Acta Pharmaceutica Sinica B 2020;10(6):979–986

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Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

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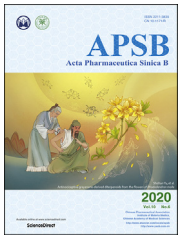

REVIEW

Delivery strategies for macromolecular drugs in cancer therapy

Qin Guo, Chen Jiang*

Key Laboratory of Smart Drug Delivery, Ministry of Education, State Key Laboratory of Medical Neurobiology, Department of Pharmaceutics, School of Pharmacy, Fudan University, Shanghai 201203, China

Received 6 August 2019; received in revised form 23 September 2019; accepted 9 December 2019

KEY WORDS

Macromolecular drugs;
Delivery strategies;
Cancer therapy;
Membrane-camouflage systems;
Exosomes

Abstract With the development of biotherapy, biomacromolecular drugs have gained tremendous attention recently, especially in drug development field due to the sophisticated functions *in vivo*. Over the past few years, a motley variety of drug delivery strategies have been developed for biomacromolecular drugs to overcome the difficulties in the druggability, *e.g.*, the instability and easily restricted by physiologic barriers. The application of novel delivery systems to deliver biomacromolecular drugs can usually prolong the half-life, increase the bioavailability, or improve patient compliance, which greatly improves the efficacy and potentiality for clinical use of biomacromolecular drugs. In this review, recent studies regarding the drug delivery strategies for macromolecular drugs in cancer therapy are summarized, mainly drawing on the development over the last five years.

Abbreviations: aPDL1, antibodies against PDL1; ChiP, multifunctional chimeric peptide; CHOL, cholesterol; CP, Cas9-sgRNA plasmid; CTCs, circulating tumor cells; CTLA4, cytotoxic T lymphocyte antigen 4; DDS, drug delivery systems; DOPE, dioleoyl phosphoethanolamine; DOTAP, (2,3-dioleoyloxy-propyl)-trimethylammonium; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; EMT, epithelial-to-mesenchymal transition; GOx, glucose oxidase; GRVs, glucose-responsive vesicles; LFA-1, lymphocyte function antigen-1; MDP, muramyl dipeptide; MFT, mifamurtide; NLR, domain-like receptors; PD1, programmed cell death protein 1; PDT, photodynamic therapy; PEG, polyethylene glycol; PEI, polyethylenimine; PAMAM, polyamidoamine; PGE2, prostaglandin E2; PMAPs, pathogen associated molecular patterns; RBC, red blood cells; rFljB, recombinant flagellin; TAT, human immunodeficiency virus-1 transcription activator; TLR, toll-like receptors; TME, tumor microenvironment; TRAIL, tumor necrosis factor related apoptosis-inducing ligand.

*Corresponding author. Tel./fax: +86 21 51980079.
E-mail address: jiangchen@shmu.edu.cn (Chen Jiang).

Peer review under responsibility of Institute of Materia Medica,Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association.

https://doi.org/10.1016/j.apsb.2020.01.009

1. Introduction

The field of macromolecular drug development (also referred as biologics, biomacromolecules, and biotechnology drugs) has grown rapidly over the past 20 years[1]. Progresses in biotechnology have contributed to the discovery and mass production of miscellaneous macromolecular drugs, such as DNA, RNA, peptides and proteins. As for applications in drug delivery, they can be applied as targeting component, active pharmaceutical ingredients, and even carrier material. According to U.S. Food and Drug Administration, macromolecules can be classified as vaccines, blood and blood products, allergen extracts for diagnosis and treatment (such as allergy vaccine injections), human cells and tissues for transplantation (such as tendons, ligaments and bones), gene therapy preparations, cell therapy preparations, and reagents for detecting infectious agents.

As drug candidates, macromolecules have attracted increased attention due to the unique affinity, satisfactory specificity and sophisticated functions[2] in the treatment of various diseases. Especially for cancer, which is a major public health problem around the world, while the world-wide incidence of cancer continues to increase.

However, a predicament to the development of macromolecule-based therapeutic delivery is the requirement for safe and effective delivery strategies. Macromolecules without modification are not stable in systemic circulation due to the degradation caused by nucleases (RNA and DNA) or proteolysis (peptides and proteins). Moreover, macromolecules cannot cross membranes lightly to enter cells on account of their big particle diameter. Thus, chemical modifications or delivery material protection are necessary to lead macromolecules to their sites of action avoiding adverse effects.

Herein, we provide a brief review of drug delivery systems (DDS) for macromolecular drugs in cancer therapy and focus on advances developing in the past five years. Firstly, according to patterns they effect on tumor treatment, macromolecular drugs will be classified into two generic groups: macromolecules which could be applied directly for tumor suppression, such as cytotoxic proteins or nucleic acids; and immunogenic macromolecules that kill malignant cells indirectly by activating the immune system, such as antigens or antibodies. Secondly, as shown in Fig. 1, some representative examples of DDS for macromolecules will be summarized. Finally, the existing challenges and prospects of macromolecule delivery for cancer treatment will be discussed.

2. Cytotoxic macromolecules

Macromolecular drugs which could directly inhibit tumor growth or kill malignant cells were widely used in cancer treatment. Miscellaneous macromolecules including oligonucleotides (such as siRNA or miRNA), long-chain nucleic acids (such as plasmid DNA), peptides and proteins (such as tumor necrosis factor related apoptosis-inducing ligand, TRAIL) have been developed in the past few decades. For the delivery of these macromolecules, our aim is to keep them stable under circulatory system, penetrate physiologic barriers effectively and accumulate more at the tumor site, in brief, as effective as possible. Corresponding to different

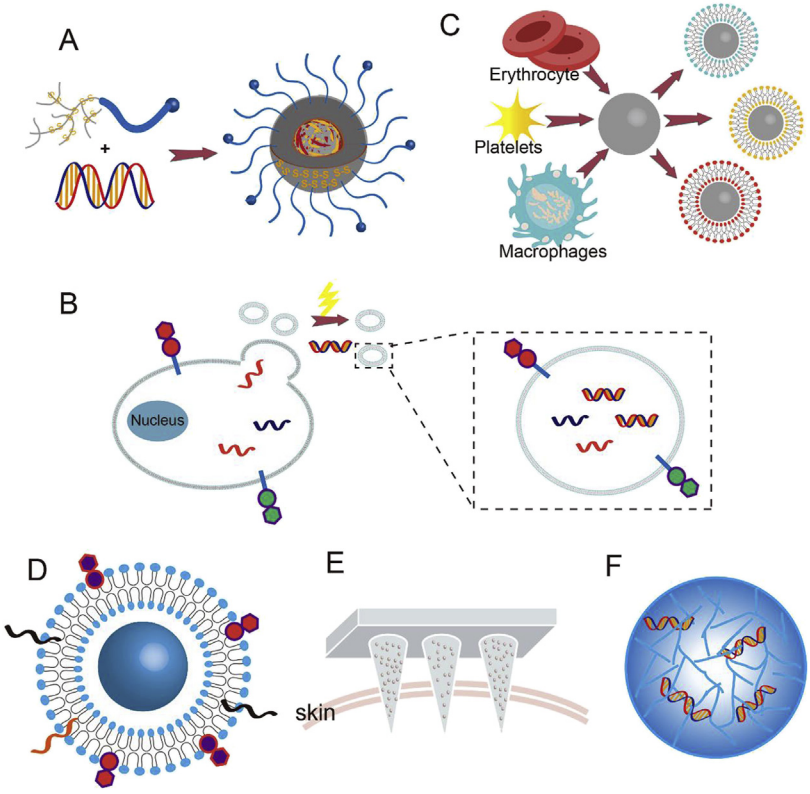

Figure 1 Novel drug delivery systems (DDS) for macromolecular drugs. (A) Cationic polymer-based delivery systems; (B) exosome-based delivery system; (C) membrane-camouflage systems; (D) lipid-based delivery systems; (E) smart patch delivery systems; and (F) nanogel-based delivery system.

properties of various macromolecules, diverse delivery strategies have their own characteristics.

2.1. *Cationic polymer-based delivery systems*

Oligonucleotides like siRNA and miRNA are anionic and hydrophilic small RNA. Their electronegativity and hydrophilicity prevent them from lightly penetrating biological membranes, which suggests that they need to be wapped in carriers to cross through cell membranes. Polycationic derivatives are the most welcome oligonucleotides carriers among non-viral systems since they can effectively condense oligonucleotides and deliver them into cells. Therefore, cationic polymer-based delivery systems have been investigated for the non-viral delivery of oligonucleotides specifically. In cationic polymer-based delivery systems, oligonucleotides are condensed within various kinds of polycationic derivatives such as polyethylenimine (PEI), cyclodextrin, and dendrimers to form nanoparticles. Furthermore, the surface of the nanoparticles is modified with polyethylene glycol (PEG) and targeting ligands.

2.1.1. *Cyclodextrins-based delivery systems*

Cyclodextrins are natural high polymer material can be applied to construct water soluble inclusion complexes with macromolecules, and involved in the first reported targeted siRNA delivery system of nanoparticles *via* systemic injection in humans named CALLA-01. This system was designed to inhibit tumor growth *via* reducing expression of the M2 subunit of ribonucleotide reductase. In this system, besides the cyclodextrin-based polymer and PEG, human transferrin was applied as targeting ligands[3].

2.1.2. *PEI-based delivery systems*

PEI is a commonly used synthetic cationic polymer for antitumor oligonucleotides delivery and commercially available as branched or linear formation in varieties of molecular weight. It has been applied as a golden standard polymer due to its high buffering capacity for endosomal escape of oligonucleotides, and ability to form small and compact nanoparticles with oligonucleotides due to its high cationic charge density. However, PEI with high molecular weight has several weaknesses such as non-degradability and high cytotoxicity *in vivo*. Thus, degradability is the most critical feature because it can reduce cytotoxicity by degrading the polymers into small molecules which are easily eliminated by *in vivo* excretion pathway. Jiang and colleagues[4,5] applied tumor cell degradable disulfide-bonded PEI for nanoball optimization and protecting the nanosphere (constructed by rolling circle transcription and containing plenty of RNAi sequences) from degradation by Dicer or other RNase in normal cells. This strategy has been explored for both siRNA[4] and miRNA[5] delivery in cancer therapy as shown in Fig. 2.

2.1.3. *Dendrimers-based delivery systems*

Dendrimers are artificial highly branched polymer with well-defined 3D nano-sized structure[6]. These unique structural properties, such as flexible size, modifiable terminal groups and favorable cargo encapsulate capacity, make them attractive as carriers for oligonucleotides delivery applications. Just like PEI, dendrimers like polyamidoamine (PAMAM) also have a high density of positive charge. The transfection efficiency of PAMAM is largely depends on the generation of the dendrimer[7]. In summary, G_3−G_{10} PAMAM dendrimers constitute more steady dendriplexes with oligonucleotides and the transfection efficiency raising with the rise of generations. Usually, PAMAM and oligonucleotides attract each other by electrostatic interaction, and form steady complexes, such as PAMAM−siRNA or PAMAM−miRNA. These “dendriplexes” showed us a high efficiency of transfection and a favorable capacity to preserve the miRNA or siRNA from degradation in battles with a variety of cancers[8−10].

As the most widely studied oligonucleotides carrier in cancer therapy, polycationic derivatives have their unique properties and advantages. They have shown excellent results *in vitro*, the future researches need to concentrate on achieving accurate delivery of siRNA to the malignant cells *in vivo*, avoiding off-target effects and immune responses.

2.2. *Exosome-based delivery system*

Cell-derived exosomes have been considered as a new therapeutic carrier for the *in vivo* delivery of functional macromolecules recently, especially oligonucleotides-like siRNA[11]. Exosomes can be favorable carriers for macromolecules because of their ideal biocompatibility and negligible biotoxicity due to the almost identical composition and structure with cytomembrane. Moreover, exosomes can penetrate in tissues deeply while escaping from immune surveillance, allowing them to deliver therapeutic drug to the cell directly[11]. Cancer cells[12−14], fibroblast cells[15,16], mesenchymal stem cells[17−19], astrocytes[20] and other cells[21] have all been reported to release exosomes. Exosomes from different sources can chase down their unique target cells or organs, making them desirable for targeting specific cells and tissues[22]. Diverse strategies have been explored for purifying exosomes from cell culture media or biological fluids, *e.g.*, gradient centrifugation (shown in Fig. 3). Therefore, exosomes emerge as favorable candidate carriers for therapeutics of cancer and other diseases. High drug loading efficiency, controlled or sustained release, unique tissue-targeting and protein protection against proteases can be achieved simultaneously through exosome.

Another important application of exosomes is loading short RNA sequences. Up to date, several non-specific methods for loading siRNA or miRNA into exosomes were developed, including electroporation[23,24], transfection methods, and passive diffusion of components. Studies of exosome-based strategies for short RNA sequences delivery are summarized in Table 1[23,25−31].

Furthermore, exosomes are not only applied for oligonucleotides delivery, their compliance to cytomembrane modification can be a highly desirable attribute to targeted macromolecule delivery. A common tactic for targeted exosome-based nanoparticles is ground on the utilization of genetically engineered cell lines expressing fusion proteins, which is mainly constitutive of extravesicle transmembrane domain and targeting domain[32]. For example, Yang and coworkers[33] developed the multifunctional chimeric peptide (ChiP)-modified exosomes for nucleus-targeted photodynamic therapy (PDT). The ChiP consisted of a C16 alkyl chain for exosomes modification, a photosensitive drug of PpIX for PDT and a peptide (PKKKRKV) for nuclear translocation. Significant tumor inhibition was confirmed in both *in vitro* and *in vivo* experiments.

2.3. *Membrane-camouflage systems*

As for many other macromolecular drugs expect oligonucleotides, burgeoning strategies have been explored. It is to be mentioned that, since first reported in 2011[34], technology of cell membrane

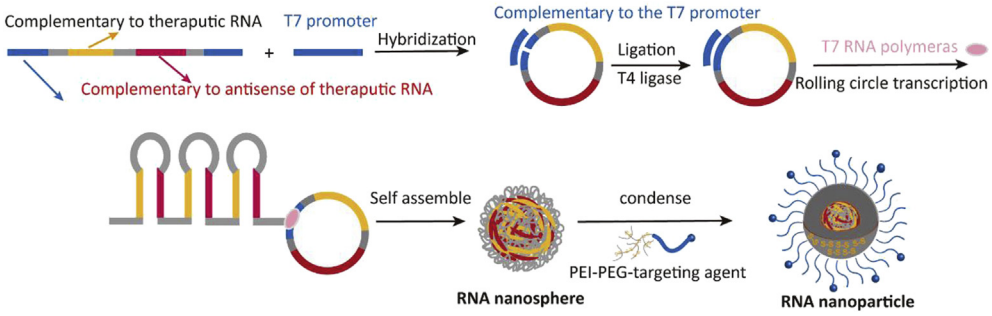

Figure 2 SiRNA and miRNA delivery in cancer therapy with the use of rolling circle transcription and PEI.

coating on nanoparticles have been applied into drug delivery systems in many studies to acquire miscellaneous advantages of natural cells (*e.g.*, biocompatibility, biodegradability, and non-immunogenicity)[35]. This rising delivery platform combines the benefits of natural cellular entities for long circulation and targeting abilities, and synthetic biomaterials for controlled drug retention, penetration and releases[36]. Various sources of cell membranes, such as erythrocytes[37–39], leukocytes[40,41], and subcellular platelets[42–44], have been applied to camouflage macromolecular delivery systems and different strategies have been investigated for cancer therapy specifically.

2.3.1. *Erythrocyte membrane camouflage systems*

As natural long-circulating delivery vehicles with limited immune cell clearance, erythrocyte or red blood cells (RBC) membrane was a satisfactory first choice for cell membrane coating onto nanoparticles[45–47]. At present, it is the most well-investigated in the field. The sharply development of this platform is partially due to membrane collection and purification of RBC, which is convenient for the lack of intracellular organelles in mature erythrocytes[47]. As for macromolecule delivery, RBCs are often applied to coat onto surfaces of synthetic nanoparticle-containing macromolecules[39]. Since interfacial interactions between natural RBC membranes and synthetic polymeric nanoparticles were well-examined, macromolecules such as aptamers, peptides and proteins, could be easily inserted on the RBC membrane-coated nanoparticle[48].

2.3.2. *Leukocytes membrane camouflage system*

Unlike erythrocyte, majority of leukocytes could make amoeboid movements, making them readily move to and from the vessels to the tissues[35]. Specifically, leukocytes could zealously be brought to gather around tissue with chronic inflammatory by the innate inflammation chemotactic ability, which also known as inflammation chemotaxis, providing promising potential for orthotopic therapies, especially for tumor treatment while tumor cells generate miscellaneous cytokines and chemokines which attract leukocytes[49].

A large part of tumor-associated leukocytes are macrophages. Zhang et al.[39] further tried to figure out the mechanism of macrophage membrane-lidded nanoparticles targeting tumor. In their opinion, inflammatory-associated receptors on cytomembrane was the accuse for tumor homing effect, because the blocking of lymphocyte function antigen-1 or CXCR1 and CXCR2 on the cytomembrane could effectively restrain the recruitment of nanoparticles by inflammatory tissue. Different from previous macrophage camouflage systems deliver macromolecular drug directly, Jiang and coworkers[41] applied macrophage as a unique bioreactor for effective tumor reservation and *in situ* manufacture of active macromolecular protein drug to affect nearby tumor cells. The antitumor transactivator-derived positively charged peptide (TAT) was integrated into the plasmid of TRAIL to penetrate the stroma barrier of carcinomatous matrix, and achieve the delivery of TRAIL to the deep site of tumor tissue.

2.3.3. *Platelet membrane camouflage systems*

Various receptors are found on the platelets to keep their "self-recognized" situation for prolonged circulation. Compared with bare systems, coated systems with platelet membrane could effectively prolong circulation time probably due to the transfer of membrane proteins such as CD47 as a "don't eat me" sign to reduce the phagocytosis of phagocytes[44]. Recent studies indicated that platelets played a pivotal role in tumor development and metastasis[50,51]. In tumor microenvironment (TME), platelets could be activated by tumor cells through secreting prostaglandin E2 (PGE2). Platelets release TGF-β in turn for epithelial-to-mesenchymal transition (EMT) impetus to induce tumor cell survival and metastasis. Furthermore, platelets could preserve circulating tumor cells (CTCs) from the immune surveillance and facilitate CTCs penetrating from vessels through reinforced adhesion of platelets with vascular endothelium cells[44]. Up till now, multiple strategies based on platelets have been investigated for the delivery of macromolecular drugs to kill or inhibit tumor cells and inhibit local recurrence or metastasis.

One example is that King and coworkers[52] developed silica particles coated with platelet membranes modified with TRAIL to target CTCs. This attempt could chase and hunt metastasizing malignant cells avoiding escaping from their TME. In another study, King and colleagues[41] uniquely remolded platelets to produce TRAIL by a genetic modification in hematopoietic stem cells. Since

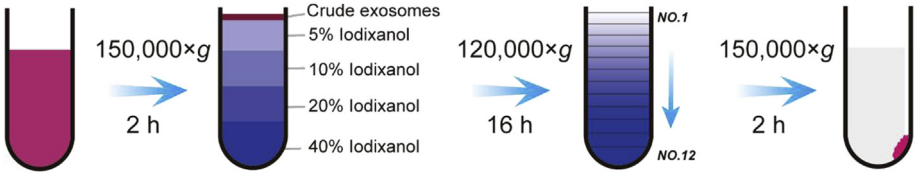

Figure 3 Schematic protocol of isolation and purification of exosomes by gradient centrifugation.

Table 1 Summary of exosome-based strategies for short RNA sequences delivery.

Cell source	Cell line	Cargo	Loading method	Disease/target	Ref.
Cancer cells	U87	Hydrophobically modified siRNA	Co-incubation	Huntington's disease	25
Epithelial cells	MCF-10A	CDK4 siRNA	Electroporation	Cancer	26
	HEK293	Luci−siRNA	HiPerFect reagent	Cancer	27
Mesenchymal stem cells	BM-MSCs	miRNA124	Gene engineering	Ischemic stroke	28
	hBMSCs	Anti-Fas receptor siRNA	Xfect transfection reagent	Pancreas islet	29
Dendritic cells	BM-DCs	Luci−siRNA	Electroporation	Central nervous system	30
	Bone marrow from C57BL/6 mice	BACE1−siRNA	Electroporation	Alzheimer's disease	23
Mechanocyte	NIH3T3	GFP−shRNA	Electroporation	Cancer	31

about 40% of the circulating platelets could express TRAIL after the genetic engineering, this system could significantly reduce the frequency and intensity of liver metastasis of prostate cancer. As certified in all the above studies, platelet membrane camouflage systems have a broad prospective in cancer treatment.

Prolonged lifetime in circulation and ability to selectively targeting are two biggest benefits we could obtain from membrane camouflage systems. Future researches could draw more attention to controllable macromolecule release which is also critical for an ideal drug delivery system in cancer therapy.

2.4. *Artificial lipid-based delivery system*

Artificial lipid-based delivery system such as liposomes are the most regular and well-studied nanocarriers for macromolecular drug delivery because they could be advantageous in biomedical applications for stabilizing active compounds, penetrating barriers and ameliorating biodistribution *in vivo*[53]. As a DDS, liposomes provide several benefits for drug delivery including favorable biocompatibility, ability to self-assemble, capacity to load macromolecular drugs, and a wide variety of physicochemical and biophysical properties which can be revised to in charge of their characteristics. Moreover, the big aqueous core and biocompatible lipid shell allow the delivery of many kinds of macromolecules, such as oligonucleotides, peptides, proteins, or even a combination of all of them.

Up to today, since various modification methods for liposomes are well-studied, multi-functional modified liposomes have attracted interests of scientists throughout the world due to the potential in combination delivery of different biomacromolecules. CRISPR-Cas9 system is a powerful toolbox for gene editing, but often arises as complex combination of DNA/RNA and peptide/protein. Therefore, many delivery strategies for CRISPR-Cas9 system are lipid-involved. Jiang et al.[54] presented a lipid/AuNPs complex based vehicle for Cas9-sgRNA delivery. Cas9-sgRNA plasmid (CP) was condensed on TAT peptide-modified AuNPs *via* electrostatic interaction to form AuNPs/CP (ACP). Lipid-encapsulated ACP was prepared by coating lipid consist of (2,3-dioleoyloxy-propyl)-trimethylammonium (DOTAP), dioleoyl phosphoethanolamine (DOPE) and cholesterol (CHOL), and further PEG2000-DSPE on the ACP. This delivery system shows high efficiency in gene editing.

Due to the strong capacity of macromolecules encapsulation, artificial lipid-based delivery system is not only used in cytotoxic macromolecules delivery, but also in immunogenic macromolecules delivery.

3. Immunogenic macromolecules

Immunotherapy has taken center stage as an emerging cancer treatment attempt. Multitudinous promising immunotherapeutics designed to generate tumor-directed immune responses are available. Different macromolecules such as antigens, cytokines, chemokines, oligonucleotides, and Toll-like receptor (TLR) agonists targeting various immune cells have been successfully demonstrated in many preclinical settings[55]. For instance, cytotoxic T lymphocyte antigen 4 (CTLA4) targeting the co-inhibitory receptors or programmed cell death protein 1 (PD1) on T cells which was known as immune-checkpoint blockade both have induced a remarkable long-lasting survival benefit in patients with various kind of tumor through systemic administration[56].

Different from cytotoxic macromolecules, it is not essential for immunogenic macromolecule to reach the tumor site, as long as the systemic anti-tumor immune response of host is activated[57]. For the delivery of these macromolecules, the ultimate aim is to generate robust and durable immune response, and reduce side effects at the same time. A wide variety of delivery strategies have been investigated.

Durable host antitumor immune responses without the elevated reactogenicity or adverse effects are expected in anticancer vaccine development. Inspired by traditional inactivated bacteria and their derivative, lipid-based system is a favorable vehicle, as the load of various pathogen-associated molecular patterns (PAMPs) from different cellular structures. Zheng and colleagues[58] constructed a synthetic vector consists of various TLRs and oligomerization domain-like receptors (NLR) agonists in a single system organically which mimicking bacterium to orchestrate potent antitumor immunity while effectively reducing the toxicity with minimized side effects.

Self assembled nanosized single strand DNA composed of two class A CpG motifs which were discovered in bacterial genomes was encapsulated into the liposomes to simulate the bacterial nucleoid. The whole liposome-based system could elicit long term protective T cell memory responses with minimized side effects.

4. Smart patch delivery systems

Smart patch that uses microneedles to deliver macromolecular drugs is one of the most popular drug delivery systems because of its excellent patient compliance and readily to use. Moreover, it has been vastly explored in response to environmental stimuli signals for delivery of their cargoes in an on-demand fashion[59]. Gu and coworkers[60] constructed an artificial pancreas-like, closed-loop and glucose-responsive vesicles (GRVs), which are charged up with glucose oxidase (GOx) enzyme and insulin providing a favorable way of regulating glycemia with minimized patient effort, potential upgrades in hyperglycemia and quality of life. Since the successful application of microneedles in insulin delivery, similar microneedles which comprised of four components: acid-degradable polymeric matrix, polyelectrolyte-based

surfactant, GOx/CAT enzymatic system and PD1 were recently applied in anti-PD-1 antibody delivery to assist cancer immunotherapy for melanoma treatment recently[61]. Although there were not many attempts applying microneedles in macromolecules delivery, it is undeniable that it has obvious advantages and potentials in the near future.

5. Nanogel-based delivery system

Nanogels are also well-studied as one ideal macromolecular drug carriers in recent years. Compared with other polymeric nanocarriers, nanogel could incorporate both hydrophilic and hydrophobic drugs simultaneously within their 3D polymer networks by fine-tuning the chemical compositions. The capacity of nanogels to encapsulate biomolecules has been applied in the development of cancer vaccines and sustained delivery of cytokines[62]. Song and colleagues[63] designed erythrocyte membrane-coated nanogels to achieve the delivery and controlled release of IL-2, which is an important cytokine in regulating the survival, proliferation of T cells and natural killer cells in response to TME. Moreover, it is reported that nanogel-delivered antigens could induce robust DC activation and T cell response at lower antigen doses than soluble antigens; therefore, making the nanogels a safer immunotherapy approach[64].

6. Future perspectives and challenges

The advantages of macromolecular therapeutics have been recognized for their diverse functionality, targetability and potential to benefit the treatment of a variety of diseases. In comparison with small chemical drugs, researches of drug delivery systems for biological macromolecules are mostly in the early stage. Due to the peculiar molecular structure, physicochemical properties and biostability characteristics, elaborate delivery strategies were required in biomacromolecular drugs delivery.

As being discussed in this review, various novel drug delivery strategies, including but not limited to cationic polymer-based delivery systems, lipid-based delivery systems, smart patch delivery systems, membrane-camouflage systems, exosome-based delivery system and gel-based delivery system, have been developed to meet macromolecules with different delivery requirements. In preclinical studies, these latest studies had their unique design and addressed some of the problems associated with the delivery of macromolecular drugs like stability, targeting and selectivity. For instance, nanoparticles coated with cell membrane could significantly prolong circulation time compared with uncoated bare particles.

However, cautions must be given to the great distance from the clinical application. A large number of studies have indicated that the delivery of biological macromolecules *in vivo* is a systematic cascade process. Major difficulties include:

(1) Biomacromolecules require certain spatial configurations to keep pharmacodynamic activities, and therefore the activity and stability need to be ensured during the preparation process and *in vivo* delivery.
(2) Some macromolecules (cytotoxic macromolecules) require to function at tumor site precisely.
(3) Biomacromolecules are difficult to cross various physiological barriers *in vivo*, such as the gastrointestinal tract barrier, blood−brain barrier, lung−blood barrier, blood−pancreatic barrier, cell membrane barrier, and even karyotheca barrier.
(4) Most macromolecules need to undergo intracellular transport and release to enter the cell.

To achieve efficient delivery of macromolecules, the above four interrelated difficulties must be overcome synchronously. The absence of any essentials will result in the failure of the overall strategy or the reduction of efficacy, and it is difficult to overcome this multiple predicament. All in all, how to maintain the activity of biomacromolecules in the process of delivery, making them successfully penetrate various biological barriers *in vivo* and achieve accurate targeted delivery into cells, are still the research directions and difficulties in the future.

Acknowledgments

The work was supported by the grants from National Science Fund for Distinguished Young Scholars (81425023, China) and Program of Shanghai Academic Research Leader (18XD1400500, China).

Author contributions

The manuscript of this review was composed by Qin Guo under the guidance and advice of Prof. Chen Jiang.

Conflicts of interest

The authors have no conflicts of interest to declare.

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