowing the recurrent growth of post-surgical glioma and significantly improving survival rates [10]. Platelet

* Corresponding author.
E-mail addresses: jucaoyun@cpu.edu.cn (C. Ju), zhangcan@cpu.edu.cn (C. Zhang).

https://doi.org/10.1016/j.jconrel.2020.08.063
Received 1 June 2020; Received in revised form 26 August 2020; Accepted 29 August 2020
Available online 01 September 2020
0168-3659/

Table 1
Pros and cons of nanomedicine and cyto-pharmaceutical.

Ideal TDDSs	Nanomedicine	Cyto-pharmaceutical
Long circulation time	**Cons:** Rapid clearance by RES. **Solutions**: i) Stealth coating by PEG or zwitterionic polymers [12]; ii) Cloaking by circulatory cell membrane [13]. **Problems associated with solutions:** Inducing immune response against PEG [14] or complex preparation procedures [15].	**Pros:** Natural stealth properties accompany with a long circulation time.
Superior targeting efficiency	**Cons:** Poor targeting efficiency due to the multiple physiological/pathological barriers [16]. **Solutions:** Modification with peptides, proteins, aptamer or antibodies [2]. **Problems associated with solutions:** Limited improvements [6].	**Pros:** Intrinsic tropism towards pathological sites and capacities crossing physiological barriers.
High drug loading efficiency	**Pros:** Easily turning the compositions or preparation parameters of nanomedicine.	**Cons:** Limited drug loading capacity to maintain the innate functions of carrier cell. **Solutions:** Utilizing nanomedicine with high drug loading efficiency.
Biocompatibility	**Cons:** Nanotoxicology [17] **Solutions:** Using biomimetic materials instead.	**Pros:** Self-components with great biocompatibility.
Controlled drug release	**Pros:** Easily controlled drug release by turning the compositions of nanomedicine.	**Cons:** Highly dependent on the disease status, carrier cell and payloads. **Solutions:** Introducing controllable switches, such as light or temperature. **Problems associated with solutions:** Need more development for clinic applications.

cytopharmaceuticals of programmed death ligand-1 (PD-L1) improved the half-life of PD-L1 from 5.5 to 35 h, thereby improving the accumulation of PD-L1 in tumor [11]. We also summarized the benefits and drawbacks of nanomedicines and cytopharmaceuticals to better understand the challenges to their future development (Table 1).

Despite the promising therapeutic benefits of cytopharmaceuticals, no such product has been approved since the first red blood cell (RBC)-based cytopharmaceutical of enzymes was investigated in 1973 [18]. Subsequently, more and more RBC-based cytopharmaceuticals have been developed, some of which are currently under clinical development. Later, immune cells-based cytopharmaceuticals, such as monocytes/macrophages, T cells, NK cells and neutrophils have been developed, aiming at a library of diseases (Fig. 1) [19-23]. The current stages of various cytopharmaceuticals including RBC-, T cell-, neutrophil-, macrophage-, and natural killer (NK) cell-based ones are reviewed in Table 2. In particular, RBC-based cytopharmaceuticals of drugs, enzymes, or peptides have been applied to treat various malignancies, such as pancreatic adenocarcinoma, acute lymphoblastic leukemia, and triple negative breast cancer. Most of these studies are currently in clinical trials. While cytopharmaceuticals based on other cell types, such as T cells, neutrophils, macrophages, and NK cells, are all in preclinical developments for the treatment of cancer [26], human immunodeficiency virus (HIV) [27], Alzheimer's disease [28] *etc.* The advantages and applications of cell-based formulations have been thoroughly discussed in several excellent reviews [7–9]. In this review, we intend to discuss possible obstacles hindering the clinical translation of cytopharmaceuticals depending on the whole workflow including sources, preparation techniques, and *in vivo* fate. It is our hope that this review will elucidate the bottlenecks of cytopharmaceutical preparation, which could lead to the acceleration of future industrialization of cell-based formulations.

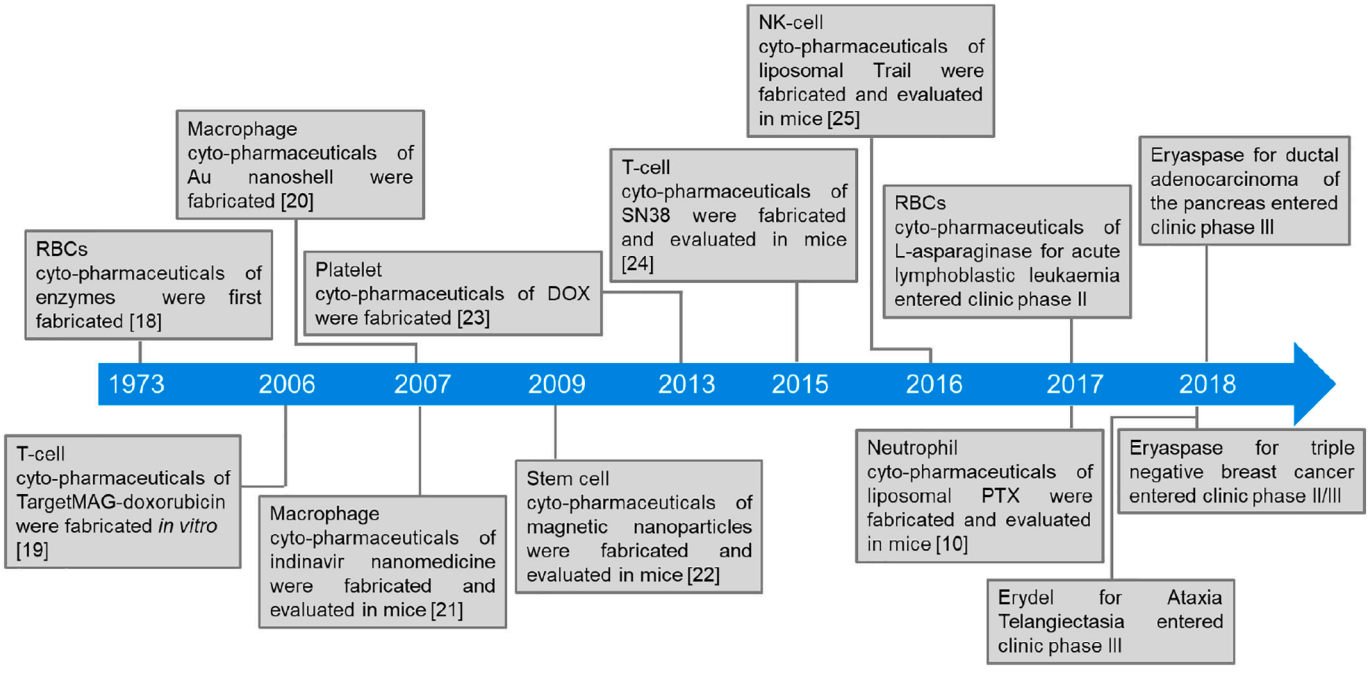

Fig. 1. The development timeline for various cytopharmaceuticals.

Table 2
Representative pre-clinical and ongoing clinical trials of cytopharmaceuticals.

Carrier-Cell	Name/Company	Drugs/Nanomedicines	Application	NCT number	Status
RBCs	Eryaspase	Asparaginase	Pancreatic adenocarcinoma	NCT03665441	Phase III
	Eryaspase	Asparaginase	Acute lymphoblastic leukemia	NCT03267030	Phase II
	Eryaspase	Asparaginase	Triple negative breast cancer	NCT03674242	Phase II/III
	Erydel	Dexamethasone sodium phosphate	Ataxia telangiectasia	NCT03563053 NCT02770807	Phase III
	Orphan Technologies	Thymidine phosphorylase	Mitochondrial neurogastrointestinal encephalomyopathy	NCT03866954	Phase I/II
	–	*Escherichia coli* L-asparaginase, autoantigens	Immunotolerance/spleen	–	Preclinical
	–	PLGA DOX	Lung metastasis	–	Preclinical [61]
Stem cells	–	Silica purpurin-18 NPs	breast cancer/cancer	–	Preclinical [62]
	–	Silica DOX	–	–	Preclinical [63]
Platelets	–	anti-PD-1 antibody	Post-surgical breast and melanoma tumor	–	Preclinical [11]
	–	anti-PD-1 antibody	Acute myeloid leukemia	–	Preclinical [64]
	–	Gold nanorods	Head and neck squamous carcinoma	–	Preclinical [65]
Neutrophils	–	Liposomal PTX	Post-surgical glioma	–	Preclinical [10]
	–	Liposomal PTX	HepS-tumor	–	Preclinical [66]
	–	Abraxane	SNU719 tumor	–	Preclinical [67]
	–	Silicon DOX and magnetic nanoparticles	Post-surgical glioma	–	Preclinical [68]
	–	Nanoparticulated indinavir	HIV	–	Preclinical [27]
	–	Catalase	Parkinson's disease	–	Preclinical [28]
	–	Cellular backpack	Inflammation/breast tumor	–	Preclinical [69]
Monocytes/macrophages	–	Prodrug	Lung metastasis	–	Preclinical [70]
T cells	–	IL 15 nanogel	Breast tumor	–	Preclinical [71]
	–	Liposomal SN38	Disseminated lymphoma	–	Preclinical [24]
Natural Killer Cell	–	Liposomal Trail	Lymphatic metastasis	–	Preclinical [25]
	–	Micelle DOX	Breast tumor	–	Preclinical [72]

2. A typical workflow of cytopharmaceuticals

Cytopharmaceuticals are formulated with *ex vivo* living cells and drugs/nanomedicines in a specific manner, followed by infusion back into patients to take effect. In brief, carrier cells are initially harvested from autologous patients or allogeneic donors with unperturbed physiological behaviors, such as migration and responsiveness to microenvironmental signals. Then, with or without an *ex vivo* expansion, drugs/nanomedicines are integrated into carrier cells *via* a facile and benign “loading into” or “loading onto” strategy under a strictly sterilized environment, thereby obtaining living cytopharmaceuticals. Finally, after a standardized quality control including but not limited to potency, purity and contamination, the prepared living cytopharmaceuticals are subsequently infused back into patients. The infused cytopharmaceuticals can circulate in circulation as a self-component that avoids recognition by the RES, cross physiological/pathological barriers, migrate into sites of disease owing to their intrinsic tropism, and unload their cargos in a designed manner after which the carrier cells die or persist according to their corresponding physiology. Of note, the fate of infused carrier cells must be considered given that the biodistribution, proliferation, and colonization of living cells could influence the efficacy and biosafety of cytopharmaceuticals. In this section, we will discuss the entire workflow of cytopharmaceuticals as indicated in Fig. 2 for a better understanding.

2.1. Source of cytopharmaceuticals

The sources of cytopharmaceuticals include both carrier cells and drugs/nanomedicines, which are both critical for a successful translation. In specific, the *in vivo* behavior of cytopharmaceuticals, such as circulation, biodistribution *etc*, is predominantly determined by carrier cell. For example, RBC-based cytopharmaceuticals exhibit a long circulation time [8], whereas neutrophil-based cytopharmaceuticals display rapid accumulation at inflammatory sites [10]. By contrast, drugs/nanomedicines loaded into or onto carrier cells are the final effector agents. Thus, in this case, we will discuss each source, respectively, regarding their advantages and disadvantages as sources of cytopharmaceuticals, as well as the rational selection.

2.1.1. Living cell carriers

The introduction of living cell carriers endows cytopharmaceuticals with cell behaviors, *e.g.* long circulation time, high motility, and tropism towards specific tissues. There are two main categories of living cell carriers based on their intended functions: carriers that prolong the blood circulation time of the drug, such as erythrocytes; and carriers that improve the accumulation of drugs into hard-to-reach sites of disease *via* the homing ability of cells involved in various disease processes, including immune cells (T cells, neutrophils, monocytes, macrophages, and NK cells), stem cells, and platelets. Notably, most carrier cells are autologous or homologous to minimize the risk of patient rejection. Additionally, the criteria, *e.g.* human leukocyte antigen (HLA) type, should be also matched between the donor and patient. In particular, the isolation of living carrier cells is tricky regarding the cellular purity and viability.

Erythrocytes. Erythrocytes (RBCs) comprise the largest population of blood cells (> 99%). Approximately 2 million new erythrocytes are continuously produced per second in the human body, and simple centrifugation can completely isolate RBCs. This simple, cost-effective acquisition method makes RBCs preferred among all carrier cells regarding convenience, and this might explain why most cytopharmaceuticals under clinical development are RBC-based. Moreover, another attribute of RBCs as carriers is that they can circulate for approximately 3 months in humans and approximately 40 days in mice. Additionally, biconcave RBCs lack organelles with a surface area of up to 160 μm^2. The drug-loading capacity of RBCs is theoretically large because the entire inner space and the extended cell surface can be used as a drug reservoir [8,29–31].

Platelets. Platelets are fragments of cytoplasm derived from

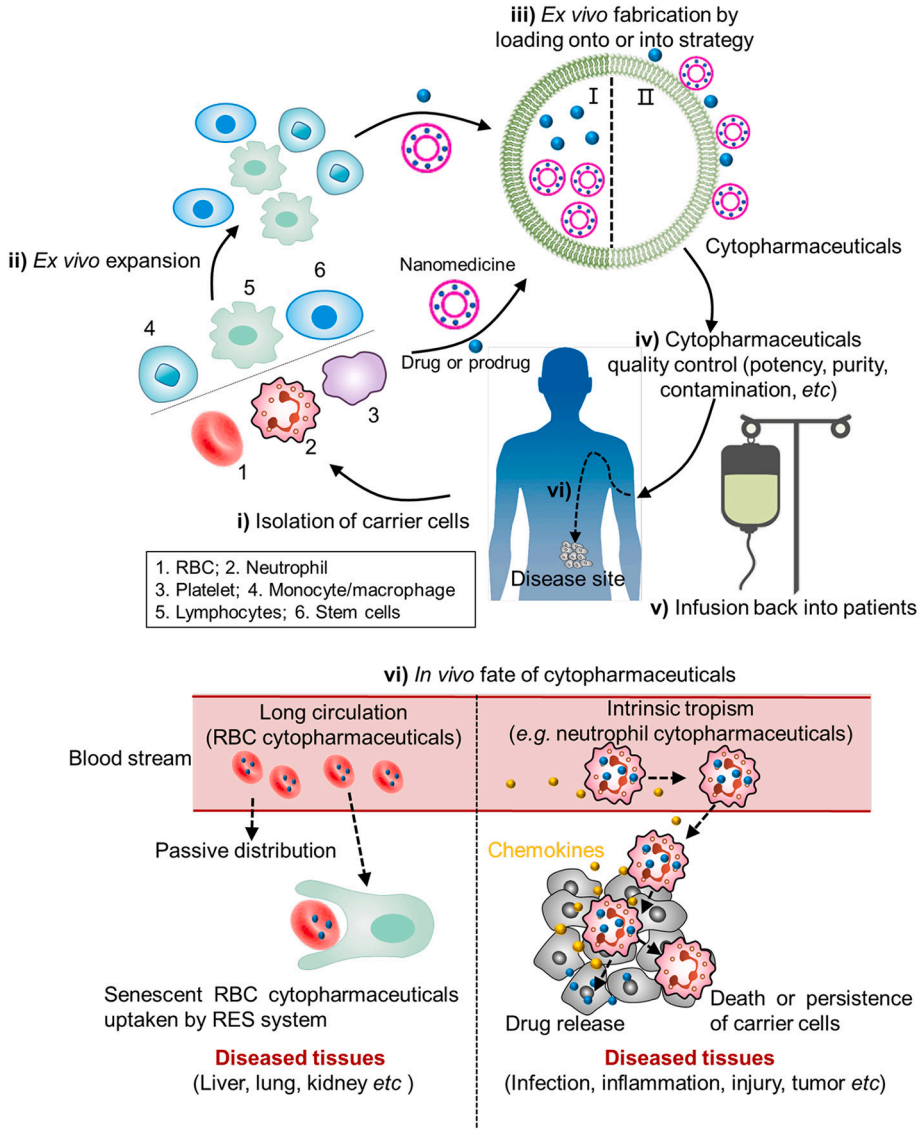

Fig. 2. A typical workflow for cytopharmaceuticals.

megakaryocytes within the bone marrow [32], and they can be harvested from the peripheral blood of autologous patients or allogeneic healthy donors. In addition to their abundance, platelets exhibit rapid responses to vascular injury induced by stroke, myocardial infarction, tumor progression, and surgery *via* their role in hemostasis [33]. Although these favorable merits of platelet make them preferred candidates for preparing cytopharmaceuticals, the positive contribution of platelets to disease progression should be addressed prior to proceeding. For example, studies revealed that the activation and adherence of platelets to tumor cells can promote tumor growth and metastasis [34,35]. Additionally, the easy activation of platelets during extraction, purification, and loading processes might represent obstacles for the future application of platelet-based cytopharmaceuticals.

Leukocytes. Leukocytes (white blood cells) represent a key component of the immune system because they clear cellular debris and foreign materials to defend the body against infections and diseases. They consist of neutrophils, eosinophils, basophils, monocytes, and lymphocytes [36,37]. Although the lifespan of leukocytes (up to 20 days) is shorter than that of RBCs [37], their intrinsic features, such as the ability to home to specific tissues (inflammatory tissues and lymph nodes) and immune responses [37–39], make them attractive as drug carriers. Specifically, neutrophils, monocytes/macrophages, and lymphocytes have exhibited promise in delivering therapeutics to various sites of disease.

Neutrophils, also known as polymorphonuclear granulocytes, are the most abundant leukocyte type in humans (comprising 50%–70% of leukocytes) [39]. They are critical components of the innate immune system, and they can migrate quickly to sites of infection or inflammation, after which they can kill invading pathogens *via* phagocytosis, degranulation, or the formation of neutrophil extracellular traps (NETs), accompanied by the release of an array of pro-inflammatory cytokines [40,41]. In addition, neutrophils have been demonstrated to highly infiltrate tumor sites and pre-metastatic niches [42,43]. Their tropism to diseased sites supports their promising targeting potential in various diseases. As an abundant leukocyte type, neutrophils can be harvested from autologous patients or allogeneic healthy donors and transfused into patients. Moreover, the adoptive infusion of neutrophils for treating neutropenia has been safely performed in the clinic for decades [44–47]. While, due to their relatively short lifespan, isolated neutrophils must be quickly transferred to avoid any decay of viability, which makes their *ex vivo* manipulation tricky. However, the short lifespan of neutrophils can be advantageous from the perspective of biosafety because carrier neutrophils might positively contribute to the progression of disease after systemic transfer [48].

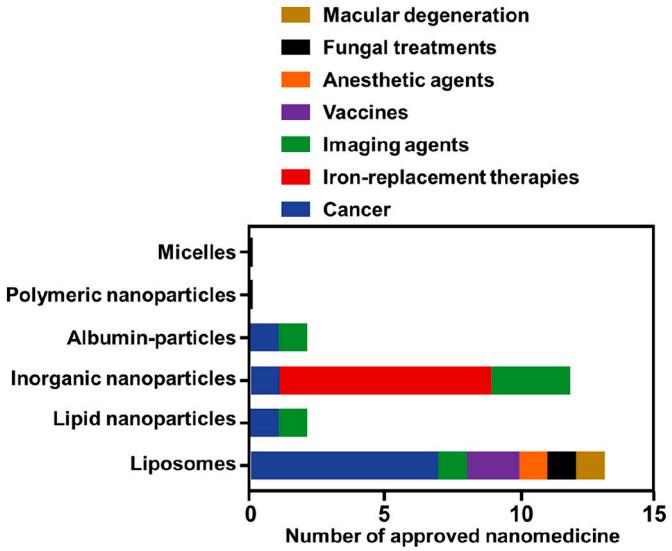

Fig. 3. Clinically approved nanomedicine for therapy and diagnostic till 2019.

Finally, the intrinsic phagocytosis of foreign materials enables the simple and feasible fabrication of neutrophil cytopharmaceuticals.

Monocytes are mononuclear leukocytes and the precursors of macrophages. Monocytes circulate in the bloodstream, migrate to diseased sites in association with infection or inflammation, and differentiate into *macrophages* [49]. Macrophages play multiple roles in inflammation, including the secretion of pro-inflammatory cytokines and clearance of bacteria/cellular debris [50]. The ability of monocytes/macrophages to access hard-to-reach tissues [51], such as hypoxic/necrotic areas, makes them ideal carriers for drugs to treat hypoxic diseases. Like neutrophils, their superior phagocytic capability enables the spontaneous preparation of monocyte/macrophage cytopharmaceuticals. Given the phenotype reversal of macrophages at sites of disease, which means the inflammatory macrophages (M1) will polarize into alternatively activated macrophages (M2) in immunosuppressive microenvironments and contribute to the progression of disease, manipulation of the fate of macrophage cytopharmaceuticals appears necessary for their further development. Notably, the clinical translation of the adoptive transfer of chimeric antigen receptor macrophages makes the translation of macrophage-based cytopharmaceuticals more promising.

Lymphocytes, consisting of T cells, B cells, and NK cells, are primarily found in the bloodstream and central lymphoid organs [52]. Lymphocytes display multiple functions in human immunity. For instance, cytotoxic T cells can recognize and reach abnormal cells and directly kill them *via* cytotoxic effectors including perforin and granzyme [53]. Obviously, lymphocytes could serve as potential carriers for cytopharmaceuticals because of their superior ability to penetrate certain physiological barriers. However, vulnerable autologous lymphocytes are difficult to harvest because of the poor conditions of patients and their labor-intensive *ex vivo* expansion [54]. In addition, allogeneic T cells carry the risk of graft-*versus*-host unless the human leukocyte antigen barriers are removed *via* complicated gene edition [55]. Notably, the approved adoptive transfer of chimeric antigen receptor T cells demonstrates the potential translation of T cell-based cytopharmaceuticals.

Stem cells. Stem cells, including induced pluripotent stem cells, neural stem cells (NSCs), and mesenchymal stem cells (MSCs), can be harvested from patients, cultured, and expanded *ex vivo*, after which they can be infused back into patients. The isolation and expansion of stem cells are generally labor-intensive. These cells have been of interest because of their applications in regenerative medicine and tissue engineering [56]. However, their use as carrier cells for cytopharmaceuticals, particularly tumor-targeted treatments, is also attractive. Like leukocytes, MSCs can penetrate many tissues after systemic administration and exit blood vessels *via* expression of cell adhesion molecules [57]. MSCs have several merits as ideal carrier cells including i) self-renewal and expansion when cultured *ex vivo* and ii) substantial disease tropism, including but not limited to tumors. It should be stated that stem cells can home to tumors and other pathological sites, *e.g.* areas of neurodegeneration, which offers the opportunity for stem cell-based cytopharmaceuticals to treat various diseases [58,59]. However, concerns regarding the malignant potential of stem cells after *in vivo* transfusion might limit their use in cytopharmaceuticals [60].

2.2. Drugs/nanomedicines

In a pursuit of sheer convenience for clinical application, approved drugs are considered the first choice of cytopharmaceuticals preparation. A library of approved drugs, including PTX, doxorubicin (DOX), dexamethasone sodium phosphate, alcohol dehydrogenase, aldehyde dehydrogenase, and therapeutic enzymes, have been formulated into cytopharmaceuticals (Table 2). However, to avoid direct interactions between drugs and carrier cells, which might lead to altered cell behavior or drug degradation, approved nanomedicines would be better choices. Specifically, liposomal doxorubicin (Doxil™/Caelyx™) was the first anti-cancer nanomedicine approved by the Food and Drug Administration (FDA) in 1995 [73]. Subsequently, several nanomedicines, such as Myocet™, DaunoXome™, Depocyt™, Abraxane™, Genexol-PM™, and Onivyde™, have been improved by the FDA for treating multiple cancer types, and investigations of other clinical uses are ongoing. In total, 29 nanomedicines gained approval through the end of 2019 [74]. Of these drugs, liposomal formulations represented 44.8% (13 products) of treatments, followed by inorganic nanoparticles (12 products [41.4%]) and other nanoparticles (polymers and proteins; four products [13.8%]; Fig. 3) [75]. Notably, the limited number of approved nanomedicines might be one of the bottlenecks for the further development of cytopharmaceuticals.

2.3. Techniques for preparing cytopharmaceuticals

To obtain cytopharmaceuticals, the aforementioned drugs/nanomedicines should be loaded into or onto living cells. Notably, the preparation of cytopharmaceuticals differs largely from that of novel pharmaceuticals because the carriers are living cells. The improved drug delivery capacity of cytopharmaceuticals is largely dependent on the physiologic functions of carrier cells. Therefore, it is essential to develop loading methods that do not disrupt the physiologic functions of carrier cells. That is, feasible and facile loading processes as well as drugs/nanomedicines with no or low toxicity are required. In addition, the drug-loading capacity of cytopharmaceuticals should be considered because this property determines the dosage and therapeutic regimen for various diseases. To date, two methods for achieving successful drug loading have been developed: i) leveraging the intrinsic uptake capacity of carrier cells or the passive diffusion of drugs to load drugs into the intercellular space and ii) backpacking the drugs onto the cell surface *via* adsorption or conjugation (Fig. 4).

2.3.1. "Loading into" strategy

Cellular compartments offer an opportunity to store drugs inside the cytoplasm. The most convenient method for loading drugs inside the cytoplasm is to utilize the phagocytic activity of carrier cells. This method is likely limited to certain phagocytic cells, such as neutrophils or macrophages. Our group has leveraged the natural phagocytic capacities of neutrophils to fabricate neutrophil cytopharmaceuticals of liposomal PTX or albumin-bound PTX nanoparticles (Abraxane) without a loss of cellular viability during circulation (Fig. 5A) [10,66,67].

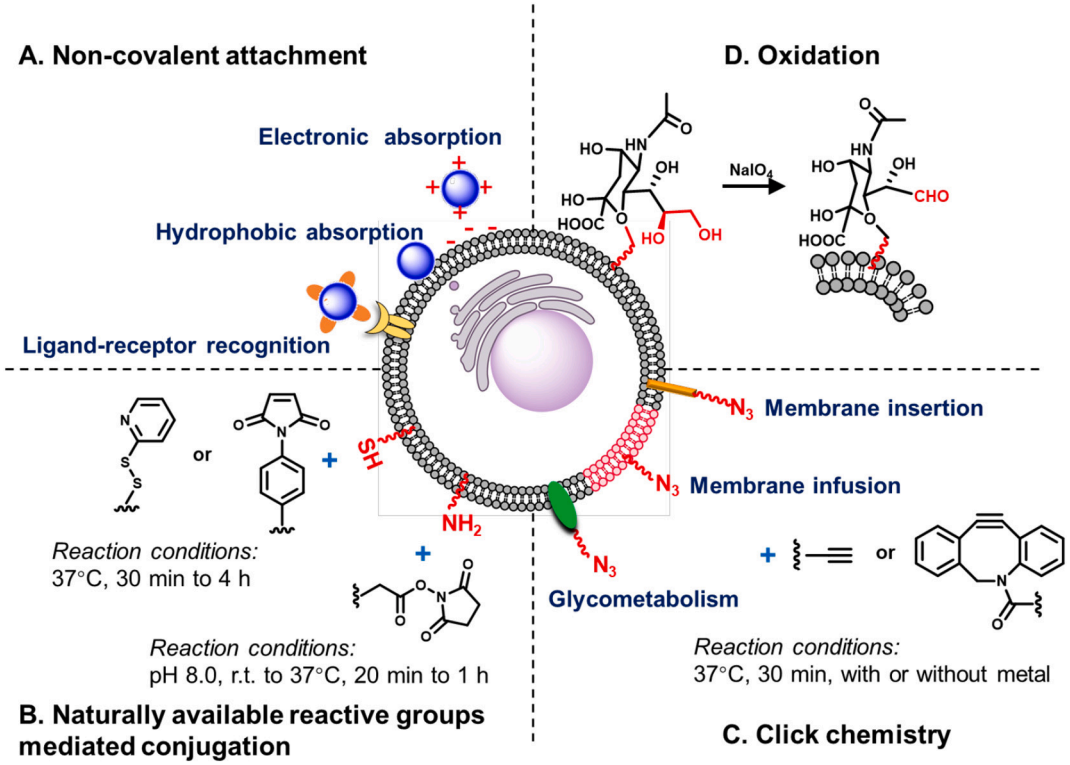

Fig. 4. Natural or engineered cell-surface properties for fabrication of cell-membrane-attached cytopharmaceuticals. (A) The non-covalent attachment sites mediated by hydrophobic and negatively charged cell surface along with the ligand. (B) Naturally available reactive groups generating from lysine and cysteine. (C) Manually introduced biorthogonal reactive groups by lipid insertion, glycometabolism as well as membrane fusion. (D) Oxidation of cell surface saccharides generating reactive aldehydes.

Unfortunately, most cells lack phagocytic activity. For these cells, the common strategy is to load the cells with drugs *via* passive diffusion or endocytosis, which is usually limited by the physical/chemical properties of drugs, resulting in relatively low drug encapsulation efficiency. Using hypotonic solutions to enable the consequent reflux of drugs into the cytoplasm is thus a promising method. For instance, hypotonic solution-treated RBCs swell and form transient pores in the plasma, allowing the entry of drugs into the cytoplasm [8]. Notably, several RBC-based cytopharmaceuticals under clinical development use this *hypotonic loading* strategy. Additionally, *electroporation* is another method to instantly open pores on cell membranes to enable the entry of high-molecular-weight drugs. For example, nearly 5%–7.5% of rIL-2 is consistently encapsulated into RBCs *via* electroporation [76]. Although improved loading efficiency of drugs can be achieved, the method of disrupting the cell membrane integrity might cause permanent damage to carrier cells, which is unfavorable for maintaining the vitality of cytopharmaceuticals. Alternatively, the use of *cell-penetrating peptides or ligands* to load therapeutics into carrier cells is under development. Specifically, hyaluronic acid-modified iron oxides are massively loaded into macrophages *via* the interaction of hyaluronic acid and CD44 receptors on macrophages [77]. The cell-penetrating peptide-mediated loading of the enzyme L-asparaginase into RBCs is another proof-of-concept for efficient drug loading without disturbing the physiology of carrier cells [78]. Last, after the membrane infusion of drugs loaded into *fusogenic liposomes*, the drugs are transferred from liposomes to carrier cells [79]. Of note, hydrophilic drugs are more favorably encapsulated in these liposomes for the membrane fusion method.

Despite the aforementioned advantages, there are two possible restrictions regarding this method: i) the degradation of internalized drugs by carrier cells because of the harsh intracellular microenvironment, including abundant enzymes and the acidic pH of endosomes/lysosomes in carrier cells; and ii) the restricted retention of drugs inside the cytoplasm because most drugs are cell-permeable, permitting their rapid escape from carrier cells, even during circulation. Considering the first restriction, the cytoplasm of RBCs is an ideal cellular container for drugs because they lack intracellular components [8]. Thus, several drugs including chemical drugs and proteins have been loaded into the cytoplasm of RBCs. However, this strategy is limited by the rapid leakage of encapsulated drugs. For instance, only 20% of encapsulated PTX remains inside RBCs after 48 h of circulation in blood [80]. Therefore, a prodrug strategy to reduce the cell permeability of drugs is necessary. For example, vitamin B12 prodrugs have a prolonged retention time because of their cell-impermeable characteristics [81]. Another prodrug strategy chemically modifies drugs with ionic groups, such as phosphate groups. In this manner, a single injection of RBC-based cytopharmaceuticals of phosphorylated DEX provides a therapeutic concentration of the drug for more than 30 days [82]. Meanwhile, the use of nanomedicines is a promising strategy to eliminate the interactions between carrier cells and drugs in this scenario. Notably, transforming drugs into nanomedicines can also improve the drug-loading capacity of cytopharmaceuticals. For example, porous silica nanoparticles with high loading efficiency of DOX are phagocytosed by macrophages to yield a macrophage-based cyto-pharmaceutical with enhanced drug contents [83]. Although nanoparticles provide a protective shell for encapsulated drugs, the engulfed nanomedicine must survive the acidic pH and/or enzymes in endosomes/lysosomes, which are the inevitable organelles involved in phagocytosis/endocytosis. Moreover, the confined intracellular compartment of carrier cells limits the drug-loading capacity of cytopharmaceuticals. In this respect, attaching drugs/nanomedicines onto the cell membrane would be more beneficial.

Collectively, the “loading into” strategy is an easy-to-use pathway for loading drugs/nanomedicines into living cells, which is preferred for industrial production. However, the complexity associated with differences among individual cells, various intracellular microenvironments,

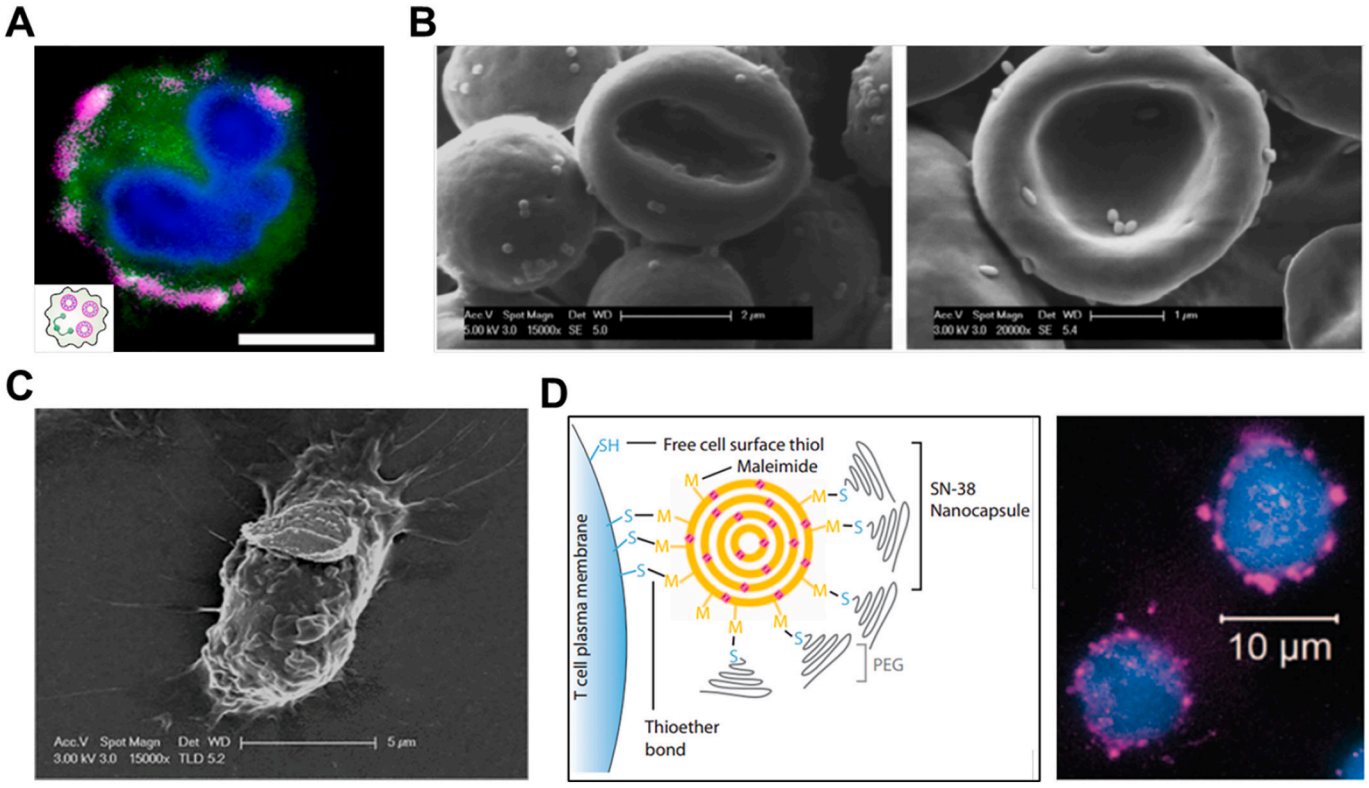

Fig. 5. Examples of cytopharmaceuticals with different drug/nanomedicines locations. (A) Fluorescent image of cytoplasm-laden cytopharmaceuticals based on neutrophil. Scale bar, 4 μm. The nanomedicines are indicated by green signals [10]. (B) Scanning electronic microscope images of cell-membrane-attached cytopharmaceuticals prepared from non-specific absorption of nanoparticles with different shapes. Adapted with permission from [108]. Copyright (2015) Elsevier Ltd. (C) Membrane-attached cytopharmaceuticals mediated by ligand-receptor interaction, observed by Scanning electronic microscope. Red arrow indicates the cargos. Adapted with permission from [109]. Copyright (2011) WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. (D) Fluorescent image of membrane-attached cytopharmaceuticals based on covalent conjugation. Pink fluorescence indicates the cargos. Adapted with permission from [24]. Copyright (2015) American Association for the Advancement of Science. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and the interference between drugs/nanomedicines and cells, might compromise the reproducibility and stability of cytopharmaceuticals. New techniques to precisely control the "loading into" process and fine-tune the properties of nanomedicines while selecting suitable carrier cells are required to develop cytoplasm-laden cytopharmaceuticals (Table 3).

2.3.2. "Loading onto" strategy

The cell membrane is a double layer of lipids, proteins, and polysaccharides that features a range of reactive modules (*e.g.* amines, thiols) and surface properties (*e.g.* negative charge, hydrophobicity) for surface attachment. Two general strategies, including *noncovalent attachment* and *covalent conjugation*, are available for attaching drugs/nanomedicines to the surface of carrier cells (Fig. 4).

Noncovalent attachment generally relies on the surface properties of carrier cells, including negative charges for the adsorption of positively charged nanoparticles, hydrophobicity for hydrophobic interactions between drugs/nanomedicines and cells, as well as overexpressed receptors for the specific binding of corresponding ligand-modified nanoparticles. Specifically, the membrane surface contains negatively charged phosphate, carboxylate, and sialic acid groups that contribute to a negative outer surface charge [84]. Cationic drugs/nanomedicines can adsorb onto carrier cells *via* electrical attraction. However, positively charged drugs/nanomedicines could be harmful to carrier cells. Rather than electrostatic interactions, the attachment of drugs/nanomedicines onto the cell surface *via* hydrophobic interactions (van der Waals forces and hydrogen bonding) is utilized in several cases of erythrocyte-based cytopharmaceuticals (Fig. 5B) [85,86]. Alternatively, the modification of drugs using membrane-mimicking lipids is another strategy for loading drugs onto the cell surface [87]. It should be noted that the drugs attached on the cell surface *via* non-specific adsorption might dissociate from carrier cells in circulation. Considering the specific interaction between ligands and receptors, overexpressed receptors on the carrier cell surface can be utilized as anchorage sites for the corresponding ligand-functionalized drugs/nanomedicines. For example, CD45 on the surface of T cells is used as the anchorage site for anti-CD45–modified nanogel containing T cell activity enhancers [71]. The CD44–hyaluronic acid interaction has been used to mediate the attachment of cellular backpacks to the surface of macrophages (Fig. 5C) [69]. In this ligand–receptor-mediated attachment strategy, if the affinity is too strong, it is likely to trigger downstream signals, leading to the unexpected activation of carrier cells. In turn, if the affinity is weak, the drugs/nanomedicines are prone to detach from carrier cells before reaching the site of disease. A balance must be established to achieve the intended delivery efficiency of cytopharmaceuticals.

In regard of stability, the *covalent conjugation* of drugs/nanomedicines onto the cell surface appears more practical. Primary amine residues (lysine) and thiols (cysteine) are abundant on the cell surface, making them readily available for the conjugation of drugs/nanomedicines. Various cross-linkers are used in this conjugation. In the case of primary amines, N-hydroxysuccinimide ester is the most commonly used linker to form stable amide bonds between drugs/nanomedicines and the cell surface. The reaction conditions are mild and cytocompatible. For instance, sulfosuccinimidyl-4-(*N*-maleimidomethyl)-cyclohexane-1-carboxylate–modified PD-L1 is mixed with platelets in Tyrode's buffer (with 1 μM PGE1) for 2 h at room temperature to complete the conjugation [11]. In the case of thiols, maleimide and 2-

Table 3
Summary of techniques to prepare cytopharmaceuticals.

Types	Methods	Pros	Cons	Ref
"Loading into"	Endocytosis, Phagocytosis, Hyponic loading, Electroporation	Facile, high drug loading capacity	High requirements on the integrity of drugs/nanomedicines during circulation	[10,66,67,76–80]
"Loading onto"	Non-covalent	i) Nonspecific adsorption (van der Waals, electrostatic interactions, hydrogen bonds, hydrophobic interactions); ii) Ligand-receptor interactions (such as CD44-Hyaluronic acid).	Facile	Unstable during circulation, risks of triggering the undesired downstream cellular signals	[69,71,84–87]
	Covalent	i) Coupling to native amine or thiol groups; ii) Coupling to metabolically introduced or membrane-inserted biorthogonal groups; iii) Coupling to aldehydes resulting from oxidized glycans.	Stable, general	Risks of disturbance on carrier-cell physiology	[11,24,88,89]

pyridyldithio groups are generally used because of the high reactivity and cytocompatibility. For example, the T cell-based cytopharmaceutical of 7-ethyl-10-hydroxycamptothecin (SN38) is fabricated *via* the covalent conjugation of nanoparticulated SN38 onto the plasma membrane of T cells *via* a reaction between cell-surface thiols and maleimide-functionalized nanomedicines (Fig. 5D) [24]. Although corresponding experiments demonstrated the cytocompatibility of the aforementioned chemical conjugations, the long-term impact of the occupation of the natural amines and/or thiols of cell-surface proteins on cell physiology requires further characterization. Rather than the natural amines and/or thiols on the cell surface, cell-surface sugars have been oxidized into aldehydes for subsequent conjugation [88]. In addition, the introduction of biorthogonal reactive groups, such as azides, onto the cell surface *via* sugar metabolism has been explored for covalent conjugation [89]. For certain carrier cells, especially those with a relatively short lifespan, the metabolic introduction of reactive sites might not be suitable. Alternative facile and general strategies for introducing exogenous reactive sites onto the cell surface, such as membrane insertion, are thus intriguing.

Therefore, the "loading onto" strategy might circumvent the possibilities of drug degradation in the scenario of cytoplasmic-loaded cytopharmaceuticals. However, the outside backpacks face new challenges, such as the undesired detachment of drugs/nanomedicines during circulation, especially when the carrier cells deform during the transendothelial migration, or the likeness of endocytosing the drugs/nanomedicines by carrier cells. Advances in the "loading onto" strategy with the discoveries of new cell-surface markers and binding ligands as well as establishment of benign biorthogonal chemistry, are beneficial for further development of backpacked cytopharmaceuticals. Moreover, the overattachment of foreign agents onto cells might change the surface properties of carrier cells, leading to deviated *in vivo* behavior. Thus, the amount and distribution of drugs/nanomedicines attached *per* cell should be controlled, which might be in contradiction to the requirements on the drug-loading capacity of cytopharmaceuticals.

2.4. *In vivo* fate of cytopharmaceuticals

The *in vivo* fate of cytopharmaceuticals, including drugs with pharmacological activities and carrier cells, predominantly determines the *in vivo* potency and biosafety. Most current studies are focusing on the fate of drugs, especially drug distribution and release, which largely contribute to the final efficacy of cytopharmaceuticals. To the best of our knowledge, studies on the *in vivo* fate of carrier cells, which could possibly result in long-term safety hazards because of the proliferation and/or differentiation capabilities of living cells, have been limited. In this section, we will discuss the *in vivo* fate of cytopharmaceuticals, especially drug release (Fig. 6) as well as the fate of carrier cells.

2.4.1. *Drug release from cytopharmaceuticals*

The timely release of drugs from cytopharmaceuticals is critical, and this process requires both limited drug release to maintain stability during circulation and a disease-preferred release profile to achieve therapeutic effects at sites of interest. It is worth noting that the prevention of premature drug leakage from cytopharmaceuticals is in great need, in order to avoid off-target toxicity and leaky drug-induced damage to carrier cells. Thus, modalities for controlling drug release are required to improve the effectiveness of cytopharmaceuticals.

Degradation is one of the most well-recognized sustained-release mechanisms in conventional nanodrug delivery systems [90], because most nanoparticulated carriers are composed of biodegradable polymers that undergo enzymatic and/or hydrolytic degradation after internalization into cells. This appears contradictory to the desired stability of cytopharmaceuticals (especially the nanomedicine-based, cytoplasm-laden ones) because large amounts of enzymes are present in carrier cells. As a result, control over the degradation rate inside carrier cells is of great importance to enable timely drug release from

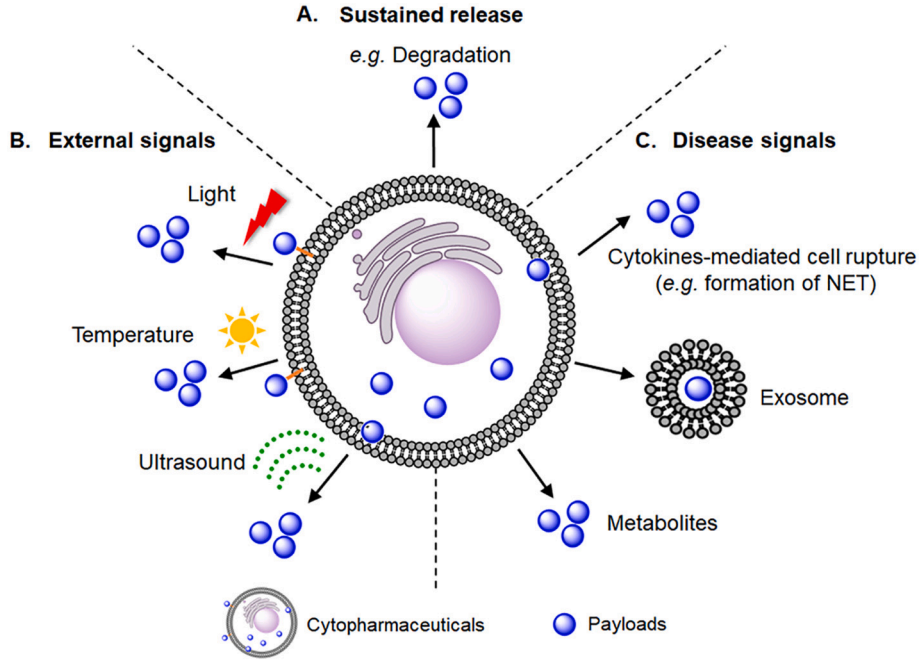

Fig. 6. The drug release behavior of cytopharmaceuticals.

cytopharmaceuticals. For instance, drugs were released from DOX/liposome-laden macrophages at tumor sites owing to macrophage death induced by leaky DOX *via* liposomal degradation [91]. However, this degradation-mediated drug release profile might not be satisfactory, since drug diffusion is inevitable and independent of degradation to a certain extent. Triggered drug release is thus of great significance.

By leveraging the responses of carrier cells to disease signals, cytopharmaceuticals can be designed to release drugs in response to these signals. For example, our group has used inflammatory cytokines in tumors to trigger the release of anti-tumor drugs from neutrophil cytopharmaceuticals *via* the formation of NETs accompanied by rupture of the cellular membrane [10,66,67]. Leveraging the overexpressed protease legumain during the differentiation of monocytes into macrophages, Li and colleagues loaded legumain-sensitive nanoparticulated mertansine into monocytes to achieve on-demand drug release in the case of monocyte differentiation into macrophages inside metastatic tumors [70]. In addition to direct release from carrier cells, cytopharmaceuticals can be designed to release drugs/nanomedicines in an indirect manner, such as that mediated by exosomes [82] or metabolites. For example, adipocyte-based cytopharmaceuticals containing DOX–fatty acid prodrugs can release the prodrug as a metabolite that is subsequently accumulated by tumor cells [92]. It is worth noting that disease signal-triggered drug release is highly dependent on the patient condition, which might be variable among individuals. Concerning good controllability, external stimulus-mediated drug release is favorable.

Stimulus-sensitive nanomedicine has been well documented [93,94], which is featured by a temporal and spatial drug release profile in response to light, pH, ultrasound, and temperature. In the cytopharmaceutical settings, stimulus responsiveness has been explored recently. For example, a peptide therapeutic can be introduced onto the surface of erythrocytes *via* a photo-cleavable lipid anchorage, and after the local application of a light source at the site of disease, the peptide is released to act on its biological target [95]. Monocyte-based cytopharmaceuticals containing echogenic polymer/C5F12 bubbles and DOX formed pore-like defects after ultrasound treatment, which led to the liberation of drugs [96]. Similarly, heat generated from co-encapsulated iron oxide has been utilized to release payloads [97]. Although external stimulation responsiveness models exhibited unique advantages concerning the control of drug release, the further application of this model requires the resolution of certain limitations [98], such as the poor tissue penetration of light and the modest spatial resolution of magnetic fields.

Taken together, multiple strategies have been explored for well-controlled drug release from cytopharmaceuticals. However, the complex environment *in vivo* might comprise the presupposed release behavior. In addition, the sophisticated design of nanoparticles for controlled release might impede the feasibility for the large-scale industrial production. Moreover, the non-invasive and precise *in vivo* detection methods should be established to permit the non-invasive real-time monitoring of drug distribution.

2.4.2. The in vivo fate of carrier cells

The *in vivo* fate of carrier cells is closely related to the potency and safety of cytopharmaceuticals, which also requires a comprehensive study. Concerning the *in vivo* fate of carrier cells, studies should include i) examine whether drug/nanomedicine loading alters the *in vivo* fate of carrier cells, and ii) assess the distribution, colonization, proliferation, differentiation, and persistence of carrier cells.

For the first issue, the rational choice of loaded therapeutic agents as well as the loading method largely contributes to the unperturbed physiological functions of carrier cells, as discussed in previous sections. For example, PTX-resistant MSCs were used to load PTX (~0.10 pg PTX per cell) without causing any loss of cellular viability [99]. The half-life of cytopharmaceuticals based on liposomal nanomedicine displayed a similar circulation time as that of its naive counterparts because of the biocompatibility of liposomes. Moreover, the loading amount is another determinant, especially for cytopharmaceuticals fabricated using the “loading onto” strategy [67]. It has been reported that approximately 3–4 polystyrene nanoparticles with a size of 200 nm on the surface of RBCs have no influence on the circulation time. However, when the amount of loading reached approximately 24 nanoparticles per RBC, the cells are cleared from the circulation more rapidly than their naive counterparts [100].

Regarding the latter issue, the positive contributions of carrier cells to the targeted disease should be avoided, especially for carrier cells that can proliferate and persist for prolonged periods at the diseased site. Several strategies have been developed to kill carrier cells after

they deliver drugs to the sites of disease. Monocyte-based cytopharmaceuticals containing echogenic polymer/C5F12 bubbles and DOX can form pore-like defects after ultrasound treatment, which might induce the loss of carrier cell viability as a result of perturbation of the cell membrane [96]. Similarly, heat generated from co-encapsulated iron oxide has been utilized to kill carrier cells [97].

Additionally, the current technologies for monitoring the *in vivo* fate of carrier cells mainly focus on labeling the cells with fluorescent, isotope, or contrast agents. For example, taking advantage of a ^{51}Cr RBC labeling technique, the infused RBCs have a near-physiological survival time with a cell life of 89–131 days [101]. However, labeling carrier cells with such tags might change the *in vivo* behavior of cytopharmaceuticals. Moreover, the current analytic methods cannot monitor the entire life cycle of carrier cells, thus necessitating further improvement.

3. Application of cytopharmaceuticals

Considering the advantages of cytopharmaceuticals, their applications include various diseases with several products under clinic development. As stated previously, several excellent reviews discussed the various applications of cytopharmaceuticals [7–9]. In this section, we will discuss several considerations before initiating a clinical evaluation.

Cytopharmaceuticals are considered personalized treatment modalities because the used carrier cells are obtained from autologous patients or allogeneic donors with matched HLA types. In addition, the application of various cytopharmaceuticals is primarily governed by the pathological status of the disease. Thereby, the translation of cytopharmaceuticals for different indications should be strictly dependent on the thorough understanding of the disease.

Solid tumors are highly heterogeneous and inflamed tissues that recruit almost all types of circulatory cells, such as neutrophils, monocytes/macrophages, NK cells, T cells, and platelets, to orchestrate an immunosuppressive microenvironment [102]. Although this property of tumors largely limits the therapeutic benefits of several treatment modalities, such as chemotherapy and immunotherapy, it can be leveraged to design cytopharmaceuticals for improved therapeutic outcomes. For example, our group reported neutrophil-based cytopharmaceuticals containing PTX nanomedicines, which can effectively deliver the loaded nanomedicines to the tumor site following the inflammatory signals of local surgery, radiotherapy or thermotherapy, thus leading to augmented anti-tumor effect [10,66,67]. In addition, macrophage-based cytopharmaceuticals of DOX provided enhanced DOX accumulation in solid tumors and thus an improved anti-tumor efficacy [83]. Human MSC-based cytopharmaceuticals of silica nanoparticulated DOX displayed enhanced tumor accumulation after systemic transfusion and resulted in elevated anti-tumor efficacy [62]. Platelet-based cytopharmaceuticals of gold nanorods were developed to suppress the growth of head and neck squamous cell carcinoma in a feedback manner [65].

Tumor metastasis, including colonization, and proliferation of disseminated tumor cells in secondary organs, contribute largely to the death of tumor patients [103]. The primary tumor orchestrates a metastatic niche by mobilization several suppressive cells [104], such as monocytes and neutrophils, into the niche to support the growth of tumor cells. Taking advantage of this property of metastasis, NSCs can be exploited to deliver drugs to inhibit brain metastasis [105]. Moreover, T cell-based cytopharmaceuticals can actively deliver anti-cancer drugs to disseminated tumors in lymph nodes [24].

Disease related to inflammation, infection, and tissue damage can release chemokines and inflammatory cytokines and generate a gradient that attracts circulating leukocytes and platelets to sites of disease. This inspired the exploration of cytopharmaceuticals based on leukocytes and platelets. For example, idiopathic pulmonary fibrosis, which is initiated by injury of type II alveolar epithelial cells, releases chemotactic factors that specifically recruit chemokine receptor-positive cells including monocyte-derived multipotent cells (MOMCs). Bearing this in mind, MOMC-based cytopharmaceuticals were developed to deliver programmed therapeutics to synergistically reverse pulmonary fibrosis [106]. Macrophage-based cytopharmaceuticals of antiretroviral drug (indinavir) nanoparticles were targeted to the brain to effectively reduce HIV replication [27]. In addition, macrophage-based cytopharmaceuticals were employed to reduce inflammation in the brain to alleviate Parkinson's disease [28].

Diseases that require prolonged drug circulation in blood can be treated using RBC-based cytopharmaceuticals with reduced dosages. For example, a single systemic injection of RBC-based cytopharmaceuticals of phosphorylated DEX maintained a therapeutic DEX concentration for more than 30 days in the treatment of ataxia telangiectasia [82]. Moreover, RBC-based cytopharmaceuticals of certain enzymes have been widely used to detoxify exogenous chemicals, such as lead, paraoxon, methanol, and ethanol [107].

Despite the great promise cytopharmaceuticals have for the treatment of various diseases, the costs associated with their acquisition and manipulation must be reduced before their broad and affordable clinical application.

4. Future perspectives

In an effort to translate academic findings from the bench to the bed, various translational hurdles depending on the workflow and *in vivo* fate of cytopharmaceuticals, including the simple isolation of carrier cells and standardized preparation protocols, must be addressed. To do so, we might make efforts from several aspects: i) develop integrated and automated production equipment to realize large-scale production with minimal possibilities of contamination by bacteria, mycoplasma, and endotoxins; ii) optimize the preparation techniques to improve the yield of cytopharmaceuticals considering sample loss during purification and preservation; iii) establish quality standards for various cytopharmaceuticals and the corresponding rapid detection methods during each production step given that cytopharmaceuticals are living products; and iv) commit to the continuous development of nanomedicines, which are key sources of cytopharmaceuticals. In addition, the drug-loading capacity of cytopharmaceuticals requires further optimization to ensure an expected efficacy of loaded drugs, with fewest biosafety concerns.

5. Conclusions

The emergence of cytopharmaceuticals has expanded the repertoire of TDDSs, offering new treatment modalities for patients, especially those with hard-to-reach pathological sites. Moreover, cytopharmaceuticals have exhibited unique benefits for drug delivery, such as active targeting to sites of disease, prolonged circulation, and biocompatibility. We can envision that *bona fide* personalized medicine can be developed through the application of cytopharmaceuticals because various autologous cells can be loaded with an array of drugs for patients with different conditions. Despite the aforementioned challenges, we firmly believe that through joint efforts of biologists, chemists, and physicists, the translation of paradigm-shifting cytopharmaceuticals will be realized.

Notes

The authors declare no competing financial interests.

Credit author statement

Li Weishuo wrote the draft manuscript and drew the draft figures. Ju Caoyun, Hao Meixi and Su Zhigui polished the figures. Zhang Can and Ju Caoyun reviewed and edited the manuscript.

Acknowledgement

This work was supported by the National Natural Science Foundation of China (81930099, 81773664, 81473153, 81803465), National Major Scientific and Technological Special Project for "Significant New Drugs Development" (2019ZX09301163), China Postdoctoral Science Foundation (2018M630639, 2019T120487), 111 Project from the Ministry of Education of China and the State Administration of Foreign Expert Affairs of China (No. 111-2-07, B17047), the Open Project of State Key Laboratory of Natural Medicines (No. SKLNMZZ202017), "Double First-Class" University project (CPU2018GY47, CPU2018GF10), and the Natural Science Foundation of Jiangsu Province (BK20180552).

References

[1] J. Fang, H. Nakamura, H. Maeda, The EPR effect: unique features of tumor blood vessels for drug delivery, factors involved, and limitations and augmentation of the effect, Adv. Drug Deliv. Rev. 63 (3) (2011) 136–151.
[2] Z. Zhao, A. Ukidve, J. Kim, S. Mitragotri, Targeting strategies for tissue-specific drug delivery, Cell. 181 (1) (2020) 151–167.
[3] J.I. Hare, T. Lammers, M.B. Ashford, S. Puri, G. Storm, S.T. Barry, Challenges and strategies in anti-cancer nanomedicine development: an industry perspective, Adv. Drug Deliv. Rev. 108 (2017) 25–38.
[4] S. Wilhelm, A. Tavares, Q. Dai, S. Ohta, J. Audet, F.H. Dvorak, W.C.W. Chan, Analysis of nanoparticle delivery to tumours, Nat Rev Mater 1 (2016) 16014.
[5] K. Greish, A. Mathur, M. Bakhiet, S. Taurin, Nanomedicine: is it lost in translation? Ther. Deliv. 9 (4) (2018) 269–285.
[6] B. Chen, W. Dai, B. He, et al., Current multistage drug delivery systems based on the tumor microenvironment, Theranostics. 7 (3) (2017) 538–558.
[7] Y. Su, Z. Xie, G.B. Kim, C. Dong, J. Yang, Design strategies and applications of circulating cell-mediated drug delivery systems, ACS Biomater Sci Eng. 1 (4) (2015) 201–217.
[8] C.H. Villa, A.C. Anselmo, S. Mitragotri, V. Muzykantov, Red blood cells: Supercarriers for drugs, biologicals, and nanoparticles and inspiration for advanced delivery systems, Adv Drug Deliv Rev 106 (Pt A) (2016) 88–103.
[9] L.A.L. Fliervoet, E. Mastrobattista, Drug delivery with living cells, Adv Drug Deliv Rev. 106 (Pt A) (2016) 63–72.
[10] J. Xue, Z. Zhao, C. Zhang, et al., Neutrophil-mediated anticancer drug delivery for suppression of postoperative malignant glioma recurrence, Nat. Nanotechnol. 12 (7) (2017) 692–700.
[11] C. Wang, W. Sun, Y. Ye, Q.Y. Hu, H.N. Bomba, Z. Gu, In situ activation of platelets with checkpoint inhibitors for post-surgical cancer immunotherapy, Nat Biomed Eng 1 (2017) 0011.
[12] S.Y. Fam, C.F. Chee, C.Y. Yong, K.L. Ho, A.R. Mariatulqabtiah, W.S. Tan, Stealth Coating of Nanoparticles in Drug-Delivery Systems, Nanomaterials (Basel) 10 (4) (2020) 787.
[13] B.T. Luk, L. Zhang, Cell membrane-camouflaged nanoparticles for drug delivery, J Control Release 220 (Pt B) (2015) 600–607.
[14] R.P. Garay, R. El-Gewely, J.K. Armstrong, G. Garratty, P. Richette, Antibodies against polyethylene glycol in healthy subjects and in patients treated with PEG-conjugated agents, Expert Opin Drug Deliv. 9 (11) (2012) 1319–1323.
[15] R. Li, Y. He, S. Zhang, J. Qin, J. Wang, Cell membrane-based nanoparticles: a new biomimetic platform for tumor diagnosis and treatment, Acta Pharm. Sin. B 8 (1) (2018) 14–22.
[16] Principles of nanoparticle design for overcoming biological barriers to drug delivery, Nat Biotechnol 33 (9) (2015) 941–951.
[17] S. Arora, J.M. Rajwade, K.M. Paknikar, Nanotoxicology and in vitro studies: the need of the hour, Toxicol. Appl. Pharmacol. 258 (2) (2012) 151–165.
[18] G.M. Ihler, R.H. Glew, F.W. Schnure, Enzyme loading of erythrocytes, Proc. Natl. Acad. Sci. U. S. A. 70 (9) (1973) 2663–2666.
[19] U. Steinfeld, C. Pauli, N. Kaltz, C. Bergemann, H.H. Lee, T lymphocytes as potential therapeutic drug carrier for cancer treatment, Int. J. Pharm. 311 (1–2) (2006) 229–236.
[20] M.R. Choi, K.J. Stanton-Maxey, J.K. Stanley, et al., A cellular Trojan Horse for delivery of therapeutic nanoparticles into tumors, Nano Lett. 7 (12) (2007) 3759–3765.
[21] H. Dou, J. Morehead, C.J. Destache, et al., Laboratory investigations for the morphologic, pharmacokinetic, and anti-retroviral properties of indinavir nanoparticles in human monocyte-derived macrophages, Virology. 358 (1) (2007) 148–158.
[22] M.R. Loebinger, P.G. Kyrtatos, M. Turmaine, et al., Magnetic resonance imaging of mesenchymal stem cells homing to pulmonary metastases using biocompatible magnetic nanoparticles, Cancer Res. 69 (23) (2009) 8862–8867.
[23] S. Sarkar, M.A. Alam, J. Shaw, A.K. Dasgupta, Drug delivery using platelet cancer cell interaction, Pharm. Res. 30 (11) (2013) 2785–2794.
[24] B. Huang, W.D. Abraham, Y. Zheng, S.C. Bustamante López, S.S. Luo, D.J. Irvine, Active targeting of chemotherapy to disseminated tumors using nanoparticle-carrying T cells, Sci Transl Med. 7 (291) (2015) 291ra94.
[25] S. Chandrasekaran, M.F. Chan, J. Li, M.R. King, Super natural killer cells that target metastases in the tumor draining lymph nodes, Biomaterials. 77 (2016) 66–76.
[26] Z. Xie, Y. Su, G.B. Kim, et al., Immune cell-mediated biodegradable Theranostic nanoparticles for melanoma targeting and drug delivery, Small. 13 (10) (2017) 1603121.
[27] H. Dou, C.J. Destache, J.R. Morehead, et al., Development of a macrophage-based nanoparticle platform for antiretroviral drug delivery [published correction appears in Blood], 109 (2007), p. 1816 5.
[28] E.V. Batrakova, S. Li, A.D. Reynolds, et al., A macrophage-nanozyme delivery system for Parkinson's disease, Bioconjug. Chem. 18 (5) (2007) 1498–1506.
[29] C.H. Villa, D.C. Pan, S. Zaitsev, D.B. Cines, D.L. Siegel, V.R. Muzykantov, Delivery of drugs bound to erythrocytes: new avenues for an old intravascular carrier, Ther. Deliv. 6 (7) (2015) 795–826.
[30] Y. Godfrin, F. Horand, R. Franco, et al., International seminar on the red blood cells as vehicles for drugs, Expert. Opin. Biol. Ther. 12 (1) (2012) 127–133.
[31] A. Krantz, Red cell-mediated therapy: opportunities and challenges, Blood Cells Mol. Dis. 23 (1) (1997) 58–68.
[32] K.R. Machlus, J.N. Thon, J.E. Italiano Jr., Interpreting the developmental dance of the megakaryocyte: a review of the cellular and molecular processes mediating platelet formation, Br. J. Haematol. 165 (2) (2014) 227–236.
[33] Y. Lu, Q. Hu, C. Jiang, Z. Gu, Platelet for drug delivery, Curr. Opin. Biotechnol. 58 (2019) 81–91.
[34] M. Haemmerle, R.L. Stone, D.G. Menter, V. Afshar-Kharghan, A.K. Sood, The platelet lifeline to cancer: challenges and opportunities, Cancer Cell 33 (6) (2018) 965–983.
[35] P. Mehta, Potential role of platelets in the pathogenesis of tumor metastasis, Blood. 63 (1) (1984) 55–63.
[36] R. Mukthavaram, G. Shi, S. Kesari, D. Simberg, Targeting and depletion of circulating leukocytes and cancer cells by lipophilic antibody-modified erythrocytes, J. Control. Release 183 (2014) 146–153.
[37] X. Dong, D. Chu, Z. Wang, Leukocyte-mediated delivery of nanotherapeutics in inflammatory and tumor sites, Theranostics. 7 (3) (2017) 751–763.
[38] K. Shen, F.A. DeLano, B.W. Zweifach, G.W. Schmid-Schönbein, Circulating leukocyte counts, activation, and degranulation in dahl hypertensive rats, Circ. Res. 76 (2) (1995) 276–283.
[39] L.M. Coussens, Z. Werb, Inflammation and cancer, Nature. 420 (6917) (2002) 860–867.
[40] P. Niethammer, C. Grabher, A.T. Look, T.J. Mitchison, A tissue-scale gradient of hydrogen peroxide mediates rapid wound detection in zebrafish, Nature. 459 (7249) (2009) 996–999.
[41] A. van der Vliet, Y.M. Janssen-Heininger, Hydrogen peroxide as a damage signal in tissue injury and inflammation: murderer, mediator, or messenger? J. Cell. Biochem. 115 (3) (2014) 427–435.
[42] W. Lee, S.Y. Ko, M.S. Mohamed, H.A. Kenny, E. Lengyel, H. Naora, Neutrophils facilitate ovarian cancer premetastatic niche formation in the omentum, J. Exp. Med. 216 (1) (2019) 176–194.
[43] P. Charoentong, F. Finotello, M. Angelova, et al., Pan-cancer Immunogenomic analyses reveal genotype-immunophenotype relationships and predictors of response to checkpoint blockade, Cell Rep. 18 (1) (2017) 248–262.
[44] S.J. Stanworth, E. Massey, C. Hyde, et al., Granulocyte transfusions for treating infections in patients with neutropenia or neutrophil dysfunction, Cochrane Database Syst. Rev. 3 (2005) CD005339.
[45] C. Peters, M. Minkov, S. Matthes-Martin, et al., Leucocyte transfusions from rhG-CSF or prednisolone stimulated donors for treatment of severe infections in immunocompromised neutropenic patients, Br. J. Haematol. 106 (3) (1999) 689–696.
[46] J.J. Lee, I.J. Chung, M.R. Park, et al., Clinical efficacy of granulocyte transfusion therapy in patients with neutropenia-related infections, Leukemia. 15 (2) (2001) 203–207.
[47] J. Gea-Banacloche, Granulocyte transfusions: a concise review for practitioners, Cytotherapy. 19 (11) (2017) 1256–1269.
[48] M.A. Giese, L.E. Hind, A. Huttenlocher, Neutrophil plasticity in the tumor microenvironment, Blood. 133 (20) (2019) 2159–2167.
[49] S. Gordon, P.R. Taylor, Monocyte and macrophage heterogeneity, Nat Rev Immunol. 5 (12) (2005) 953–964.
[50] A. Shapouri-Moghaddam, S. Mohammadian, H. Vazini, et al., Macrophage plasticity, polarization, and function in health and disease, J. Cell. Physiol. 233 (9) (2018) 6425–6440.
[51] C. Murdoch, A. Giannoudis, C.E. Lewis, Mechanisms regulating the recruitment of macrophages into hypoxic areas of tumors and other ischemic tissues, Blood. 104 (8) (2004) 2224–2234.
[52] H. von Boehmer, Positive selection of lymphocytes, Cell. 76 (2) (1994) 219–228.
[53] M. Barry, R.C. Bleackley, Cytotoxic T lymphocytes: all roads lead to death, Nat Rev Immunol. 2 (6) (2002) 401–409.
[54] M. Daher, K. Rezvani, Next generation natural killer cells for cancer immunotherapy: the promise of genetic engineering, Curr. Opin. Immunol. 51 (2018) 146–153.
[55] Y. Yang, E. Jacoby, T.J. Fry, Challenges and opportunities of allogeneic donor-derived CAR T cells, Curr. Opin. Hematol. 22 (6) (2015) 509–515.
[56] D. Confalonieri, A. Schwab, H. Walles, F. Ehlicke, Advanced therapy medicinal products: a guide for bone marrow-derived MSC application in bone and cartilage tissue engineering, Tissue Eng Part B Rev. 24 (2) (2018) 155–169.
[57] A. Uccelli, L. Moretta, V. Pistoia, Mesenchymal stem cells in health and disease, Nat Rev Immunol. 8 (9) (2008) 726–736.
[58] X.C. Jiang, J.J. Xiang, H.H. Wu, et al., Neural stem cells transfected with reactive oxygen species-responsive Polyplexes for effective treatment of ischemic stroke, Adv. Mater. 31 (10) (2019) e1807591.

[59] F. Pisati, P. Bossolasco, M. Meregalli, et al., Induction of neurotrophin expression via human adult mesenchymal stem cells: implication for cell therapy in neurodegenerative diseases, Cell Transplant. 16 (1) (2007) 41–55.
[60] J.R. Bagó, K.T. Sheets, S.D. Hingtgen, Neural stem cell therapy for cancer, Methods. 99 (2016) 37–43.
[61] Z. Zhao, A. Ukidve, Y. Gao, J. Kim, S. Mitragotri, Erythrocyte leveraged chemotherapy (ELeCt): Nanoparticle assembly on erythrocyte surface to combat lung metastasis, Sci Adv. 5 (11) (2019) eaax9250.
[62] B. Cao, M. Yang, Y. Zhu, X. Qu, C. Mao, Stem cells loaded with nanoparticles as a drug carrier for in vivo breast cancer therapy, Adv. Mater. 26 (27) (2014) 4627–4631.
[63] L. Li, Y. Guan, H. Liu, et al., Silica nanorattle-doxorubicin-anchored mesenchymal stem cells for tumor-tropic therapy, ACS Nano 5 (9) (2011) 7462–7470.
[64] Q. Hu, W. Sun, J. Wang, et al., Conjugation of haematopoietic stem cells and platelets decorated with anti-PD-1 antibodies augments anti-leukaemia efficacy, Nat Biomed Eng. 2 (11) (2018) 831–840.
[65] L. Rao, L.L. Bu, L. Ma, et al., Platelet-facilitated Photothermal therapy of head and neck squamous cell carcinoma, Angew Chem Int Ed Engl. 57 (4) (2018) 986–991.
[66] C. Ju, Y. Wen, L. Zhang, et al., Neoadjuvant chemotherapy based on Abraxane/human neutrophils Cytopharmaceuticals with radiotherapy for gastric Cancer, Small. 15 (49) (2019) e1905688.
[67] L. Zhang, Y. Zhang, Y. Xue, et al., Transforming weakness into strength: Photothermal-therapy-induced inflammation enhanced Cytopharmaceutical chemotherapy as a combination anticancer treatment, Adv. Mater. 31 (5) (2019) e1805936.
[68] M. Wu, H. Zhang, C. Tie, et al., MR imaging tracking of inflammation-activatable engineered neutrophils for targeted therapy of surgically treated glioma, Nat. Commun. 9 (1) (2018) 4777.
[69] C.W. Shields, M.A. Evans, L.L.W. Wang, N. Baugh, S. Iyer, D. Wu, Z.M. Zhao, A. Pusuluri, A. Ukidve, D.C. Pan, S. Mitragotri, Cellular backpacks for macrophage immunotherapy, Sci Adv. 6 (18) (2020) eaaz6579.
[70] X. He, H. Cao, H. Wang, et al., Inflammatory monocytes loading protease-sensitive nanoparticles enable lung metastasis targeting and intelligent drug release for anti-metastasis therapy, Nano Lett. 17 (9) (2017) 5546–5554.
[71] L. Tang, Y. Zheng, M.B. Melo, et al., Enhancing T cell therapy through TCR-signaling-responsive nanoparticle drug delivery, Nat. Biotechnol. 36 (8) (2018) 707–716.
[72] S. Im, D. Jang, G. Saravanakumar, et al., Harnessing the formation of natural killer-tumor cell immunological synapses for enhanced therapeutic effect in solid tumors, Adv. Mater. 32 (22) (2020) e2000020.
[73] Y. Barenholz, Doxil®–the first FDA-approved nano-drug: lessons learned, J. Control. Release 160 (2) (2012) 117–134.
[74] A.C. Anselmo, S. Mitragotri, Nanoparticles in the clinic: an update, Bioeng Transl Med. 4 (3) (2019) e10143.
[75] Islam Ahmed Hamed Khalil, Islam A. Arida, Mohamed Ahmed, Introductory Chapter: Overview on Nanomedicine Market, Current and Future Aspects of Nanomedicine, Islam Ahmed Hamed Khalil, IntechOpen, 2020.
[76] D.H. Mitchell, G.T. James, C.A. Kruse, Bioactivity of electric field-pulsed human recombinant interleukin-2 and its encapsulation into erythrocyte carriers, Biotechnol. Appl. Biochem. 12 (3) (1990) 264–275.
[77] C.X. Li, Y. Zhang, X. Dong, et al., Artificially reprogrammed macrophages as tumor-tropic immunosuppression-resistant biologics to realize therapeutics production and immune activation, Adv. Mater. 31 (15) (2019) e1807211.
[78] H. He, J. Ye, Y. Wang, et al., Cell-penetrating peptides meditated encapsulation of protein therapeutics into intact red blood cells and its application, J. Control. Release 176 (2014) 123–132.
[79] M.E. Favretto, J.C. Cluitmans, G.J. Bosman, R. Brock, Human erythrocytes as drug carriers: loading efficiency and side effects of hypotonic dialysis, chlorpromazine treatment and fusion with liposomes, J. Control. Release 170 (3) (2013) 343–351.
[80] G.I. Harisa, M.F. Ibrahim, F. Alanazi, G.A. Shazly, Engineering erythrocytes as a novel carrier for the targeted delivery of the anticancer drug paclitaxel, Saudi Pharm J. 22 (3) (2014) 223–230.
[81] H.G. Eichler, W. Raffesberg, S. Gasic, A. Korn, K. Bauer, Release of vitamin B12 from carrier erythrocytes in vitro, Res Exp Med (Berl). 185 (4) (1985) 341–344.
[82] G. Mambrini, M. Mandolini, L. Rossi, et al., Ex vivo encapsulation of dexamethasone sodium phosphate into human autologous erythrocytes using fully automated biomedical equipment, Int. J. Pharm. 517 (1–2) (2017) 175–184.
[83] W. Zhang, M. Wang, W. Tang, et al., Nanoparticle-laden macrophages for tumor-tropic drug delivery, Adv. Mater. 30 (50) (2018) e1805557.
[84] D.L. Gilbert, G. Ehrenstein, Membrane surface charge, Curr. Top. Membr. Transport 22 (1984) 407–421.
[85] E. Chambers, S. Mitragotri, Prolonged circulation of large polymeric nanoparticles by non-covalent adsorption on erythrocytes, J. Control. Release 100 (1) (2004) 111–119.
[86] E. Chambers, S. Mitragotri, Long circulating nanoparticles via adhesion on red blood cells: mechanism and extended circulation, Exp Biol Med (Maywood). 232 (7) (2007) 958–966.
[87] H. Cao, H. Wang, X. He, et al., Bioengineered macrophages can responsively transform into Nanovesicles to target lung metastasis, Nano Lett. 18 (8) (2018) 4762–4770.
[88] S.M. Ong, L. He, N.T. Thuy Linh, et al., Transient inter-cellular polymeric linker, Biomaterials. 28 (25) (2007) 3656–3667.
[89] L. Xu, O.Y. Zolotarskaya, W.A. Yeudall, H. Yang, Click hybridization of immune cells and polyamidoamine dendrimers, Adv Healthc Mater. 3 (9) (2014) 1430–1438.
[90] M.K. Gupta, J.R. Martin, B.R. Dollinger, M.E. Hattaway, C.L. Duvall, Thermogelling, ABC triblock copolymer platform for Resorbable hydrogels with tunable, Degradation-Mediated Drug Release, Adv Funct Mater. 27 (47) (2017) 1704107.
[91] J. Choi, H.Y. Kim, E.J. Ju, et al., Use of macrophages to deliver therapeutic and imaging contrast agents to tumors, Biomaterials. 33 (16) (2012) 4195–4203.
[92] D. Wen, J. Wang, G. Van Den Driessche, et al., Adipocytes as anticancer drug delivery depot, Matter. 1 (2019) 1203–1214.
[93] P. Lavrador, V.M. Gaspar, J.F. Mano, Stimuli-responsive nanocarriers for delivery of bone therapeutics - barriers and progresses, J. Control. Release 273 (2018) 51–67.
[94] Y. Lu, W. Sun, Z. Gu, Stimuli-responsive nanomaterials for therapeutic protein delivery, J. Control. Release 194 (2014) 1–19.
[95] R.M. Hughes, C.M. Marvin, Z.L. Rodgers, et al., Phototriggered secretion of membrane compartmentalized bioactive agents, Angew Chem Int Ed Engl. 55 (52) (2016) 16080–16083.
[96] W.C. Huang, W.H. Chiang, Y.H. Cheng, et al., Tumortropic monocyte-mediated delivery of echogenic polymer bubbles and therapeutic vesicles for chemotherapy of tumor hypoxia, Biomaterials. 71 (2015) 71–83.
[97] W.C. Huang, I.L. Lu, W.H. Chiang, et al., Tumortropic adipose-derived stem cells carrying smart nanotherapeutics for targeted delivery and dual-modality therapy of orthotopic glioblastoma, J. Control. Release 254 (2017) 119–130.
[98] P. Davoodi, L.Y. Lee, Q. Xu, et al., Drug delivery systems for programmed and on-demand release, Adv. Drug Deliv. Rev. 132 (2018) 104–138.
[99] L. Pascucci, V. Coccè, A. Bonomi, et al., Paclitaxel is incorporated by mesenchymal stromal cells and released in exosomes that inhibit in vitro tumor growth: a new approach for drug delivery, J. Control. Release 192 (2014) 262–270.
[100] A.C. Anselmo, V. Gupta, B.J. Zern, et al., Delivering nanoparticles to lungs while avoiding liver and spleen through adsorption on red blood cells, ACS Nano 7 (12) (2013) 11129–11137.
[101] B.E. Bax, M.D. Bain, P.J. Talbot, E.J. Parker-Williams, R.A. Chalmers, Survival of human carrier erythrocytes in vivo, Clin Sci (Lond). 96 (2) (1999) 171–178.
[102] E. Elinav, R. Nowarski, C.A. Thaiss, B. Hu, C. Jin, R.A. Flavell, Inflammation-induced cancer: crosstalk between tumours, immune cells and microorganisms, Nat. Rev. Cancer 13 (11) (2013) 759–771.
[103] P.S. Steeg, Targeting metastasis, Nat. Rev. Cancer 16 (4) (2016) 201–218.
[104] Y. Wang, Y. Ding, N. Guo, S. Wang, MDSCs: key criminals of tumor pre-metastatic niche formation, Front. Immunol. 10 (2019) 172.
[105] K.S. Aboody, J. Najbauer, N.O. Schmidt, et al., Targeting of melanoma brain metastases using engineered neural stem/progenitor cells, Neuro-Oncology 8 (2) (2006) 119.
[106] X. Chang, L. Xing, Y. Wang, et al., Monocyte-derived multipotent cell delivered programmed therapeutics to reverse idiopathic pulmonary fibrosis, Sci. Adv. 6 (22) (2020) e3167.
[107] V. Agrahari, V. Agrahari, A.K. Mitra, Next generation drug delivery: circulatory cells-mediated nanotherapeutic approaches, Expert Opin Drug Deliv. 14 (3) (2017) 285–289.
[108] A.C. Anselmo, S. Kumar, V. Gupta, et al., Exploiting shape, cellular-hitchhiking and antibodies to target nanoparticles to lung endothelium: synergy between physical, chemical and biological approaches, Biomaterials. 68 (2015) 1–8.
[109] N. Doshi, A.J. Swiston, J.B. Gilbert, et al., Cell-based drug delivery devices using phagocytosis-resistant backpacks, Adv. Mater. 23 (12) (2011) H105–H109.