Cell-Based Bio-Hybrid Delivery System for Disease Treatments

Chu-Xin Li, Yong-Dan Qi, Jun Feng,* and Xian-Zheng Zhang*

Live cells are implicated in diverse biological processes, including nutrient transport and removal of foreign substances. Their intelligent biofunctions with complex mechanisms cannot be replicated at all in man-made materials despite the significant advance in material design. Taking advantages of their biocompatibility and biotropic capability, various live cells have been developed as a kind of special carriers for the on-demand delivery of therapeutic and diagnostic agents in recent years. Furthermore, synthetic materials can be integrated with live cells to provide bio-hybrid systems that inherit advantages from both the synthetic particles and the natural cells. Herein, recent strategies and advances in cell-based bio-hybrid delivery systems for disease treatment are summarized. Challenges and opportunities in this field are also discussed.

1. Introduction

Synthetic delivery systems such as liposome and polymeric particles provide advantages in improving the solubility, biocompatibility, and pharmacokinetics of free drugs. However, considerable effort is still needed to improve their properties to satisfy clinical expectations. Low delivery efficiency is still one of the major problems for therapeutics administration. It is reported that only 0.7% of the injected nanoparticles can reach solid tumor in general. For tumor-tropic delivery, synthetic delivery systems, especially nanoscale materials, may rely on passive targeting effects such as the enhanced permeability and retention (EPR) effect, which is a special character of solid tumors due to the aggressive angiogenic environment. Nevertheless, the EPR effect for tumor-selective delivery is to some extent controversial and researchers reported that active transport process via endothelial cells contributed to the majority of nanoparticles’ entrance into solid tumors. In case of inflammatory diseases, current therapeutic delivery take advantages of extravasation through leaky vasculature to cross the endothelial barrier, or hitchhiking the inflammatory cell-mediated sequestration activity. The transport processes of injected particles are usually heterogeneous and unpredictable, which may lower the bioavailability of therapeutics. In addition, intravenously injected particles will face multiple biological barriers, such as the well-known mononuclear phagocyte system, during circulation and upon entering extra-cellular microenvironment.

The innate functions of autologous cells have thus attracted great interest as new constituent for developing powerful therapeutics. For example, red blood cells (RBCs) participate in metabolic exchange and nutrients transport throughout the human body. Phagocytes like monocytes and neutrophils circulate in peripheral blood, survey the inflammatory cues, and migrate toward pathologic tissues to elicit pathogen eradication and tissues remodeling. The question naturally arises that how to integrate drugs and therapeutic particles with cells. As known, exotic intruders can be cleared by immune cells through multiple mechanisms. This physiological barrier has been widely exploited as opportunity to develop bio-hybrid cell-based delivery system, also called cellular vehicle or cellular carrier, where drugs and particles are integrated with natural cells. Taking advantages of the loading capacity, phagocytosis activity, and natural optimized transport patterns of live cells, therapeutic drugs and synthetic materials loaded into cell carrier display improved pharmacokinetics and active targeting capacity.

With the increasing understanding of cellular biology and the advance of nanotechnology, intelligent functions of live cells as well as interactive design of therapeutic materials have also been considered for developing novel bio-hybrid delivery systems. The plasticity, diverse functional subtypes, and ingenious changes in response to different pathological stimuli of intrinsic cells enable the feasibility of using synthetic materials to organically integrate with cells and to regulate their diversity for multiple diagnostic and therapeutic purposes. By harnessing advantages of synthetic materials as well as the special cellular activities of natural cells, novel bio-hybrid delivery systems display multiple functionalities and biological intelligence. As shown in Figure 1, we concisely summarize several interesting characters of natural cells and attractive strategies that benefit from synthetic materials for constructing live cell-based bio-hybrid delivery systems. We will provide a brief introduction of recent studies in this field for disease treatment. Different types of intrinsic cells display distinct traffic patterns in response to different pathology and disease process.

C.-X. Li, Y.-D. Qi, Prof. J. Feng, Prof. X.-Z. Zhang
Key Laboratory of Biomedical Polymers of Ministry of Education & Department of Chemistry
Wuhan University
Wuhan 430072, P. R. China
E-mail: fengjun@whu.edu.cn, xz-zhang@whu.edu.cn

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Thus, we divide recent studies according to cell types, including RBC, neutrophil, monocyte/macrophage, lymphocyte, and platelet. The in vivo cell hitchhiking strategy is also introduced and compared with ex vivo cell engineering. We also discuss about the advantages and limitations of different types of live cell-based carrier, and provide our insights into future development of novel bio-hybrid delivery systems.

2. Strategies for Constructing Bio-Hybrid Delivery Systems

2.1. RBC Engineering

RBC, also named erythrocyte, is the most abundant cell type and important cellular component in blood. Learning from its transporter role for oxygen and carbon dioxide transfer, researchers have made considerable efforts in manipulating RBCs for in vivo drug and particle delivery. RBCs display multiple advantages when serving as a carrier, including prolonged circulation, benign biocompatibility, wide availability, and flexible adaptability. One of the major purposes of using RBC carriers is to improve circulation half-life and to elongate in vivo retention period of laden cargoes. Taking advantage of the biodistribution of RBCs, several studies have reported erythrocyte-hitchhiking strategy for lung-targeting delivery. Ukidve et al. exploited another biological traffic pattern of erythrocyte to deliver polystyrene nanoparticles loaded with ovalbumin (OVA) to the spleen, rather than lung, for immunization (Figure 2A). It has been verified that erythrocytes are able to capture pathogens during circulation and transport them to Kupffer cells in the liver and professional antigen-presenting

Figure 1. Schematic depicting advances in live cell-based bio-hybrid delivery system. Natural cells have been widely explored as biological delivery vehicles in recent years, including erythrocyte, monocyte, macrophage, neutrophil, lymphocyte, and platelets. Special biological processes that provide opportunities for intelligent drug delivery and release are summarized at the top. Synthetic biomaterials such as magnetic/optical/acoustic responsive particles have been exploited for endowing bio-hybrid systems with additional functions.
cells (APCs) to the spleen. By adjusting ex vivo feeding ratio of nanoparticles and erythrocytes, the authors defined a preferable feeding ratio of 300:1 (nanoparticles-to-erythrocytes), which displayed enhanced accumulation in spleen and reduced accumulation in lung (Figure 2B). In addition, this formulation also promoted its internalization by APCs possibly due to the high density of conjugated nanoparticles, which could increase the phosphatidylserine and physically mask CD47, a “do not eat” signal molecule, on the surface of erythrocytes. This OVA-loaded nanoparticles-conjugated erythrocytes displayed therapeutic potential as a vaccine for tumor that expressed OVA antigens.

Researchers also cleverly manipulated the surface receptor proteins on RBCs to construct smart delivery system. Gu and co-workers linked glucose derivative-modified insulin with abundant glucose transporter molecule on RBCs. This interaction is reversible, making it possible to release insulin under high glucose conditions due to the competitive interaction of free glucose with glucose transporter molecule (Figure 2C). Hotz et al. demonstrated the potentials of RBCs in binding and clearing cell-free RNA for preventing lung injury, which are benefited from surface receptors. Efforts in excavating the protein components of RBCs may help advance current erythrocyte-based therapeutics and promote their clinical translation.

There are some studies that also adopted the biomaterial-assisted/engineered RBCs strategy while aimed at other purposes. Zhao et al. have reported a strategy of shielding surface antigens of RBCs by chemical frameworks for blood transfusion (Figure 2D). They anchored horseradish peroxidase (HRP) on cellular surface by using biocompatible anchor molecule (BAM) that contained an oleyl chain for membrane insertion. In the presence of substrates including polysialic acid (PSA), tyramine, and hydrogen peroxide, HRP catalyzed the formation of a 3D framework encompassing the RBC. This method did not affect the fluidity and meanwhile shield the immunogenic antigens on RBCs, providing a universal strategy for modifying RBCs and adoptive transfer. Zhu et al. developed a strategy of using zirconium-based metal-organic framework (MOF) nanoparticles for cryopreservation of RBCs, without the need of any toxic agents and solvents. Herein, the authors adjusted different parameters of MOF nanoparticles to optimize the cryopreservation effect. They found that MOF nanoparticles improved the cryopreservation by inhibiting ice recrystallization meanwhile catalyzing the melting of ice crystals, which may destroy the structure of RBCs. Some researchers also excavated the potential of RBCs as a versatile scaffold for generating microreactors and immune modulatory agents.

We can summarize from these studies that current RBC engineering mainly involves three directions: 1) generating delivery system with biocompatibility and prolonged circulations, 2) excavating smart responsiveness and therapeutic potentials of erythrocytes’ components, and 3) serving as a special scaffold with unique shape and characteristics for artificial modification.

2.2. Neutrophil Carriers

Neutrophil is the major effector immune cell in response to acute inflammation, which is usually occurred in infectious site and sterile injured tissues such as burn and hypoxia. Neutrophils also participate in chronic inflammation, adaptive immune responses, and tumor development. The recruitment...
and infiltration cascade of neutrophils are initiated by the endothelium following the gradient of inflammatory factors like cytokine and chemokine. Therefore, neutrophil is usually taken as an indicator of inflammation and relevant diseases, and has been widely studied as a drug delivery system in recent years. For example, neutrophil-based delivery system can cross the blood–brain barrier (BBB) and overcome the blood–brain–tumor barrier (BBTB) for malignant glioma treatment. Hou et al. integrated neutrophils and monocytes with cyclo-(Arg-Gly-Asp-D-Tyr-Lys) (cRGD) peptide-modified drug-loaded liposomes, realizing successful comigration across BBB for delivering therapeutic molecules for cerebral ischemia. Wu et al. incubated neutrophils with drug-loaded magnetic mesoporous silica nanoparticles for magnetic resonance (MR) imaging tracking of their in vivo traffic toward inflamed glioma tissues. Zhang and co-workers reported a neutrophil-based chemotherapy delivery system that targeted inflammatory signals in the brain after surgical resection of glioma. In addition, they also utilized similar neutrophil-based carrier to deliver chemotherapeutics toward inflamed tumor tissues after radiotherapy.

An additional advantage of the neutrophil-based delivery system is that in response to inflammatory stimuli, neutrophils will form neutrophil extracellular traps (NETs), and release the loaded drug to extracellular environment, which overcomes a delivery problem of cellular vehicles that the loaded drug is usually trapped in the carrier cell and cannot reach the preferred targets.

Zhang et al. integrated neutrophils with photocatalytic nanoparticles Fe₃O₄@TiO₂ (Figure 3B), containing an iron oxide core and a titanium dioxide shell, to target infection nidus and activate innate immune system for eradicating pathogens. Natural neutrophils displayed certain antibiotic effect in vitro, which was further boosted after Fe₃O₄@TiO₂ integration (Figure 3C). Upon enriching at infectious tissues, photocatalytic nanoparticles produced abundant reactive oxygen species (ROS) under near-ultraviolet irradiation, which could not only eradicate surrounding micro-organisms but also induced the apoptosis of the transporter neutrophils. The apoptotic neutrophils produced and released a series chemokine such as MCP-1 and MIP-1β, recruiting macrophages and amplifying subsequent innate immune responses of the host for further elimination of pathogens. The therapeutic potentials of this platform were verified in mouse model with necrotizing fasciitis and intra-abdominal infection.

### 2.3. Monocyte/Macrophage Carriers

Monocyte and macrophages are important components of innate immune system, participating in homeostasis and inflammation

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**Figure 3.** Neutrophil, monocyte, and macrophage-based delivery systems. A) Schematic of using neutrophils loaded with doxorubicin-loaded magnetic mesoporous silica nanoparticles (ND-MMSNs) for crossing BBB to reach glioma tumor and activation-triggered drug release. Reproduced with permission. Copyright 2018, Springer Nature. B) Preparation of photocatalytic Fe₃O₄@TiO₂-loaded neutrophils for antibiotic therapy. Reproduced with permission. Copyright 2020, ACS Publications. C) Photo of the methicillin-resistant bacterial colonies treated with different groups with or without near-ultraviolet (NUV) irradiation. Reproduced with permission. Copyright 2020, ACS Publications. D) Molecule structure of functional membrane-anchoring PpIX conjugate and schematic illustration of integration of PpIX conjugate onto macrophage. Reproduced with permission. Copyright 2017, Wiley. E) Schematic of macrophages internalized with Dox–silica nanocomplex (DSN) for tumor-tropic drug delivery. Reproduced with permission. Copyright 2018, Wiley. F) Schematic illustration of monocytes loaded with legumain-responsive nanoparticles for targeting lung metastatic breast cancer and realizing activation-responsive drug release. Reproduced with permission. Copyright 2017, ACS Publications. G) Schematic of ultrasound-responsive macrophage-mediated drug delivery and release system. Reproduced with permission. Copyright 2020, Wiley.
development. Monocytes, the blood-borne precursor of both macrophage and dendritic cell (DC) lineages, can migrate following the stimulus gradients, emigrate from blood vessels, infiltrate into tissues, and differentiate into macrophages to elicit various functions to control infection and disease progression.\[^{44,45}\] While macrophages, which distribute throughout the body and all organs, mainly reside in tissues and monitors the surrounding physiological changes. Both monocyte and macrophage lineages display heterogeneity and can divide into various subsets in response to specific microenvironment, making them hot candidates for site-specific targeting delivery. For example, it has been verified that macrophages can be recruited deep into hypoxic area of tumor.\[^{46}\] Hence, the specific while multifarious distribution patterns and homing capacities of monocytes and macrophages are widely exploited for diagnosis and therapy for tumors and inflammatory diseases.\[^{47–49}\]

Our group previously reported a contact-cell-enhanced photodynamic therapy (PDT) by anchoring functional molecule containing protoporphyrin X (PpIX) on the plasma membrane of macrophages for tumor treatment (Figure 3D).\[^{50}\] The engineered macrophages actively accumulated at tumor tissues, recognized, and interacted with cancer cells. This interaction formed macrophage-to-cancer cell contact, which concentrated PpIX and ROS production around cancer cells, and improved the therapeutic outcomes than free PpIX. This membrane insertion strategy for loading cargoes onto surface of macrophage-based delivery system cleverly exploited the cell–cell contact for concentrating cytotoxic agents onto cancer cells, taking advantages of natural cancer cell recognition capacity of macrophages meanwhile circumventing the problem of drug release from cell-based carriers.

Zhang et al. reported a macrophage-based chemotherapy delivery system with high drug dosage.\[^{51}\] They engineered a silica-based nanoparticles (DSN) for loading doxorubicin (DOX) and coordinated DSN with the macrophage carrier, with a high loading efficacy up to 16.6 μg per cell (Figure 3E).\[^{51}\] DSN possessed a robust silica sheath that degraded quite slowly in the first few hours during circulation, preventing drug release in lysosomes of macrophages before reaching targets. Upon reaching tumor tissues, the silica core was more susceptible to degradation and released DOX for tumor inhibition. The authors took notice of the intracellular lysosomal activity, which is also a troublesome limitation for cell-based delivery system, and prudentially designed nanoparticle carrier to guarantee efficient drug preservation during transport meanwhile realized rapid drug release upon reaching tumor tissues.

Li and co-workers loaded inflammatory monocytes with legumain-responsive nanoparticles for treating lung metastatic breast tumors.\[^{52,53}\] Cytotoxic agent mertansine was linked to poly(ethylene-co-maleic anhydride) (SMA) via legumain-sensitive peptide to obtain SMA-AANK-Mertansine conjugate. This conjugate self-assembled into nanoparticles (SMNs) and were loaded into inflammatory monocytes to prepare monocyte-based delivery system (M-SMNs) for targeting cancer cells with lung metastasis (Figure 3F).\[^{52}\] Upon settle down in metastatic niche, monocytes activated, and switched into macrophages, which expressed a high level of legumain, which triggered the release of mertansine. The authors found that the anticancer drug of mertansine was released in the form of free drug as well as microvesicles that were derived from monocyte carriers. This cell-based delivery system exploited not only the homing capacity but also biological changes of monocytes as an off–on switch to realize tumor-specific drug transport and release.

Researchers also took advantage of cues in disease-associated microenvironment to realize stimuli-responsive drug release. Chang et al. loaded polymeric nanoparticles with astaxanthin (AST) and trametinib (TRA) and modified this nanomedicine with matrix metalloproteinase-2 (MMP-2) cleavable peptide, which was overexpressed in idiopathic pulmonary fibrosis (IPF) nidus, for pathology-responsive drug release.\[^{54}\] They collected peripheral monocytes from mice of IPF to isolate and obtain multipotent cells, which possessed homing capacity toward damaged lung areas and participated in lung normalization. Then, they adhered to the as-designed nanomedicine onto cellular surface through interaction with CXCR4 on the multipotent cells. Upon active homing toward injured lung tissues, both the laden drugs and the carrier multipotent cells would elicit therapeutic effects and synergized with each other to reverse IPF.

The strategy of combining cell-based delivery system with enzyme-mediated catalytic reactions to trigger drug release provides intelligent responsiveness toward different stimuli, including pathological changes in microenvironment and natural immune cells recognition and activation. However, considering that current studies mostly utilized enzymatic reaction to realize drug release from synthetic particles rather than live cell carriers, there are some dilemma that needs to be noticed and settled. Intracellular enzymatic catalysis does not directly solve the problem of transporting drugs from intracellular compartment of live cell carriers to extracellular environment for acting on target cells. This problem may require the extracellular secretion activity or artificial destroy of live cells carriers. Meanwhile, extracellular enzymatic activity-mediated drug release requires the conjugation and retention of laden drugs and cargoes on the surface of live cell carriers. This is to some extent difficult and requires technical live cell modification, as live cells possess phagocytic activities, especially for monocytes and macrophages, which are important components of the mononuclear–phagocyte system.

Xu et al. have utilized ultrasound-mediated rupture to realize drug release from macrophage carrier (Figure 3G).\[^{55}\] Chemotherapy DOX and perfluoropentane (PFP) were loaded into hollow mesoporous organosilica nanoparticles (HMONs), which were further internalized into macrophages for active homing to tumor tissues. PFP functioned as an ultrasonography imaging-agent for tracking macrophage migration, and evaporated to disrupt macrophage vehicle upon ultrasound sonication for releasing chemotherapy drugs. This method provided both real-time ultrasound imaging capability as well as on-demand controllability with spatial precision.

By harnessing the phenomenon that macrophages can phagocytize and excrete oligonucleotides into the surrounding environments, Wayne et al. utilized macrophages to delivery small interfering RNA (siRNA) for inhibiting breast tumor growth.\[^{56}\] They utilized several coincubation models to demonstrate the feasibility of siRNA horizontal transfer from macrophages to cancer cells. They further studied the siRNA transfer dynamics, and explored the associations between the transfection efficacy in cancer cells and the phenotypes of macrophages. They found that the alternatively activated macrophages (also called M2...
macrophages) were better gene carrier for transferring siRNA to cancer cells, due to their special activities in siRNA uptake and enhanced exosomal secretion for siRNA release. This study linked the siRNA transfer efficacy to phenotypes of macrophages and provided a special insight in the field of excavating underlying functionalities of macrophage carriers. Guo et al. directly loaded DOX with macrophages via electrostatic and hydrophobic interactions for treating metastatic ovarian carcinoma.\(^{57}\) They discovered that the classically activated macrophages, also known as proinflammatory M1 macrophages, can form tunneling nanotube network between themselves and cancer cells. This specific nanotube functioned as a drug transportation expressway, facilitating ultrafast transfer of the laden drug DOX from cell carrier toward cancer cells. In addition, relying on the intrinsic recognition of cancer cells by macrophages, this delivery system display preferable intelligent cancer cell-specific drug delivery capacity. We can notice from these studies that heterogeneity of macrophages can provide new delivery methods and strategies. Whether M2 macrophage, M1 macrophage, or macrophage of other subsets can provide the most satisfactory delivery outcome is uncertain. It seems that they are competent in delivering different types of cargoes, because their functionalities and biological behaviors display diversity and huge otherness, which also stimulate great interests and efforts in this field. Current studies in this field are far more enough, and the underlying mechanisms and potentials remain unclear. Exploiting the heterogeneity of monocyte and macrophage can be challenging meanwhile promising for developing advanced delivery system.

As monocytes and macrophages are professional phagocytes, unlike neutrophils that can form NETs for unloading cargoes, it is necessary to solve the problem of drug release when using monocyte/macrophage carriers. We summarized from recent studies that there are about four strategies to circumvent this dilemma: 1) restrict the laden cargoes on the cellular surface outside live cells and exploit environmental cues to trigger drug release, 2) take advantage of cell–cell contact to realize cytotoxic agent transfer, 3) excavate the cell-derived microvesicles and exosomes to transmit drugs, and 4) utilize external stimuli such as ultrasound to disrupt cellular carriers. These strategies are also adaptable for other types of cell carrier.

### 2.4. Lymphocyte Carriers

Trafficking of lymphocytes, including B lymphocyte and T lymphocyte, is important for integrating and controlling the systemic immune responses and homeostasis.\(^{58}\) The homing process of lymphocytes is also regulated by inflammatory and homeostatic chemokines.\(^{59}\) In terms of chemokine receptors, lymphocyte is the most complex group of leukocytes and can differentiate into multiple subsets in response to specific microenvironment. Researchers have taken advantages of the diversity of lymphocytes to generate cellular vehicle with specific phenotype for on-demand drug delivery. Rubner and co-workers synthesized functional polymer patches as cellular backpack to load on both B lymphocytes and T lymphocytes.\(^{60}\) They demonstrated that these cellular patches did not alter the viability and migration capacity of live lymphocytes, while could serve as a platform for monitoring their homing to lymphoid tissues, tumors, or infectious sites. In addition, these patches could be doped with magnetic nanoparticles for manipulating lymphocytes’ in vivo distribution. While lately, they discovered that the primarily freely suspended synthetic backpack can also lead to lymphocytes’ aggregation, which was dependent on the ratio of cell-to-backpack and the size of the synthetic backpack.\(^{61}\) This can be a hindrance that makes it difficult to prepare homogeneous cell-based delivery system, whereas it can be taken as an opportunity for regulating the functional aggregation of cells.

Irvine and co-workers developed several types of cellular backpack loaded onto T lymphocytes for tumor-tropic chemotherapy drug delivery and T cell-mediated immunotherapy.\(^{62-65}\) Specifically, they generated a type of activated T cells, which can efficiently target lymph node, for drug loading and active targeting toward lymphoma.\(^{63}\) Considering that during activation, T cells usually downregulated lymph node homing molecules, such as CD62L and CCR7, and reduced lymph node-tropism through mTOR signaling, researchers expanded T cells in vitro via IL-2 stimulation and retained their targeting moiety through the mTOR inhibitor rapamycin to inhibit mTOR signaling. Liposome-based nanocapsules loaded with SN-38, an active form of camptothecin derivative, were conjugated to the surface of these lymph node-homing T cells via maleimide-thiol interaction with free cell surface thiol groups. The authors demonstrated enhanced delivery efficacy and therapeutic performance by T cell-mediated delivery strategy than free SN-38 and free nanocapsules loaded with SN-38. This study indicates that specific migration patterns of lymphocytes can be manipulated through targeting relevant signaling pathways. Further study using this strategy can stem from exploiting the basic understanding of lymphocytes’ migration, and may provide multifarious cell-based delivery modalities.

Addressing the problem of drug release, researchers also exploited cellular processes like enzyme catalysis of lymphocytes to realize specific event-responsive drug release. For example, Irvine and co-workers linked immunostimulatory drugs release to T-cell activation for strengthening T-cell functions in vivo.\(^{66}\) It is noteworthy that biochemical processes in live cells can be taken advantages for other purposes more than drug release, such as conversing prodrug into active forms. Our group has reported a simply modified lymphocyte without genetic intervention for tumor elimination.\(^{66}\) Herein, both tumor-targeting as well as biosynthesis capacity of lymphocytes were taken advantage to promote in vivo therapeutic efficacy of the clinically used drug molecule, δ-aminolevulinic acid (δ-ALA). First, microfluidic device was used to incubate lymphocytes with δ-ALA and to isolate δ-ALA-loaded lymphocytes [Figure 4A].\(^{66}\) After ex vivo loading with δ-ALA, the adoptively transferred lymphocytes generated antineoplastic drug PpIX through a series of enzymatic reactions and delivered the activated drug to tumor tissues (Figure 4B).\(^{66}\) Upon light irradiation, these engineered lymphocytes underwent apoptosis and disassembled into smaller vesicle, which is apoptotic body loaded with PpIX. The generated apoptotic body facilitated further penetration deep into tumor tissues and improved internalization of PpIX by cancer cells. In this study, by taking advantages of the biochemical processes of lymphocytes, therapeutic agent experienced two transformation stages: 1) inactive prodrug δ-ALA to active photosensitizer
PpIX and 2) PpIX trapped in cells to PpIX loading in penetrable small vesicles.

As lymphocytes possess potent immunological functions including cell-mediated cytotoxicity and cytokine/antibody production, major efforts in studying lymphocytes have been directed to developing advance cell-based medicine and immunological therapeutics. Strategy of lymphocyte-based delivery system for drug and synthetic materials should combine with the novel cell therapy for improving the clinical translation of these bio-hybrid systems.

### 2.5. Stem Cell Carriers

Stem cells have many detailed classifications, and generally represent cells with self-renewal and differentiation capacities. Hematopoietic stem cells (HSCs) and mesenchymal stem/stromal cells (MSCs) that have been reported for clinical usage quite early before.[67,68] In recent years, stem cells and stem cell-based carriers have been applied in treatments for not only endogenous injury but also tumors.[69] Therapeutic stem cells also display tumor tropism,[70] therefore, they are currently taken as “Trojan horses” for delivering therapeutic agents toward malignant tumors. The tumor tropism of stem cell usually depends on their surface receptors, which sense the gradients of chemokine and cytokine, leading stem cells migrate toward tumor tissues that released multiple chemotactant. For example, Luo et al. utilized MSCs as carriers to internalize and deliver biocompatible poly(lactic-co-glycolic acid)/black phosphorus quantum dots (PLGA/BPQDs) toward subcutaneous glioma tumor for photothermal therapy.[71] This migration capacity relies on the surface receptor CXCR4 of MSCs, which directs MSCs to migrate toward tumor tissues that release high concentration of chemokine such as stromal-derived factor 1. The authors demonstrated the enhanced maintenance of PLGA/BPQDs within MSCs in vitro and improved retention within tumor tissues in vivo through using MCSs carrier. In addition to active tumor targeting, the authors also noted that the intravenously injected MSCs tended to be trapped in lungs.[71] This phenomena has also been demonstrated and exploited for lung cancer-targeted delivery by other researchers.[72]

Shah and co-workers have demonstrated that MSCs can home to tumor metastasis in brain, and have taken this property for delivering bioagent oncolytic virus.[73–76] The authors adjusted the infection ratio of oncolytic herpes simplex virus (oHSV) to MSCs to obtain the optimal delivery system MSC-oHSV.[75] They found that with a multiplicity of infection value of 1, MSC-oHSV can survive more than 4 days meanwhile displayed better oHSV loading capacity. Through internal carotid artery injection, MSC-oHSV can reach metastatic melanoma tumor in brain as early as 24 h after injection, and continuously recruited during subsequent 4 days. However, the authors also found that MSC-oHSV mostly accumulated in the lung via intravenous injection. This result suggests that the injection method and location will influence the biodistribution of reperfused cells.

To solve the problem of stem cells’ lung residence and reprogram the in vivo biodistribution, Liu et al. have developed a bispecific antibody termed PT-BsAbs to realize in situ conjugation of HSCs and platelet.[76] The authors modified the platelet binding ligand CD42b with tetrazine (TZ)–polyethylene glycol–NHS to obtain CD42b-TZ, and also conjugated the HSCs targeting moiety CD34 with trans-cyclooctene (TCO)–polyethylene glycol–NHS to generate CD34-TCO. Through TZ and TCO conjugation, CD34 and CD42b were linked to provide bispecific PT-BsAbs. Upon inhalation, PT-BsAbs mostly accumulated in the lungs and simultaneously targeted HSCs and platelets and linked these two cells together. Thereafter, platelets can help direct HSCs toward endogenous injured sites and help improve the treatment of myocardial infarction. Using platelets to improve stem cells’ biodistribution is also reported by Gu and co-workers.[77]
Zhang et al. also reported a nanomaterial-based strategy to improve the targeting capacity of MSCs. They devised a magnetosome-like 1D ferrimagnetic iron oxide nanochains (MFIONs) through self-assembly method. The as-designed MFIONs are more favorable for cellular uptake, and thus improve gene transfection in stem cells. In addition, with higher cellular uptake, MFIONs can release high concentration of ferrous iron to stimulate upregulation of homing-receptor CXCR4 in MSCs, thereby improving the accumulation and therapeutic performances of MSCs within injured brain. Furthermore, there are other research also utilized superparamagnetic iron oxide nanoparticle to integrate with stem cells for magnet-guided targeting.

Kim et al. modified human MSCs (hMSCs) with dibenzyclooctyne–polyethylene glycol (PEG)–pheophorbide A conjugates (DPP) through click chemistry for active delivery toward tumors and PDT. The authors demonstrated that after PDT stimulation, hMSCs-DPP can release proinflammatory cytokines like heat shock protein 70 and induce antitumor immune response. This study also prompted another advantage of stem cell-based carrier, which is the cell death of this cellular vehicle can be exploited for activation of immune responses and may provide therapeutic benefits. In addition, it is noteworthy that, in comparison with free nanoparticles DPP, hMSCs improved the final concentration of DPP in tumor tissues, which took a longer time of about 2−4 days. This result suggests a feature of stem cell carrier, which is, it may take a longer time for stem cell carrier to realize higher accumulation at tumor tissues than nanoscale delivery system. And this feature can be a challenge and needs delicate construction to insure longer retention as well as benign integrity of loaded cargoes in stem cell carrier.

In summary, stem-cell-based carrier possesses active migration capacity via sensing the gradients of chemokine and cytokines, thus can be taken as vehicle for delivering drugs and bioactive therapeutics and targeting toward injured tissues and tumors. Stem cell-based carrier display several preferable characters, including therapeutic potential for regenerative medicine, programmable targeting pattern, low immunogenicity, and benign biocompatibility. Recent studies also excavated the potential of PDT-induced cell death of stem cell carrier to strengthen antitumor immune responses. And there are several features for stem cell carriers, which in some condition can be troublesome and can be taken as new opportunities via clever strategy as well, including: 1) the injection method and transplantation location can significantly alter the in vivo distribution of the perfused stem cells; 2) effective targeting of stem cell usually requires more than 24 h; 3) intravenously injected stem cells tend to trap in lung and liver. These features can lead to off-target accumulation and unwanted consumption of loaded drugs within cellular lysosomes before reaching targets, thus lowering the bioavailability and therapeutic effects.

2.6. Platelet-Involved Delivery System

Platelets are anucleate cells produced by megakaryocytes and they are famous for their hemostasis and wound healing functions. Taking advantages of their recruitment and adhesion to the injured blood vessel and the exposed extracellular matrix, researchers have excavated platelet-based delivery systems. Gu and co-workers conjugated platelets with anti-PDL1 antibodies to target tissues that received surgical resection of tumor for preventative treatment. Upon reaching surgical section, platelets elicited activation process and generated platelet-derived microparticles, which were derived from plasma membrane of platelets and inherited the surface-decorated anti-PDL1 antibodies. This cell-derived microvesicle facilitated accessibility of laden antibodies to cancer cells. Li et al. exploited another biological process during platelets’ activation to realize drug release. They loaded platelets with interferon-gamma-induced protein 10 (IP10) through endocytosis and found that most of the laden proteins restricted in intracellular α granules. Upon reaching to melanoma tissues with leaky vasculature and overexpressed thrombin, platelets would aggregate and activate to release α-granules containing IP10 for subsequent recruitment of effector immune cells for tumor immunotherapy.

Apart from carrying drugs and particles, researchers have demonstrated the potential for directing live cells toward injured tissues by platelets. Tang et al. fused cardiac stem cells with platelet nanovesicles ex vivo, utilizing platelets to promote the in vivo targeting, retention, and engraftment efficacy of cardiac stem cells for treating myocardial infarction injury. It is worth noting that in another work from Gu and co-workers, which also developed a stem cell–platelet combination cellular delivery system, HSC directed the targeting and retention of antibody-decorated platelets toward bone marrow nidus for treating leukemia. Whether it is platelet or stem cell that will determine the in vivo biodistribution of the bio-hybrid system may be affected by many factors such as the amount and types of surface receptor and cell–cell incorporation ratio. Thus, the underlying mechanisms and fundamental principles in designing dual cell combination delivery systems and cellular therapeutics remains an unknown realm waiting for further exploration.

Tang et al. developed a Janus-like platelet micromotor by immobilizing urease asymmetrically on the surface of natural platelets. This platelet micromotor preserved intrinsic targeting potential toward cancer cells and pathogens, whereas with the help of artificial intervention, it also possessed autonomous self-propulsion capacity in the presence of biological fuel. By catalyzing decomposition of urea, the products ammonia and carbon dioxide were generated asymmetrically and thus formed a concentration gradient to push platelet micromotor forward. The cell-based micromotor strategy endows cell carrier with an external driving force that may satisfy additional delivery purposes such as penetration deep into solid tumors or crossing through biological barriers in brain and intestinal tract.

2.7. In Vivo Cell Hitchhiking

Ex vivo live cell engineering usually encounter challenges such as difficult harvesting and separation process as well as complex manipulation procedure. Several studies also reported the in vivo hitchhike strategy for taking advantage of cellular migration. Except from ligand–receptor interaction, some studies exploited the capacity of monocytes/neutrophil in clearing foreign materials, pathogens, and dead cell debris for successful particle internalization into circulating immune cells in vivo. Li et al.
cloaked nanoparticles with bacterial outer membrane vesicles to obtain nanopathogenoids for antitumor chemotherapy and photothermal therapy, which were easily recognized and taken up by neutrophils in peripheral blood (Figure 4C).[90] These neutrophils then actively transported laden nanoparticles to tumor tissues in response to chemokine gradient, sacrificed, and formed NETs for release of nanoparticles. In this study, transporter neutrophils provided an intelligent responsive release function for drug delivery, which is usually an unsolved problem in the field of cellular delivery system. Tan and co-workers exploited apoptotic body derived from dead cells to facilitate cellular uptake of therapeutic nanomedicine by inflammatory monocytes in vivo, realizing deep penetration into tumor tissues.[91]

It is worth noting that in the in vivo hitchhiking strategy, the interaction between injected particles and internal cells is still not clearly explored and needs further investigation.[92,93] Aizik et al. adjusted quantum dots with functionalized liposomes for active delivery toward tumors via hitchhiking monocytes in vivo, evaluating the influence of liposome modification on in vivo cell adhesion.[92] Fromen et al. explored the associations between neutrophils and intravenously injected carboxylate-modified particles of both 2 and 0.5 μm.[93] They found that particles’ adhesion onto neutrophils would alter the traffic patterns of neutrophils in acute inflammation, resulting in neutrophils’ liver-targeting division and function switch toward particle clearance. Improving the understanding of interplay between laden particles and cell carriers may provide more opportunities in updating both nanomedicine and cell-based delivery system for disease treatment.

To sum up, constructing bio-hybrid delivery systems can be achieved through ex vivo cell engineering or in vivo cell hitchhiking. Ex vivo cell engineering usually contains three steps: 1) cell carrier collection and expansion, 2) drug and particles encapsulation with live cells, and 3) in vivo reperfusion of the as-obtained bio-hybrid systems. In the second step, the operation can be either simple or complicated, depending on the cell types and the construction methods. Regarding chemical modification, anucleate cells are preferable candidates, as eukaryotic cells are vulnerable to cytotoxic chemical agents. Phagocytes like monocytes and macrophages can easily uptake particles for drug loading, but cellular internalization in turn creates barriers for drug preservation and release. Cells with less-active phagocytosis activity, such as lymphocytes, are better objects for cellular surface modification, providing opportunities for constructing responsiveness toward extracellular cues and environmental pathologic conditions. Comparing with ex vivo manipulation, in vivo cell hitchhiking strategies is relatively simple and convenient in terms of procedure, which usually includes preparation of therapeutics with cell-adhesive molecule and in vivo administration. Considering cell-binding specificity, the strategy of in vivo cell hitchhiking encounters huge uncertainty and low efficacy. Complex internal environments and shared surface proteins by distinct cells may cause significant off-target hitchhiking. In addition, the homogeneity, chemical compositions, and physical characters of injected particles also affect their in vivo cell-binding efficiency and final biodistribution. Precise technology in therapeutic particle synthesis together with advance in live cell trafficking imaging are needed for improving the translational potentials of in vivo hitchhiking.

3. Clinical Trials of Cell-Based Delivery System

Some clinical trials have taken advantages of natural cells for targeting delivery. We have shown some clinical studies on the topic of cell-based delivery in Table 1.

Advanced bio-hybrid pharmaceutics taking advantages of RBC-based carrier have been developed and promoted into clinical trials. Some of these RBC carriers have reached phase III study. For example, the EryDex System (EDS) utilizes autologous RBCs for encapsulating dexamethasone sodium phosphate (DSP) and sustained release in vivo. Dexamethasone can freely infuse into RBCs due to its chemical structure, which makes it hard to retain dexamethasone within RBC as well. Non-diffusible dexamethasone 21-phosphate has been developed and can be catalyzed into therapeutic form by resident enzymes within RBCs.[94] Through this modification strategy, the dexamethasone release behavior of EDS can be detected as long as 35 days post the single intravenous injection.[94] This bio-hybrid delivery system is designed for improving the neurological symptoms and treatment of ataxia telangiectasia, and its biosafety and scientific validity have been widely evaluated in clinical trials (ClinicalTrials.gov: NCT01255358, NCT02770807, and NCT03563053). The biosafety and pharmacokinetic of EDS have also been tested in healthy volunteers (ClinicalTrials.gov: NCT01925859 and NCT02380924). In addition, RBC-mediated delivery of dexamethasone and its derivatives have been also applied in human patients for treating other diseases, such as pulmonary disease,[95–97] bowel disease,[98–100] and Crohn’s disease.[101,102] Clinical trials have also explored the capacity of RBCs to deliver bioactive enzymes for intervention in metabolic disease and cancer, including phenylalanine ammonia lyase (PAL), thymidine phosphorylase, and i-asparaginase.

Deficiency in conversion of phenylalanine into tyrosine can lead to systemic accumulation of phenylalanine and serious phenylketonuria. PEGylated recombinant PAL had been adopted for daily administration into patients with phenylketonuria, which significantly reduced the concentration of phenylalanine in blood but accounted for severe adverse immune effects in clinical trial.[103] To improve the biocompatibility, the company Rubius Therapeutics has developed a drug named RTX-134, consisting of RBCs expressing recombinant Anabaena variabilis phenylalanine ammonia (AvPAL). Clinical trial (ClinicalTrials.gov Identifier: NCT04110496) aims at examining the biosafety, tolerability, preliminary dosage, and pharmacokinetics of RTX-134 is currently under conduct.

High concentrations of thymidine and deoxyuridine can lead to autosomal recessive disorder, also known as mitochondrial neurogastrointestinal encephalomyopathy (MNGIE). Thymidine phosphorylase can catalyze the phosphorylation of these detrimental metabolites into normal products like thymine and uracil, respectively. Several studies have preliminarily demonstrated the therapeutic potential and preferable dosage of erythrocyte encapsulated thymidine phosphorylase (EE-TP) for MNGIE treatment.[104–107] The nucleoside transporter ENT1 on cellular surface of RBCs facilitates diffusion of the harmful substrates and thus improves the catalytic outcome. Clinical trial (ClinicalTrials.gov: NCT03866954) with aims of evaluating the
Early studies have demonstrated the biosafety and effectiveness of blood RBCs loaded with L-asparaginase. Currently, researchers and doctors collect white blood cells from patients’ blood through leukopheresis, and load the obtained cells with CMV pp65-lysosomal-associated membrane protein (LAMP) messenger ribonucleic acid (mRNA). The engineered autologous cells can synthesize CMV pp65 protein modified with a LAMP moiety. The LAMP moiety can direct the translocation of CMV pp65-LAMP protein into lysosomes, thus promoting the antigen presentation process and CMV pp65-targeted immune response. Clinical trials are majorly evaluating the therapeutic efficacy of CMV pp65-LAMP mRNA-loaded cells in combination with radiotherapy and chemotherapy. In addition, a DC vaccine biological product named TriMix-DC has been developed and its therapeutic potentials are still under clinical examination (ClinicalTrials.gov Identifier: NCT00723346, NCT01518517, NCT01523808, NCT02195180, NCT02195180, NCT03267030, NCT01810705, NCT00723346, and NCT03866954).

Tumor cells, especially lymphatic tumor cells, require huge amount of L-asparagine to satisfy their growth. However, unlike normal cells, tumor cells are unable to synthesize L-asparagine by themselves and this character has been taken as therapeutic target using L-asparaginase for tumor suppression. Early studies have demonstrated the biosafety and effectiveness of blood RBCs loaded with L-asparaginase. Currently, based on encapsulating L-asparaginase into RBCs, several therapeutic products have been developed and promoted into clinical trials of different stages, including GRSPA (ClinicalTrials.gov Identifier: NCT01518517, NCT00723346, NCT01523808, NCT01523808, NCT01523822, and NCT01810705), Eryaspase (ClinicalTrials.gov Identifier: NCT02195180), and Eryaspase/ERYtech Pharma (ClinicalTrials.gov Identifier: NCT03674242 and NCT03665441) for treating leukemia, pancreatic cancer, and breast cancer, respectively.

Monocyte and DCs that are loaded with synthetic gene products and express cytomegalovirus (CMV) protein have been utilized in clinical trials (ClinicalTrials.gov Identifier: NCT04741984 and NCT03686878) for treating malignant glioblastoma that express special antigen CMV pp65 protein. Specifically, researchers and doctors collect white blood cells from patients’ blood through leukopheresis, and load the obtained cells with CMV pp65-lysosomal-associated membrane protein (LAMP) messenger ribonucleic acid (mRNA). The engineered autologous cells can synthesize CMV pp65 protein modified with a LAMP moiety. The LAMP moiety can direct the translocation of CMV pp65-LAMP protein into lysosomes, thus promoting the antigen presentation process and CMV pp65-targeted immune response. Clinical trials are majorly evaluating the therapeutic efficacy of CMV pp65-LAMP mRNA-loaded cells in combination with radiotherapy and chemotherapy. In addition, a DC vaccine biological product named TriMix-DC has been developed and its therapeutic potentials are still under clinical examination (ClinicalTrials.gov Identifier: NCT00723346, NCT01518517, NCT01523808, NCT02195180, NCT00723346, and NCT03866954).

As for stem cells, clinical study has reported their active migration capacity toward sites of inflammation (ClinicalTrials.gov Identifier: NCT03674242 and NCT03665441).
Taking advantages of the active migration capacity and intrinsic therapeutic potentials (such as reducing inflammation and promoting tissue repair) of adult human MSCs, a biological drug named PROCHYMAL has been applied for treating various diseases in clinic, including Crohn’s disease (ClinicalTrials.gov Identifier: NCT00482092 and NCT01233960), myocardial infarction (ClinicalTrials.gov Identifier: NCT00877903), diabetes (ClinicalTrials.gov Identifier: NCT00690066), graft-versus-host disease (ClinicalTrials.gov Identifier: NCT00136903), pulmonary disease (ClinicalTrials.gov Identifier: NCT00683722). These studies reperused ex vivo-cultured MSCs, which do not load with any drugs or cargoes, into patients through intravenous injection. Therefore, the localized therapeutic performance largely relies on the natural migration and biodistribution of stem cells. Up to now, most of the clinical studies on the topic of stem cell therapy major in evaluating its safety and efficacy, and stem cell-based carriers are still in preclinical study. We speculate that only when the effectiveness and safety of stem cell therapy are fully proved, the research of stem cell-based carrier will be promoted to clinical trials.

T lymphocytes, especially chimeric antigen receptor T (CAR-T) cells, arouse wide research interest in recent years due to their significant therapeutic benefits toward malignant leukemia. Similar to stem cell, most of the currently developed clinical studies are still majoring in evaluating the safety, efficacy, as well as persistence of CAR-T, and there is almost no ongoing clinical report on the topic of T lymphocyte-based drug delivery systems. The targeting capacity and specificity largely depend on the artificially engineered receptors of CAR-T cells, which are of a great variety. The CD19/CD20/CD22 CAR-T for leukemia and lymphoma are the most widely studied types of CAR-T cells in clinical trials (ClinicalTrials.gov Identifier: NCT04029038, NCT03097770, NCT04169932, and NCT04715217). Most of the ongoing clinical studies major in the treatment of hematological malignancies and have demonstrated some therapeutic significance. Up to now, there are a few completed clinical trials had explored the therapeutic potential of CAR-T toward solid tumors (ClinicalTrials.gov Identifier: NCT02107963 and NCT02761915). It is widely believed that the tumor immunosuppressive micro-environment is one of the most important factors to inhibit the proliferation and infiltration of immune cells within tumors, thus impairing therapeutic outcome of CAR-T.[115,116] To improve the abundances of CAR-T cells, direct intratumoral injection is usually adopted, displaying significant tumor suppression in some cases while transient therapeutic effect in some patients.[117–119] Modulating the immune microenvironment of solid tumors may be a key point and effective method to solve this problem. To this end, the fourth-generation CAR-T cells which can produce cytokines like interleukin 7 for strengthening T cells’ proliferation and infiltration within tumors.[120] This strategy has been promoted into clinical trials for treating both solid tumors and hematological malignancies (ClinicalTrials.gov Identifier: NCT03932565, NCT04833504, NCT04381741, and NCT03778346). Along this line, using the strategy of cellular backpack as well as T-cell-based carrier are mostly adopted in preclinical studies and, as far as we know, have not been promoted to clinical trials, which is worth looking forward to. Currently, most of the clinical trials and much efforts have been made to improve the understanding of the safety profile, efficacy, and in vivo behaviors of reperfused cells. Apart from RBCs, bio-hybrid drug delivery systems, which rely on immune cells of other types, are still in their infancy and mostly being explored in preclinical studies.

4. Conclusion

Bio-hybrid systems based on natural living cells have been exploited for drug delivery through ex vivo modification or in vivo hitchhiking since the end of 20th century.[121] The strategy of cell-based bio-hybrid delivery system mainly relies on the following biological foundations: 1) benign biocompatibility and low immunogenicity; 2) intrinsic biodistribution and tropism patterns; and 3) special interaction and behavior upon disease recognition, which are widely explored and exploited in recent studies. With the expectation that natural cells can help promote the localized drug delivery and participate in disease recovery, researchers have made many efforts in organically integrating synthetic compounds with living cells to develop intelligent delivery systems. On one hand, synthetic materials functions as drug carriers for improving loading and preservation within live cells, meanwhile they can endow natural cells with additional functions and artificial controllability by light/ultrasound. On the other hand, intrinsic functions more than active homing and migration of live cells can also help achieve drug release and improve drug delivery specificity. Special biological activities such as intelligent recognition, activation, exocytosis, enzymatic reaction, biosynthesis, cell-to-cell contact, and cellular synapse have been excavated. However, natural cells do not always participate in the rehabilitation of diseases. Sometimes they also intervene in the negative regulation of diseases and play an unexpected bad role.[122,123] For example, cytokine release syndrome is a frequently observed side effect in patients receiving lymphocytes perfusion, which can bring life-threatening effects in severe cases.[124,125] It is reported that peptidyl arginine deiminase 4 (PAD4) released by neutrophils can generates autoantigens, and NETs help present these autoantigens together with danger signals and induce severe autoimmune.[126] With the help of platelets, NETs can also form a scaffold for thrombosis development and even provoke inflammation in some pathological conditions.[127] For tumor treatment, many types of intrinsic cells display plasticity upon reaching tumor microenvironment and may promote the development and metastasis of tumors.[128–131] It is of vital importance to further evaluate the site effects and pro-tumoral potential of the cell-based delivery systems.

The strategy of cell membrane coating represents some superiorities in the field of drug delivery, taking advantages of cellular membrane receptors for on-demand targeting meanwhile circumventing the unwanted protumoral activities of living cells, which has been comprehensively introduced in other reviews.[132] Through this facile top-down method, synthetic carriers can be camouflaged to obtain biomimetic delivery system,
which inherits and concentrated the natural components of living cells for long-term circulation and site-specific targeting. However, in our point of opinion, cell membrane-based delivery systems mainly rely on passive delivery and cannot reproduce the special behaviors of living cells.

Considering this we suggest that future development and improvement of cell-based bio-hybrid delivery system can be directed in these aspects: 1) indepth study of disease prognosis after perfusion of bio-hybridized cellular carriers; 2) novel strategy for leveraging the biological functions while circumventing the unwanted effects of living cell carriers; 3) construction of interactive behaviors between synthetic materials and natural cells. Even though current studies have provided insights in excavating interactions between synthetic and natural components in cell-based delivery systems, most of these studies constructed a relatively one-way regulation interplay. In the future, it is expected that more efforts in excavating synergistic interactions between biomaterial and live cells can advance the current bio-hybrid delivery system for disease diagnosis and treatment. We expect that synthetic biomaterials can complete the efficient introduction of complex logic control into bio-hybrid systems. Development of smart materials, live cell engineering technology, and in vivo imaging techniques, in combination with advances in the understanding of cellular biology have important implications for developing novel bio-hybrid systems.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

bio-hybrid, drug delivery, immune cells, live cells, synthetic materials

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bio-hybridized systems composed of synthetic materials and natural substances, including biomolecules.

Chu-Xin Li is now a M.Phil.-Ph.D. student at the College of Chemistry and Molecular Sciences at Wuhan University (China). She received her B.Sc. degree (2016) at Wuhan University under the supervision of Professor Xian-Zheng Zhang. Her research focuses on the design and construction of bio-inspired and bio-hybridized systems composed of synthetic materials and natural substances, including biomolecules and live cells, for biomedical applications.
Yong-Dan Qi is a M.Phil.-Ph.D. student under the supervision of Professor Xian-Zheng Zhang in the College of Chemistry and Molecular Sciences at Wuhan University (China). She received her B.Sc. (2018) from the College of Chemistry, Liaoning University (China). Her research focuses on the design and function of biomaterials for immunotherapy.

Xian-Zheng Zhang is a professor at Wuhan University (China). He received his Ph.D. (2000) in chemistry from Wuhan University, China. From 2000 to 2001, he joined Institute of Materials Research and Engineering in Singapore as a research associate. From 2001 to 2004, he had been a postdoctoral associate at Cornell University, USA. His research focuses on the design and synthesis of functional biomaterials and their biomedical applications.