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Chinese Journal of Natural Medicines

"Natural-derived drug carriers (NDDCs) for precision therapy"Special Issue

•Review•

Natural endogenous material-based vehicles for delivery of macromolecular drugs

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[ABSTRACT] Natural endogenous materials (NEMs), such as cell and cell derivatives, polysaccharide, protein and peptide, and nucleic acid-derived vectors, often exhibit biocompatibility, biodegradability and natural homing ability, which can minimize adverse reactions *in vivo* and have the potential to improve drug delivery efficacy. Currently, a variety of drug delivery systems (DDSs) based on NEMs have been constructed for macromolecules to address the challenges posed by their inherent large size, intricate structure, low permeability, and susceptibility to harsh environments. The aim of this article is to provide a comprehensive overview of various delivery strategies that predominantly utilize NEMs as carriers for macromolecular delivery. By thoroughly discussing the pros and cons of NEM-based DDSs, we hope to provide valuable insights into future innovations in pharmaceutical science, with a focus on improving therapeutic outcomes through advanced drug formulations.

[KEY WORDS] Macromolecular delivery; Endogenous material; Cell and cell derivatives; Exosome; Protein and peptide-based carrier; Nucleic acid-derived vector

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Introduction

Macromolecular drugs play a crucial role in the diagnosis, prevention and treatment of multiple diseases due to their diverse attributes such as biodiversity, good tolerance, high efficacy, low immunogenicity, strong site-specific activity, and remarkable therapeutic effect [1, 2]. However, their application is hindered by challenges such as inherent large molecular size, complex structure, inferior stability both *in vitro* and *in vivo*, immune stress induced by immunogenicity, low permeability and bioavailability [3]. These challenges are particularly significant when considering the therapeutic potential of macromolecules such as proteins and peptides (PPDs), vaccines, antibodies, hormones and nucleic acids. To improve the bioavailability and ensure that these therapeutics reach their intended targets *in vivo*, the development of effective drug delivery systems (DDSs) is crucial. Currently, although several of the diverse DDSs developed for macromolecular drugs have gained approval for clinical use, majority of the DDSs are still in the preclinical study stage due to various issues [4-7]. For instance, the traditional DDSs based on synthetic materials can enhance the bioavailability of therapeutic molecules *in vivo* but face challenges such as limited targeting efficiency, insufficient tissue permeability, and potential safety risks associated with synthetic materials. Even clinically approved PEGylation liposomes carry the risk of activating the body's complement system and causing side effects [8, 9].

In contrast to the traditional DDSs, delivery vehicles based on natural endogenous materials (NEMs) have garnered increasing attention due to their enhanced efficacy and biosafety. NEMs refer to substances originating from natural living organisms such as plants, bacteria, animals, and humans, including cell and cell derivatives, biomembrane, vesicles and exosomes, polysaccharide, PPDs, nucleic acid, etc. This shift towards utilizing these endogenous biomaterials reflects an increasing understanding of how biological systems operate at a molecular level. For instance, cell-inspired systems have been developed to mimic cellular structures or functions to facilitate targeted delivery [10]. Biomem-

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These authors have no conflict of interest to declare.

brane-based carriers leverage lipid bilayers similar to those found in cell membranes have been utilized to encapsulate drugs while providing protection from degradation [11]. Vesicles and exosomes serve as promising vehicles because they naturally transport biomolecules between cells; thus, they can be engineered for specific therapeutic applications [12]. Additionally, polysaccharides such as chitosan or hyaluronic acid offer unique properties like muco-adhesiveness and controlled release profiles that enhance drug stability during transit through biological barriers [13, 14]. Even PPDs themselves can also be utilized not only as therapeutic agents but also as components of DDSs due to their ability to form nanoparticles or hydrogels that improve solubility and targeting capabilities [15]. Currently, several of the NEMs-based DDSs have been successfully converted to clinical application or enrolled in clinical trials (Table 1). For instance, at least four exosome-based DDSs have been involved in clinical trials, suggesting the promising application of such materials in drug delivery. Similarly, protein nanotechnology-based chemotherapeutics like Abraxane® have paved the way for novel treatment modalities in oncology [16]. Sirolimus protein-bound particles for injectable suspension (Fyarro®) represent another significant advancement in DDSs. Moreover, the utilization of albumin-bound drugs for tumor treatment continues to expand with promising developments. All these demonstrated the promise of PPDs-based carriers for drug delivery. Ongoing efforts are focused on harnessing NEMs based DDSs to deliver PPDs for chronic diseases treatment, antibodies for cancer immunotherapy, vaccines for infectious diseases or cancer prevention, hormones necessary for metabolic regulation or reproductive health management, nucleic acids involved in gene therapy approaches to correct genetic disorders or modulate gene expression levels [10, 17]. Ongoing clinical trials (Table 1) and development efforts are exploring the potential of NEMs as therapeutic carriers in diverse areas, showcasing the myriad possibilities that this promising field of research holds [18].

The aim of this article is to provide a comprehensive overview of various delivery strategies that predominantly utilize NEMs as carriers for macromolecular delivery (Fig. 1). It highlights the respective advantages of each category, such as enhanced pharmacokinetics for longer drug circulation, versatility of co-delivering multiple drugs for synergistic effects, and better lesion targeting for improved treatment efficacy. However, it also points out challenges like scalability in production and variability associated with natural sources used in formulation development. By deeply exploring the strengths and weaknesses of NEM-based DDSs, our aim is to shed light on potential advancements in pharmaceutical research, prioritizing the enhancement of treatment success through sophisticated DDSs.

Cell-based Carriers for Macromolecular Delivery

Cellular DDSs are the most complex and intelligent platforms to deliver drugs to the lesion area by utilizing the special physiological functions of cells. Common cellular DDSs include systems based on blood cells, immune cells, stem cells, *etc* [10]. The intricate interplay between cells and therapeutic agents has opened up new avenues for targeted and efficient drug delivery (Fig. 2). Efforts are ongoing to explore various innovative approaches to enhance the capabilities of cellular DDSs.

Immune cell derived carriers for macromolecular delivery

One promising area of research involves the engineering of immune cells such as T cells and macrophages to serve as carriers for therapeutic payloads. By leveraging the natural homing abilities of these immune cells, scientists aim to precisely target disease sites while minimizing off-target effects. Furthermore, advancements in nanotechnology have enabled the development of sophisticated nanoparticle-based strate-

Table 1 Representative cases of approved and ongoing clinical trials using NEMs as therapeutic carriers

NEMs	Agent	Disease	Approved year	Phase	Study start	Trial number
Albumine	Abraxane®	Breast cancer, NSCLC. pancreatic cancer	2005	/	/	/
Albumine	Fyarro®	PEComa	2021	/	/	/
Albumine	Nanozora®	Rheumatoid arthritis	2022	/	/	/
B cell	ASP-001	mucopolysaccharidosis	/	I	2023-04-12	NCT05682144
Protein	Influenza HA Ferritin Vaccine	Influenza	/	I	2017-10-25	NCT03186781
Protein	SARS-COV-2-Spike-Ferritin-NP	SARS-CoV-2	/	I	2021-04-05	NCT04784767
Protein	EBV gp350-Ferritin NP	EBV	/	I	2022-03-29	NCT04645147
Exosome	Plant Exosomes	Colon cancer	/	/	2011-01	NCT01294072
Exosome	iExosomes	Pancreatic cancer	/	I	2021-01-27	NCT03608631
Exosome	CSET 1437	NSCLC	/	II	2010-05-19	NCT01159288
Exosome	Tumor Cell-derived Microparticles	Malignant ascites or maligna	/	II	2013-05	NCT01854866

Abbreviation: NEMs, natural endogenous materials; HA, hyaluronic acid; EBV, epstein-barr virus; NSCLC, non-small cell lung cancer; PEComa, perivascular epithelioid cell tumor.

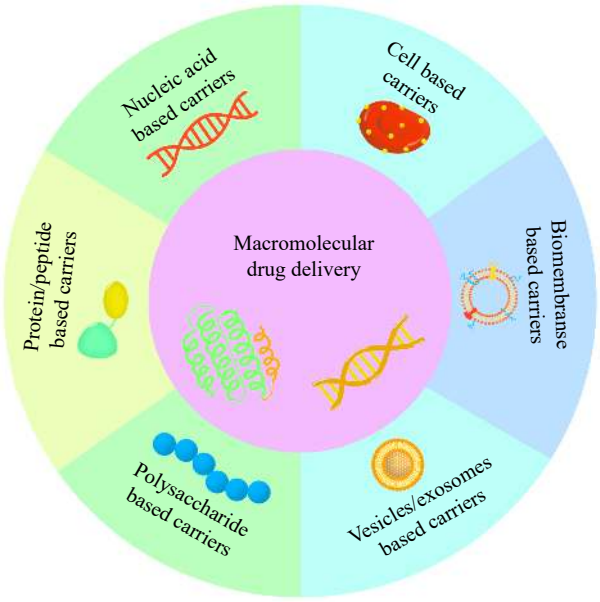

Fig. 1 Schematic diagram of the natural endogenous materials that have been employed as macromolecular delivery carriers.

gies that can be integrated with cellular drug delivery platforms. Modification of the cell surface with nanoparticles (or microparticles) can achieve targeting drug delivery by the intrinsic cell tropism to the injury sites. For instance, a study found that soft discoidal particles strongly attached to macrophages, with an 86.9% binding rate thanks to a cell-adhesive layer, a PLGA layer, and two PVA layers that loaded the cytokine IFN-γ to activate proinflammatory macrophages and exert anti-tumor effects [19]. This strategy made the particles evade phagocytosis for several days and release IFN-γ to promote the polarization of macrophages. Compared with the equal dose of macrophages and free cytokine, the "backpack" delivery system could decrease the metastatic burdens and tumor growth rate in murine breast cancer models [19]. In another study, nanogels were used to "backpack" protein drugs (interleukin-15 super-agonist complex) on T cells. These constructed cell surface-conjugated nanogels selectively released drugs at tumor antigen sites, leading to a more focused T cell expansion in tumors compared to systemic cytokine delivery. This "backpack" method enhanced the therapeutic window and improved tumor clearance through CAR-T cell therapy [20]. Similarly, in another study, a PD1-formed nanogel was anchored onto the surface of T cells by bio-orthogonal click chemistry, and the combination of aPD1 and ACT T cells achieved an excellent treatment outcome in murine solid tumor models [21].

Non-immune cell derived carriers for macromolecular delivery

In addition to immune cells, platelets are also engineered to achieve targeted delivery of macromolecular therapeutics by using its ability to target the bleeding sites. For instance, nanoparticle-anchored platelets hold great potential to act as DDSs in post-surgical cancer treatment. In a study, a tumor microenvironment-responsive nanoparticle-anchored platelet platform has been developed for intracellular protein delivery [22]. Protein nanogels loading GALA peptide and Granzyme B (GrB) were conjugated on the platelet surface by benzoic-imine linkers. This delivery system could actively accumulate at the surgical trauma and release nanogels in acidic microenvironment. After cellular uptake, the nanogels could escape from lysosome by the pore-forming GALA peptide, and then release GrB in cytoplasm by glutathione (GSH), thereby inhibiting tumor recurrence [22]. Additionally, fibroblast-based delivery vehicles can improve pharmacokinetics by extending circulation time. In one study, SHEN *et al*. attached PLGA-based microparticles to fibroblast surfaces. The resulted microparticles were loaded with peroxiredoxin-1 (Prx1) to achieve sustained drug release, potentially increasing resistance of fibroblast senescence using this backpack approach [23]. In addition to traditional cell types, emerging studies are investigating the potential of utilizing genetically modified or synthetic cells as versatile vehicles for drug transport. These engineered cell-based systems offer a customizable platform for tailored drug release kinetics and enhanced biocompatibility. Interestingly, dead cells also can be utilized as DDS carriers. In a study by Ci *et al*., a simple method was developed to obtain the dead cell-based DDSs by shocking acute myeloid leukemia (AML) cells in liquid nitrogen to decrease pathogenicity while preserving the chemotaxis toward the bone marrow. This treatment allowed the cells to be repurposed as a cancer vaccine, stimulating potent antitumor immune responses [24]. This strategy exemplifies how dead cell carriers could be harnessed for targeted macromolecule delivery in future therapeutics.

To sum up, cellular DDSs are under extensive study and provide new opportunities for *in vivo* delivery of macromolecules. However, there are still some challenges such as limited drug loading capacity, high cost and the risk of drug

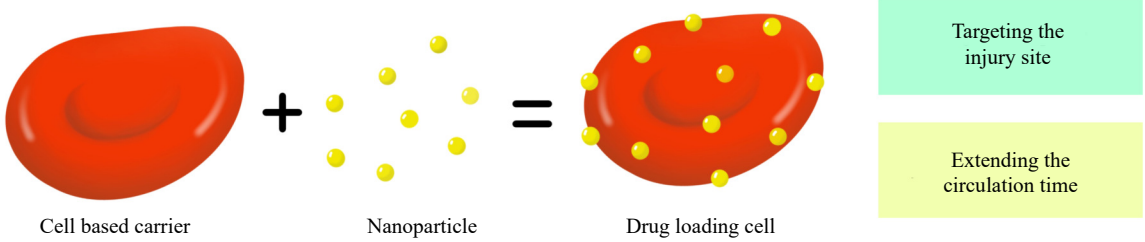

Fig. 2 Cell based carrier can targeting the lesion site and extending the circulation.

leakage during circulating transport. Researchers are tackling the challenges of cellular DDSs by employing covalent coupling technology to boost drug loading and minimize leakage. They're also integrating responsive chemical groups for the precise release of drugs at the target site. Streamlining production and utilizing more affordable, accessible cell sources can further reduce costs, making cell therapies more viable. As this field progresses, interdisciplinary collaboration is crucial for overcoming challenges and bringing these innovations from lab to the clinic, advancing personalized medicine and fulfilling unmet needs across various illnesses.

Biomembrane-based Carriers for Macromolecular Delivery

Currently, nanoparticle-based DDSs provide a potential technology for the treatment, prevention and detection of various diseases. The bionic cell membrane coating technology has been developed to enhance the targeting efficiency and therapeutic potential of nanoparticles [11]. This technology allows for the functionalization of synthetic nanoparticles with the properties of natural cell membranes, endowing them with superior biocompatibility and an ability to evade macrophage uptake, among other advantages [25]. Different types of cell membranes possess distinct components and different functions that can be harnessed for specific biomedical applications. The process of modifying nanoparticles with separated biomembranes typically involves a hybridization treatment, which can improve the drug loading efficiency and reduce the likelihood of drug leakage (Fig. 3). However, it's important to note that current coating methods may not always result in full membrane integrity, which could affect the biomedical functionality of the nanoparticles, such as cargo leakage or undesired biomolecule adsorption.

Delivery systems based on immune cell derived biomembrane

Macrophages play a crucial role in inflammation homing and immunomodulation, making their isolated membranes an attractive option for functionalizing nanoparticles. The work of Wu *et al*. on macrophage membrane-coated NPs highlights their ability to target fracture lesions and promote bone repair through siRNA delivery [26]. Additionally, the work of Cao *et al*. on modifying saALOX15-loaded mesoporous polydopamine with macrophage membranes demonstrates enhanced blood-brain barrier penetration, decreased clearance by mononuclear phagocyte system, and enhanced targeted therapy for orthotopic glioblastoma multiforme (GBM), emphasizing the potential impact of this approach on improving treatment outcomes [27]. Moreover, in a study, the use of M1-type macrophage cell membrane-coated nanoformulations has shown promise in enhancing glioma immunotherapy by penetrating the blood-brain barrier and co-delivering therapeutic agents of phosphorus dendrimer (termed AK128) and programmed cell death protein 1 antibody (aPD1) directly to tumor sites [28]. Similarly, in another study, M2 macrophage cell membrane-derived siRNA nano-delivery systems have exhibited notable biocompatibility and remarkable targeting properties and thereby reducing the inflammatory and improving tendon adhesion when used for tendon injury treatment [29]. Neutrophil-membrane-coated nano-delivery systems represent another innovative approach in targeted drug delivery. For instance, the research of Liu *et al*. on neutrophil-membrane-coated zeolitic imidazolate framework-8 (ZIF-8)-based nano-delivery system for the delivery of ASOs against microRNA-155 (miR-155) illustrates its potential in alleviating inflammation associated with atherosclerotic lesions through precise endothelial cell targeting by the interaction between CD18 (neutrophil membrane protein) and ICAM-1 (endothelial cell membrane protein) [30].

Delivery systems based on non-immune cell derived biomembrane

Red blood cell (RBC) membrane-modified nanoparticles have demonstrated remarkable capabilities in prolonging circulation time within the body. Studies by Xu *et al*. have showcased how RBC membrane camouflaged nanoparticles can prolong the circulation time and effectively combine gene therapy with chemotherapy to enhance therapeutic outcomes for drug-resistant cancer [31]. Furthermore, researchers as Fei *et al*. have developed nanoformulations (PMVs@siRNA NPs) with platelet membrane camouflage to regulate the TGF-β1 pathway through gene therapy and alleviate the inflammation and fibrosis in the kidneys, showcasing their potential in targeting organs affected by ischemia/reperfusion injury [32]. In addition to utilizing whole-cell membranes for nano-

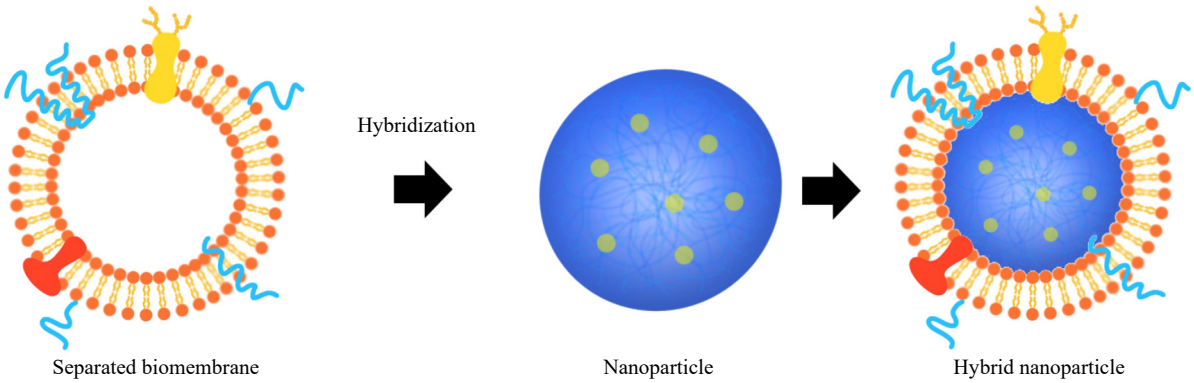

Fig. 3 The separated biomembrane can be used for nanoparticle modification through hybridization treatment.

particle functionalization, researchers like Qiu *et al*. have explored leveraging endoplasmic reticulum (ER) membranes from cancer cells to enhance siRNA transfection efficiency within hybrid formulations (EhCv/siRNA NPs). The ER membrane decoration on NPs could facilitate the siRNA transportation through the endosome-Golgi-ER pathway, resulting in evading lysosomal degradation and increasing the silencing efficiency of siRNA [33]. This approach holds promise for achieving improved anti-tumor effects through enhanced siRNA transportation mechanisms.

Overall, the biomimetic nanodelivery systems provide an effective approach of achieving macromolecular targeted delivery *in vivo* due to their unique biological properties conferred by cell membrane coating technology—such as extended circulation time, precise targeting delivery capabilities, and immune escape mechanisms [34]. However, the clinical translation of biomembrane-modified nanoparticles requires concerted efforts to overcome challenges related to large-scale production, quality control and standard protocols. Furthermore, in-depth studies elucidating the mechanism and fate of biomembrane-modified nanoparticles within living organisms will be instrumental in guiding further refinements in the design of biomimetic carriers for macromolecular targeted delivery.

Vesicles/exosome-based Carriers for Macromolecular Delivery

Extracellular vesicles (EVs), ranging in size from 30−150 nm, are secreted by cells into the extracellular space and possess remarkable characteristics such as high stability, innate and acquired targetability, good biocompatibility, and low immunogenicity [35]. EVs have garnered significant attention in the field of drug delivery due to their unique properties described above and potential applications [36]. Recent findings have revealed that EVs play a key role in cell communication and can be utilized as biomarkers in the detection of diseases [37]. EVs consist of membrane structure, transmembrane-anchored protein and a variety of nucleic acids, lipids, and cytosolic proteins contained within the vesicles. These special structures of exosomes facilitate their targeted tissue delivery and contribute to the biocompatibility. The versatility of EVs as natural drug carriers stems from their ability to encapsulate a diverse range of cargo inside them and the potential to traverse various biological barriers within the body. This cargo can include proteins, polypeptides, nucleic acids, and other biomacromolecules. According to their cellular origin, exosomes can be divided into human and animal cell derived EVs, plant derived EVs and bacterial derived EVs (Fig. 4). Considering the universality of extraction and drug loading approaches across various exosome types, this section provides an initial introduction to the techniques employed for exosome extraction and drug loading.

Exosome isolation and loading strategies

In terms of isolating EVs for drug loading purposes, several techniques including centrifugation, filtration, column chromatography and immunoprecipitation have been employed with varying degrees of success. Centrifugation remains a widely utilized method due to its simplicity and reproducibility. Additionally, advancements in exosome isolation techniques have led to the development of semi-auto-

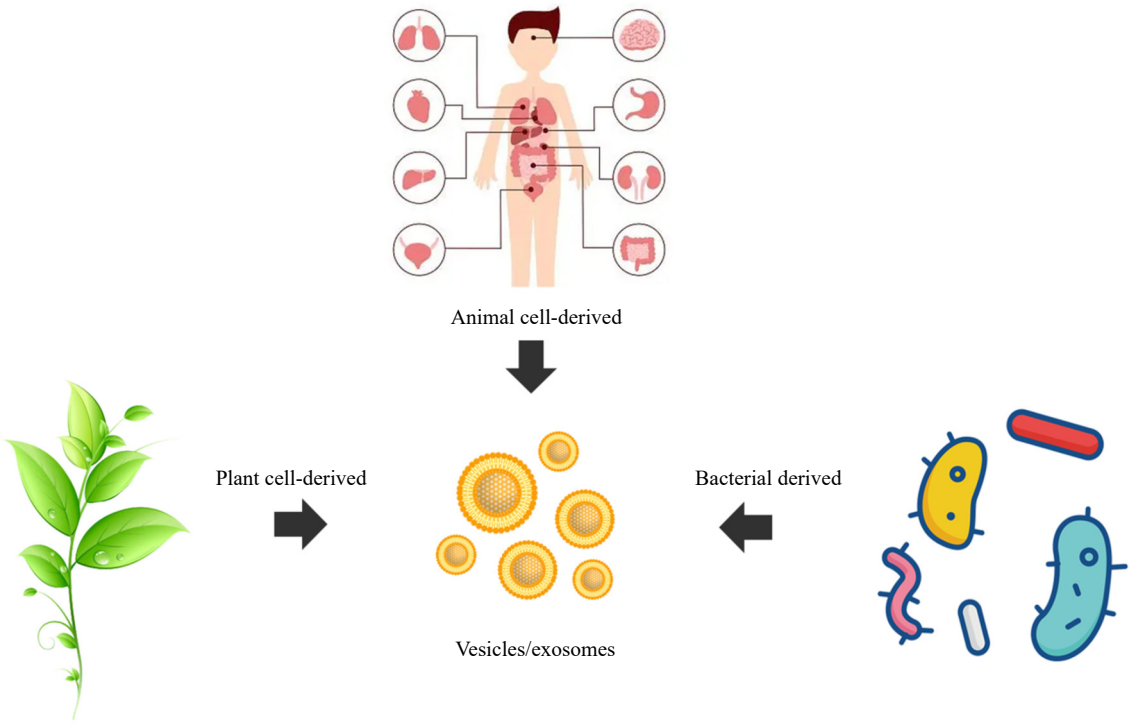

Fig. 4 Exosomes can be divided into animal cell derived EVs, plant derived EVs and bacterial derived EVs.

mated extraction instruments that offer enhanced convenience for researchers [38].

When it comes to loading macromolecules such as proteins, peptides, and nucleic acids, *etc*. into EVs, various techniques have been explored to improve the efficiency and specificity of the process. Four common methods include incubation, sonication, electroporation and endogenous loading approaches have been employed.

Incubation is a simple and relatively straightforward method where drugs and carriers are co-incubated at a specific temperature and time. However, this method may result in lower encapsulation rates for certain molecules. For instance, the encapsulation rate of catalase in exosomes from various cell origins using the incubation method was less than 20% [39]. The efficiency of this incubation method depends on the polarity and molecular weight of drug molecules. Nucleic acid drugs like siRNA and miRNA often exhibit suboptimal encapsulation rates compared to protein-based drugs using incubation method. In a study by Yuan *et al*., miR159 was loaded into THP-1 cell-derived exosomes through incubation, but the encapsulation rate in exosomes was only 1.2% without modification of miR159. Efforts are ongoing to optimize these loading processes.

Sonication has shown promise in achieving higher encapsulation rates but may impact the structural integrity of EVs. This method involves applying mechanical shear forces through an ultrasonic probe to deform the exosome membrane and facilitate drug entry. In a study by Haney *et al*., the inclusion rate of catalase in RAW 264.7 macrophage exosomes reached up to 26.1% with sonication compared to only 4.9% with incubation [39]. Sonication has also shown promising efficacy in loading nucleic acid drugs. Xiang *et al*. successfully loaded siRNA-Keap1 into milk-derived exosomes using ultrasonic treatment, with an encapsulation rate reaching 24% [40].

Electroporation is another method that uses an electric field to temporarily permeabilize the membrane, allowing macromolecules to enter the EVs. By utilizing short high-pressure pulses, electroporation induces instantaneous breakage of lipid layers and creates transient membrane permeability that allows diffusion of drug molecules into the interior of exosomes. This method can achieve high encapsulation rates but may require specialized equipment and can be more technically challenging. It also poses challenges related to maintaining the structural integrity of EVs post-loading. In Belen' s research work, insulin was successfully encapsulated within HepG2 cells' exosomes as well as HDfa and RIN-m cellular exosomes through electroporation with an encapsulation rate reaching approximately 50% [41]. Kim *et al*. achieved an encapsulation rate of only 1.68% for miRNA-21 antisense oligonucleotides loaded into HEK293T cell-derived exosomes using electroporation [42].

Endogenous approaches for mRNA drug encapsulation within exosomes are favored due to their natural biocompatibility and selective incorporation capabilities. This method typically involves the transfection of cells with specific plasmids to induce the transcription of target mRNA sequences, which are then packaged into exosomes upon cellular secretion [43]. However, the larger size and higher molecular weight of mRNA drugs compared to siRNA and miRNA present challenges for efficient loading into exosomes. For example, Surya *et al*. successfully utilized an endogenous drug-loading system called EXOtic to package the HIV-1 inhibitory protein zinc finger protein (ZFP-362) mRNA sequence into 293T cell-derived exosomes [44]. These engineered exosomes effectively suppressed HIV expression levels in mouse bone marrow, spleen, and brain tissues. However, qRT-PCR analysis revealed that only approximately 20 000 RNA copies were detected per $10^{\wedge 8}$ exosomes in this investigation. This finding indicated that while endogenous methods like EXOtic show promise, they may not be optimal for all applications and further improvements in efficiency are needed.

Each technique with its own advantages and limitations, and the choice of method may depend on the specific cargo, the source of EVs, and the desired application. As research continues to delve deeper into understanding the intricacies of exosome-based DDSs, it becomes increasingly important to tailor these approaches based on specific experimental needs while considering factors such as loading efficiency and preservation of vesicle integrity throughout the process.

Extracellular vesicle-based DDSs for biomacromolecules

EVs derived from human and animal cells

293T cells are human renal epithelial cell lines valued for their easily transfection and low expression of endogenous receptors required for extracellular ligands, which makes them ideal candidates for extracting endogenously loaded drug-carrying EVs. Researchers have successfully utilized these cells to extract EVs carrying specific mRNA and siRNA molecules, demonstrating promising results in inhibiting HIV and angiogenesis capabilities in cancer cell lines. For instance, Surya et al. achieved expression of ZFP-362 mRNA in 293T cells through plasmid transfection and obtained EVs carrying ZFP-362 mRNA for HIV inhibition research [44]. In another study, Fatma et al. transfected 293T cells to express SALL4-targeting siRNA and obtained siRNA-enriched EVs through centrifugation which successfully inhibited angiogenesis capability in gastric cancer cell line MGC-803 [45].

Moreover, the attention given to human stem cell exosomes has led to significant advancements in understanding their homing effect. Human stem cell-derived exosomes exhibit enhanced targeting capabilities towards specific organ tissues, offering immense potential for developing organ-specific drug carriers with precise targeting abilities. For instance, lung stem cell exosomes have been found to demonstrate remarkable targeting capabilities towards lung tissue, providing opportunities for exploring treatments for pulmonary fibrosis [46]. Notably, researchers have harnessed the unique properties of lung stem cell exosomes by using them as carriers for SARS-CoV-2 receptor-binding domain (RBD) in COVID-19 vaccine development [47]. This vaccine is ad-

ministered intranasally, which enhances retention of RBD in the mucosal respiratory tract and lungs compared to the liposome-based vaccines. Preliminary studies in mice have shown that this method can elicit a specific immune response against RBD and effectively clear SARS-CoV-2 pseudovirus, indicating the potential of this approach. The therapeutic efficacy demonstrated through this vaccine holds great promise for addressing severe pneumonia following live SARS-CoV-2 challenge. In addition, Mesenchymal stem cells are pluripotent stem cells that possess the fundamental characteristics of self-renewal and multidirectional differentiation. These cells can be derived from various tissues, including bone marrow, adipose tissue, umbilical cord, placenta, etc. Mesenchymal Stem Cell-Derived Exosomes (MSC-Exos) are secreted by mesenchymal stem cells and exhibit low immunogenicity and prolonged half-life, and have garnered attention for their remarkable properties as drug carriers and their ability to stimulate cellular regeneration. One area of significant promise lies in the use of MSC-Exos for targeted delivery of biomacromolecules. In the study of Feng et al., exosomes derived from bone marrow mesenchymal stem cells loaded with miR-6924-5p exhibited therapeutic effects in a mouse model of anterior cruciate ligament tendine-bone healing [48]. The findings suggest that miR-6924-5p-loaded exosomes inhibit osteoclast formation during tendon-bone healing while enhancing biomechanical strength at the tendon-bone insertion site. The ongoing exploration and refinement of MSC-Exo-based therapies hold immense potential for revolutionizing DDSs and advancing regenerative medicine practices. Overall, the continued exploration and utilization of 293T cell-derived exosomes along with stem cell-based approaches represent exciting avenues that hold tremendous potential for advancing targeted drug delivery and therapeutic interventions across various medical conditions.

Due to the issue of biocompatibility, the utilization of exosomes derived from animal cells in human disease-oriented research is limited. However, recent advancements in oral delivery of exosomes have highlighted the potential of milk exosomes, which represent animal-derived exosomes found in milk, for efficient delivery of biological macromolecules. Milk exosomes (mExos) have demonstrated significant promise as vehicles for the oral administration of protein and peptide drugs owing to their superior capacity to traverse epithelial barriers. According to the work of Wu *et al*., milk-derived exosome loaded with insulin shows superior and persistent hypoglycemic effects in the oral pathway, suggesting that milk-derived exosomes have excellent insulin delivery capacity, which also provides reference value for the oral delivery of biological macromolecules [49]. Nevertheless, certain challenges persist due to the intrinsic characteristics of natural mExos, including suboptimal drug loading efficiency, inadequate mucus penetration capability, and susceptibility to membrane protein loss. One notable advancement involves the design of hybrid vesicles with self-adaptive surface properties by confusing functionalized liposomes with natural mExos—resulting in enhanced oral bioavailability of 8.7% and notably improved pharmacological therapeutic effects [50]. As research continues to unravel the intricacies surrounding these remarkable nanovesicles, it is anticipated that they will play an increasingly pivotal role in shaping future medical interventions aimed at addressing diverse health challenges.

Plant cells derived exosomes

Plant exosomes are extracellular vesicles isolated from different parts of various plants, including roots, seeds, and leaves. Plant exosomes have garnered significant attention in the field of biotechnology and medicine due to their unique properties and potential applications. In addition to their structural similarity to mammalian exosomes, plant exosomes offer distinct advantages that make them highly promising for various therapeutic and drug delivery purposes. One notable characteristic of plant exosomes is their wide availability from diverse plant sources without the need for cell culture. This accessibility not only simplifies the isolation process but also reduces production costs, making plant exosomes a cost-effective option for DDSs. Moreover, compared to mammalian exosomes, plant-derived exosomes exhibit lower immunogenicity, minimizing the risk of triggering adverse immune responses in recipients. Furthermore, studies have demonstrated that numerous types of plant exosomes display remarkable stability in simulated gastric and intestinal fluids. This property is particularly advantageous for oral drug delivery applications as it ensures the protection and effective transport of biological macromolecules through the harsh gastrointestinal environment. Taking ginger-derived exosomes as an example, research has shown their multifunctionality in modulating intestinal flora, suppressing intestinal inflammation, and ameliorating alcoholic liver injury [17, 51-53]. Furthermore, the study conducted by Man et al. provides evidence supporting the stability of ginger-derived exosomes in gastroenteric fluid and their efficient absorption in the small intestine [54]. These findings highlight the potential therapeutic benefits of utilizing ginger-extracted exosomes as oral delivery carriers. In recent investigations by Wang et al., ginger-extracted exosomes were utilized to deliver Dmt1 siRNA orally to mouse models with encouraging results in improving iron metabolism [55]. In addition, the work of Li *et al*. focused on cancer therapy by loading survivin siRNA into ginger-extracted exomes and administering them through intravenous injection. The outcomes revealed tumor-targeting capabilities along with effective gene silencing effects while demonstrating low cytotoxicity and good biocompatibility in xenograft mouse models [56]. These findings provide evidence supporting their role as cost-effective delivery systems for cancer treatment. Overall, these advancements underscore the exceptional potential of plant-derived exosome-based delivery systems for a wide range of biomedical applications.

Bacterial derived exosomes

Bacteria-derived exosomes, also known as bacteria outer membrane vesicles (OMVs), are vesicles prepared from bac-

terial cell membranes. Due to the abundance of pathogen-associated molecular patterns (PAMPs) such as peptidoglycan, lipopolysaccharide (LPS), and flagellin, they possess a robust capacity to activate innate immune signaling pathways and are considered valuable natural vaccine adjuvants and drug carriers [57, 58]. Based on the above attributes of OMVs, when OMVs are used as drug carriers, the loaded drugs are mostly small molecule compounds of antibiotics, but there are also practices of delivering biological macromolecules through OMVs [57, 59, 60]. For instance, Gujrati *et al*. genetically engineered MSBB-mutated E. coli to produce a type of detoxification lipopolysaccharide and to express human epidermal growth factor receptor 2 (HER2) affinity for cancer cell targeting [59]. The siRNA was loaded to the prepared HER2 affibody-displaying OMVs by electro-perforation for cancer therapy. This method enhanced siRNA accumulation in tumors and inhibited tumor growth by silencing spindle protein, thus validating OMVs as an effective DDS for targeted cancer treatments [59]. Moreover, current research has explored polypeptide modification on OMVs to enhance their targeting capabilities [61]. It is anticipated that as further investigations into OMVs are conducted in the future, their potential as a vehicle for delivering biological macromolecule drugs will be further harnessed.

Polysaccharide-based Carriers for Macromolecular Delivery

Naturally derived polysaccharides have made significant progress in the field of drug delivery, showcasing unique advantages that stem from their inherent properties such as good biocompatibility, biodegradability, low toxicity, and specific biological activities [62]. Commonly used polysaccharides in drug delivery include hyaluronic acid (HA) and chitosan, among others. The diverse physical and chemical characteristics of these polysaccharides allow researchers to design a wide array of drug delivery carriers tailored specifically for biomacromolecules, ultimately enhancing treatment efficacy across different diseases.

HA, a natural mucopolysaccharide found abundantly in connective tissues, has garnered attention due to its numerous advantageous properties such as high biocompatibility, non-immunogenicity, biodegradability, and exceptional hydrophilicity [14]. These attributes render HA an ideal material for use as a carrier in DDSs. For instance, YAN et al. reported-on HA-based nanomedicines designed to reprogram synovial macrophages for treating osteoarthritis. In this innovative study, HA was chemically modified with alendronate—a bisphosphonate used primarily for bone health —and *o*-phenylenediamine to create novel polysaccharide polymer derivatives known as NAHA. This derivative served as outer shell stabilizing siCA9-loaded calcium phosphate nanoparticles (NAHA-CaP/siCA9 NPs) (Fig. 5). Following intra-articular-articular injection into affected joints, these nanoparticles facilitated the repolarization of macrophages from the pro-inflammatory M1 phenotype towards the anti-inflammatory M2 phenotype through synergistic effects achieved *via* CA9 gene silencing combined with nitric oxide scavenging mechanisms [63]. In subsequent research efforts focused on advancing treatment strategies further still within this domain; Cui *et al*. introduced a combination strategy integrating HA-based nanomedicine with cell therapy techniques. Their study involved synthesizing HA grafted with both alendronate and kartogenin—an agent known to promote cartilage regeneration—to form calcium phosphate nanoparticles loaded with siCA9 targeting osteoarthritis management effectively. Additionally, these engineered nanoparticles were combined strategically with bone marrow-derived mesenchymal stem cells (BMSCs) aiming not only at regulating inflammatory microenvironment but also promoting MSCs-oriented differentiation essential for tissue repair processes [64].

Chitosan represents another promising candidate within the realm of naturally derived polysaccharides that are extensively employed in DDSs. This is largely attributed to its non-toxic nature, cationic and unique mucoadhesive properties. Furthermore, chitosan has the ability to open tight junctions (TJs) between epithelial cells instantly, which significantly enhances the permeability of macromolecules across biological barriers. The potential applications of chitosan-

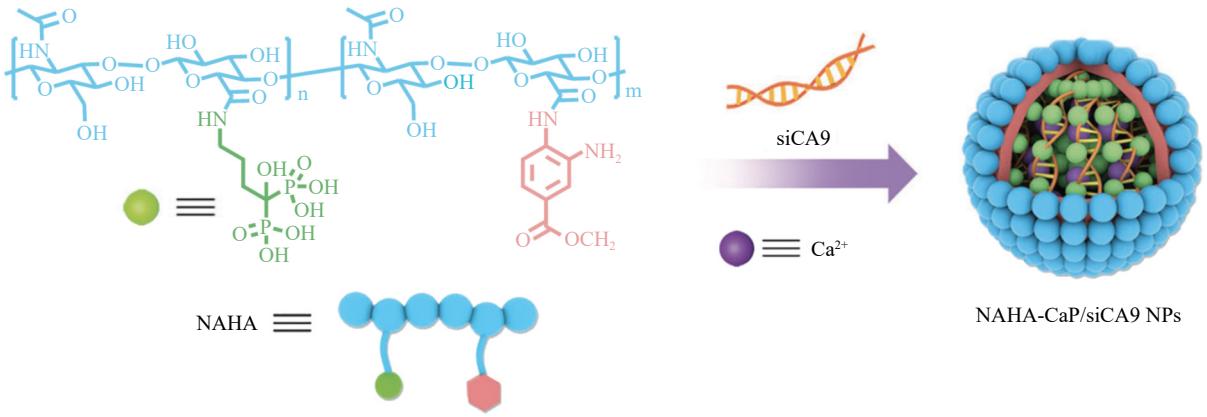

Fig. 5 Hyaluronic acid derivatives was used to form calcium phosphate nanoparticles for loading siRNA.

based nanoparticles in oral insulin administration have garnered considerable attention in recent years [65]. For instance, Sudhakar *et al*. developed thiolated chitosan nanoparticles specifically designed for oral insulin delivery. Their research demonstrated through both *in vitro* and *in vivo* experiments that these nano-systems effectively reached over the tip of the microvilli at the intestinal mucosa after oral administration. Consequently, this facilitated an increase in blood insulin levels while simultaneously decreasing glucose levels in diabetic rats induced by specific models [66]. In addition to its role in insulin delivery, the positive charge associated with chitosan allows it to efficiently load nucleic acid drugs such as siRNA. Liu *et al*., for example, explored a novel approach by grafting lactose acid (LA) and all-trans retinoic acid onto N,N,N-trimethyl chitosan for co-delivery purposes involving doxorubicin and siRNA targeting specific oncogenes or pathways involved in tumor progression. In their study, siRNA was condensed on the hydrophilic shell of these nanoparticles. Due to multiple cooperative antitumor effects, these nanoparticles demonstrated superior *in vitro* and *in vivo* antitumor efficiency compared to other formulations [67]. The synergistic action not only enhanced therapeutic efficacy but also minimized side effects commonly associated with conventional chemotherapy regimens.

Protein and Peptide-based Carriers for Macromolecular Delivery

Naturally derived proteins and peptides are favored for constructing nanocarriers due to their distinct physicochemical properties, which vary based on their amino acid composition and sequence. Their charge can be positive, negative, or neutral, influencing their drug-carrying capacity. For nucleic acid drugs and electronegative proteins, positively charged protein materials are preferred. The method of covalent coupling can ignore the influence of the physicochemical properties of proteins and peptides on drug loading efficiency.

Albumin is the most abundant plasma protein, non-toxic, low immunogenicity, biocompatible and biodegradable, and serves as a transport protein for a wide range of compounds in plasma. These properties make albumin an ideal material for the fabrication of nanoparticles for drug delivery. Human serum albumin (HSA) and bovine serum albumin (BSA) are by far the most commonly studied albumin species [68]. Han *et al*. reported a cationic BSA-based siRNA delivery system for treating lung metastatic cancer. The cationic BSA could effectively load siRNA and protect the siRNA from degradation. As the Bcl2 targeting siRNA (siBcl2) is introduced to this nano-system, the formed nanoparticles exhibited an efficient gene silencing effect, thereby inducing cell apoptosis and inhibiting tumor growth [15]. Albumin can also serve as an auxiliary material for constructing nanocarriers. Azevedo *et al*. designed a novel of albumin conjugated PLGA-PEG nanoparticles in order to address the problems of gastrointestinal tract degradation and low bioavailability for oral delivery of insulin. By modifying the nanoparticles with site-specific human albumin variants, albumin-functionalized NPs beneficially bound FcRn in a pH-dependent manner and showed enhanced transport across polarized cell layers. When diabetic mice that can express FcRn were treated by oral administration, a significant hypoglycaemic effect was found, with a reduction in blood glucose to 40% of the initial blood glucose at 1h post-delivery [69]. In addition, HSA can be directly coupled to macromolecular drugs as a potential DDS. Short fragments of insulin can initiate and accelerate hormone aggregation in the body. N-methylated hot-spot analogs have been proved inhibitory activity against insulin aggregation. Meanwhile, the functional property of HSA is its strong inhibitory activity against amyloid/peptide aggregation. Wasko *et al*. investigated whether HSA could bind N-methylated hot-spot analogues of natural insulin. The interaction of HSA with N-methylated insulin fragments was investigated by applying various research techniques. It was found that the N-methylated derivatives were able to attach to HSA. Therefore, HSA can be used as a delivery system for insulin aggregating peptide inhibitors [70].

Ferritins are spherical iron storage proteins found within cells, consisting of a combination of 24 subunits of two types: heavy-chain ferritin (HFn) and light-chain ferritin (LFn). The unique nanocage structure, excellent safety profile, and definite *in vivo* behavior are the key characteristics of native ferritin nanocages that make them uniquely attractive for the development of ferritin-based formulations in nanomedicine. These attributes position ferritin as a promising candidate for further exploration and utilization in the field of nanomedicine [71]. PEDICONI et al. specifically decorated the internal cavity of “humanized” chimeric Archaeal ferritin with novel cationic piperazine-based compounds for siRNA delivery. These systems enhanced the siRNA delivery efficiency into HeLa, HepG2, and MCF-7 cancer cells [72].

The capsid protein is the protein shell of a virus. It protects nucleic acid of virus, participates in the infection process of the virus and has good antigenicity. Choi *et al*. designed a chimeric capsid protein consisting of a capsid shell, integrin-targeting peptide and p19 RNA binding protein to achieve efficient cytoplasmic delivery of siRNA. Briefly, the investigators modified the hepatitis B virus (HBV) capsid protein to improve nanocarrier stability, and used RGD peptides to replace loop segment on the surface of the capsid protein to achieve precise targeting of the nanocarrier. The high affinity of the p19 proteins for siRNA enables capsid nanocarriers to form siRNA/capsid nanocarrier (siRNA/CN) complexes. This enhances siRNA stability and enables siRNA targeted delivery and suggests that the capsid nanocarrier system has the potential to deliver siRNAs efficiently [73, 74].

Bacterial inclusion bodies (IBs), as natural amyloids, exist in bacteria as discrete protein nanoparticles. These nanoparticles have recently been explored as functional protein-based biomaterials and adapted as nanocarrriers to deliver recombinant protein drugs into mammalian cells [75]. Meanwhile, cholera toxin chimeric proteins are used as novel cell-

penetrating carriers for delivery of biomolecule drugs to the brain. This expression vector provides a viable expression framework for the construction of several new macromolecular protein drugs [76]. Retroviral gag proteins are able to spontaneously assemble into virus-like particles (VLPs). Receptor-targeted engineered virus-like particles were used to encapsulate siRNA and deliver it into the cytosol of target cells [77].

In addition to proteins, smaller molecular weight peptides can also be used for the delivery of biomacromolecular drugs. Amphiphilic peptides can self-assemble to form nanostructures for drug loading [78]. Mazza *et al*. designed and constructed peptide nanofibers as a genetic intervention toll for siRNA delivery. The positively charged amino acids in the sequence of peptide can electrostatically bind siRNA. In this study, a surfactant-like peptide (palmitoyl-GGGAAAKRK) was designed and synthesized to form self-assembled nanofibers, thereby increasing the residence time of siRNA in the brain tissues after intracranial administration [79]. Cell penetrating peptides (CPPs) have been widely studied for 30 years due to their excellent delivery properties. They can be used in combination with other delivery vehicles to efficiently cross biological barriers (cell membranes) and further improve the delivery efficiency of macromolecular drugs. Additionally, CPP is often used as a modification for nanoparticles. Yamada *et al*. developed a liposome-based carrier modified with R8 (a cell penetrating peptide) and GALA (a pH-sensitive fusogenic peptide) for releasing antibodies into the cytosol. The experimental results showed that these nanoparticles were superior in cellular uptake and endosomal escape than commercially available reagents [80].

Although protein and peptide-derived drug delivery systems have made some achievements in improving the stability and bioavailability of biomacromolecules, there are still many challenges and problems that need to be further studied and solved, such as the safety of constructed nanocarriers and cell penetration ability. Therefore, it is necessary to further study the mechanism and optimization strategy of protein and peptide-derived drug delivery systems in the future, so as to provide more valuable reference data and treatment regimen for improving the therapeutic effect and bioavailability of biomacromolecules.

Nucleic Acid-based Carriers for Macromolecular Delivery

Nucleic acids, especially DNA molecules, have garnered significant attention in the field of drug delivery due to their unique structural properties and biocompatibility. DNA can also serve as a source of drug delivery vectors. DNA molecules could be used as excellent substrates for the construction of molecular devices, serving as intelligent vehicles for drug delivery and controlled drug release [81]. One innovative approach within this domain is DNA origami, a technique that enables the construction of intricate DNA nanostructures with dynamic functionalities. The assembly mechanism of DNA origami relies on the Watson-Crick base complementary pairing principle. DNA strands can be coupled to molecules such as nucleic acids, protein and polymers in a non-covalent/covalent manner with nanoscled precision. In addition, DNA origami exhibits the responsible ability to external stimuli such pH, small molecules and enzymes, which is advantageous for drug delivery [82]. To enhance therapeutic efficacy further, it is essential to functionalize these DNA origami constructs with bioactive ligands—molecules that can specifically bind to target cells or tissues [83]. For instance, Li *et al*. developed an autonomous DNA robot utilizing the principles of DNA origami technology, thereby specifically transporting payloads to tumor tissues. As shown in Fig. 6, this DNA robot was modified on the outside with a DNA aptamer for targeting tumor site. Inside its cavity, thrombin was loaded; upon reaching the tumor site, its release could trigger localized coagulation processes aimed at disrupting blood supply and inhibiting cancer cell proliferation. *In vivo* anti-tumor experiments demonstrated promising results where intravenously injected DNA nanorobots successfully induced intravascular thrombosis in blood vessels associated with tumors, leading to significant inhibition of tumor growth [84]. Similarly, Lee *et al*. explored self-assembled tetrahedral nanoparticles made from DNA for delivering siRNA intended for silencing genes related to disease progression in tumors. Their findings indicated that effective gene silencing required at least three folate modifications per nanoparticle—a crucial factor enhancing cellular uptake via receptor-mediated endocytosis [85]. Their work also demonstrated that DNA molecule-based nanoparticles could be utilized as a kind of non-cationic carrier for siRNA delivery. In addition, Guo *et al*. reported on an innovative strategy combining an aptamer known as PL1 and Pcsk9 siRNA using precisely defined tetrahedral nanoparticles constructed from DNA scaffolds. Following folate modification allowed these DDSs not only to target specific tumor sites effectively but also resulted in an impressive 83.48% inhibition in tumor growth observed in mouse models [86]. Furthermore, Rahman *et al*. contributed significantly by designing various shaped nanoparticles resembling nanorectangles and nanotubes through modular brick methods aimed at delivering Bcl2-targeting siRNA (siBcl2). Both their *in vitro* and *in vivo* studies confirmed substantial downregulation of Bcl2 expression levels within cancerous cells alongside notable suppression of overall tumor growth rates [87].

The integration of metals into nucleic acid frameworks has opened new avenues for creating advanced materials tailored specifically for drug delivery applications. Utilizing metal-nucleic acid coordination techniques facilitates the formation of metal-DNA nanocomposites referred collectively as Metal-Nucleic Acid Frameworks (MNFs). For example, Yan *et al*. constructed a Ca^{2+}/(aptamer-deoxyribozyme) MNFs for loading interferon regulatory factor-1(IRF-1) to target regulate glucose transporter (GLUT-1) expression in gastric cancer cells. The results of *in vivo* and *in vitro* experiments showed that these nanodevices could facilitate

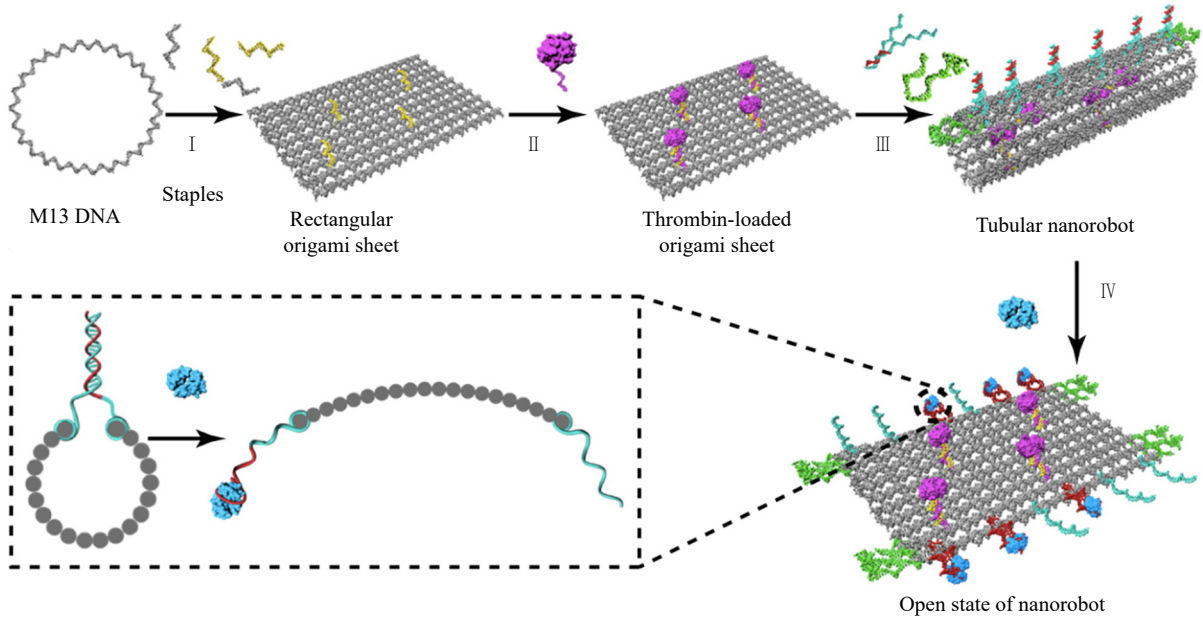

Fig. 6 An autonomous DNA robot was modified on the outside with a DNA aptamer for targeting tumor site, and loaded the thrombin within the inner cavity.

the ROS-induced DNA damage, thereby inhibiting tumor growth with a inhibitory rate of 90% [7].

The development of structural DNA nanotechnology offers an unprecedented capability to fabricate DNA nanoparticles with multiple functions. The uniform and adaptable nature of DNA nanoparticles holds significant potential for the systemic delivery of macromolecular drugs. As an emerging field, DNA nanotechnology offers powerful design techniques for DDSs with unique advantages in increasing drug targeting and decreasing drug toxicity. However, there are still certain obstacles that need to be addressed in the field of DNA nanotechnology [88]. Firstly, there is limited research regarding the pharmacokinetics of DNA nanostructures. In addition, it remains unclear how the physical and chemical properties of DNA nanostructures affect their pharmacokinetic bioavailability, such as *in vivo* circulation, distribution, metabolism and so on. Also, the current uptake of most DNA nanostructures by cells primarily depends on their inadequate endocytosis or pinocytosis pathways. Therefore, further improving the membrane penetration efficiency of DNA structure is the main means to enhance therapeutic efficiency. It is worth mentioning that RNA nanotechnology is definitely another field that is worthy to follow in the field of macromolecular delivery.

Conclusions and Future Perspective

Compared with traditional DDSs, the utilization of natural biomaterials such as cells, proteins, pathogens, nucleic acid, polysaccharide, and EVs as carriers represents a significant advancement in DDSs. These natural biomaterial-based DDSs offer unparalleled biocompatibility and demonstrate fewer side effects compared to their synthetic counterparts. Moreover, they possess inherent targeting capabilities and can be easily modified for diverse applications. Despite these remarkable advantages, challenges persist in the development and application of natural biomaterial-based DDSs. One critical obstacle is ensuring the stability and controlled release of drugs from these carriers over prolonged periods. Additionally, there is a pressing need to optimize manufacturing processes to ensure scalability and reproducibility while maintaining the desired properties of the carrier materials. To address these limitations, ongoing research efforts are focused on exploring innovative strategies for enhancing the performance of DDSs based on NEMs. Scientists are leveraging advanced technologies such as nanotechnology and bioengineering to revolutionize DDSs based on natural biomaterials. It is anticipated that continued advancements in this area will lead to breakthrough developments in treating human diseases using innovative DDSs based on NEMs. With their potential to overcome limitations associated with conventional approaches, these innovative DDSs hold great promise for transforming the landscape of medical treatment in the future.

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