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REVIEW

Advances and Prospects of Prolamine Corn Protein Zein as Promising Multifunctional Drug Delivery System for Cancer Treatment

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Abstract: Zein is a type of prolamine protein that is derived from corn, and it has been recognized by the US FDA as one of the safest biological materials available. Zein possesses valuable characteristics that have made it a popular choice for the preparation of drug carriers, which can be administered through various routes to improve the therapeutic effect of antitumor drugs. Additionally, zein contains free hydroxyl and amino groups that offer numerous modification sites, enabling it to be hybridized with other materials to create functionalized drug delivery systems. However, despite its potential, the clinical translation of drug-loaded zein-based carriers remains challenging due to insufficient basic research and relatively strong hydrophobicity. In this paper, we aim to systematically introduce the main interactions between loaded drugs and zein, administration routes, and the functionalization of zein-based antitumor drug delivery systems, in order to demonstrate its development potential and promote their further application. We also provide perspectives and future directions for this promising area of research.

Keywords: zein, nanomedicine, drug delivery, antitumor

Introduction

Cancer is a growing concern globally, with an estimated 19.3 million new cases and 10 million cancer-related deaths reported in 2020 alone. Unfortunately, the situation may worsen in the future, with projections indicating a 47% rise in global cancer cases by 2040, to reach 28.4 million.[1] Currently, various therapeutic approaches are employed to treat cancer, including chemotherapy, surgery, and radiotherapy.[2] While chemotherapy remains the primary treatment for cancer, it comes with several drawbacks. Although it can improve the survival and quality of life for cancer patients, it also affects blood-forming cells in the bone marrow, hair follicles, and cells in the digestive tract and reproductive system.[3,4]

Nanoparticle technology is a promising new approach for cancer therapy, offering several advantages over traditional methods. For one, it can improve the bioavailability of insoluble drugs, control drug release, and reduce side effects.[5,6] Additionally, nanoparticles can passively target cancer cells thanks to the enhanced permeability and retention (EPR) effect, which leads to increased accumulation of drug-loaded particles within tumors.[7] Recent studies, however, have shown that nanoparticles primarily enter tumors through endothelial cells, rather than through gaps between them.[8] Although nanoparticles are typically administered intravenously, there are now oral and pulmonary-inhalation methods

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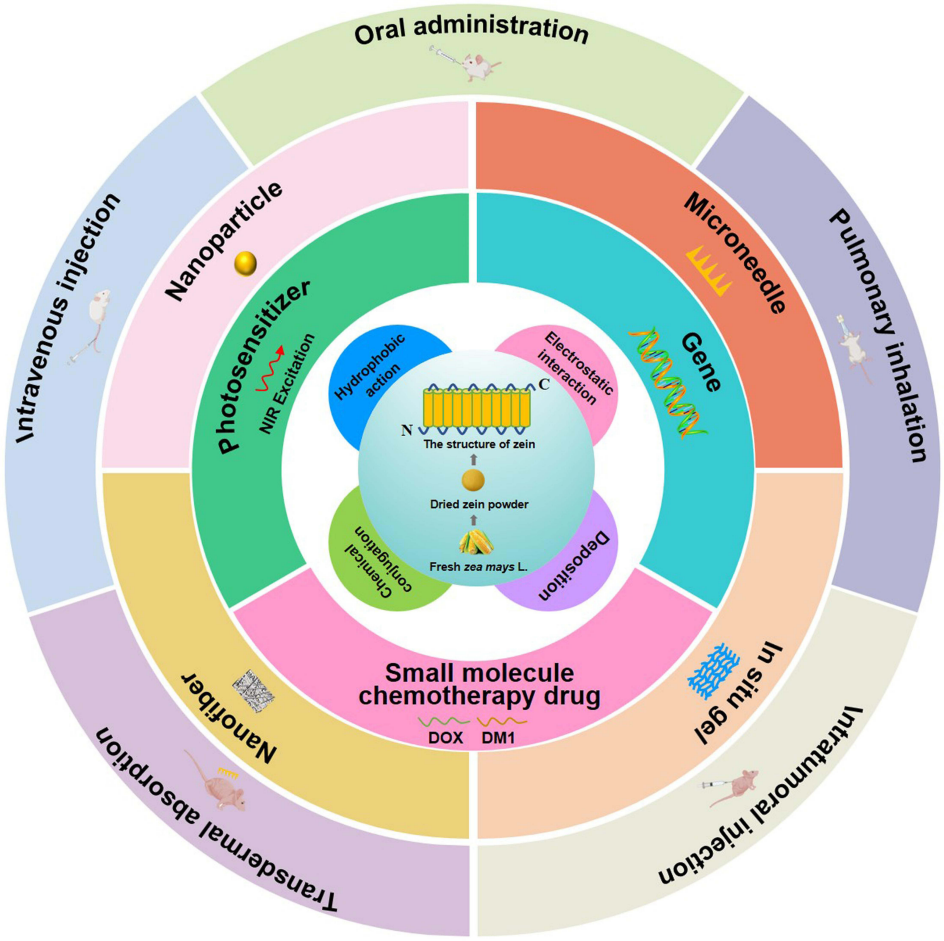
Graphical Abstract



available, which can improve patient compliance.[9,10] To enhance the effectiveness of nanoparticle-based therapies, researchers have designed functionalized nanoparticles that can precisely deliver drugs to cancer cells based on the unique characteristics of the tumor microenvironment. These nanoparticles can be engineered to be pH-sensitive or GSH-sensitive, or to target highly expressed receptors on the tumor surface.[11–13] Nanoparticles can combine phototherapy, chemotherapy, and immunotherapy to further inhibit drug resistance and tumor cell migration.[14] Overall, nanoparticle technology offers a promising new approach to cancer therapy, with the potential to improve patient outcomes and reduce side effects.

In the past few years, researchers have explored a variety of inorganic and organic materials as micro/nano delivery carriers for anticancer drugs. These materials include nonmetallic and metal inorganic materials, as well as natural polymers, liposomes, exosomes, and dendrimers.[15] Currently, 16 anti-tumor nano drugs have been approved for marketing, not including polymer drug conjugates or antibody drug conjugates.[16] Additionally, there are nearly 200 clinical trials involving a large number of nano drugs in various stages of development. In completed studies, the success rate of Phase I was about 94%, but dropped to 53% in Phase II and 18% in Phase III. Low effectiveness is the primary reason for clinical failure, and the toxicity and side effects of nano drugs are also major concerns.[17,18] For example, liposomal encapsulation of doxorubicin with surface-bound methoxypolyethylene glycol (Caelyx®) is known to be less cardiotoxic and nephrotoxic than unbound doxorubicin, but it produces more dermal lesions primarily on the feet and legs.[19] Side effects on the immune system were reported for three out of the four nanomedicinal liposome products, including

liposomal amphotericin B, pegylated liposomal doxorubicin, and liposomal daunorubicin.[19] To address these challenges, active-targeting nanomedicine drugs have become a research hotspot for drug delivery systems, offering a new way to clinically treat cancer.[16] Therefore, it's essential to find a new delivery carrier for anti-tumor drugs that is highly efficient, has low toxicity and side effects, and can be easily functionalized.

Zein is a type of prolamine protein derived from corn and has been recognized by the US FDA as one of the safest biological materials. It is also the most extensively researched plant protein due to its low immunogenicity, amphipathy, edibility, biodegradability, biocompatibility, and gastrointestinal resistance. Because of these advantages, zein is highly favored in the research of improving oral bioavailability and achieving sustained and targeted drug delivery.[20–23] Its brick-like structure (Figure 1) allows for the interception and encapsulation of drugs, while the N-terminal region of γ-zein can interact with the cell membrane, making it a useful carrier for drugs that cross the cell membrane. Zein also contains free hydroxyl and amino groups that can serve as more modification sites, making it a versatile material for functionalized drug delivery.[24–27] Currently, zein-based drug delivery systems include nanoparticle,[28] nanofiber,[29] microneedle[30] and hydrogels[31] prepared by hydrophobic interaction, chemical conjugation, and electrostatic interaction with drugs. These systems have successfully delivered small-molecule chemotherapeutic drugs, anti-tumor genes, and photosensitizers through various administration routes such as intravenous injection, oral administration, pulmonary inhalation, transdermal absorption, and intratumoral injection. Additionally, due to its hydrophobicity, zein gel can also be used to form 4D-printing drug delivery systems that can change shape, property, and function over time when stimulated.[32]

This paper aims to systematically introduce the advantages and disadvantages of current antitumor drugs and how zein-based drug delivery systems can improve their effectiveness (Figure 1). It also discusses the administration routes and functionalization of zein-based nanoparticles, in order to promote further development and application of zein as an antitumor drug carrier.

Drug Loading

Zein has shown promising results in delivering various types of antitumor drugs, including small-molecule chemotherapeutic drugs, genes, and photosensitizers. Due to its amphiphilic nature, zein can interact with drugs through

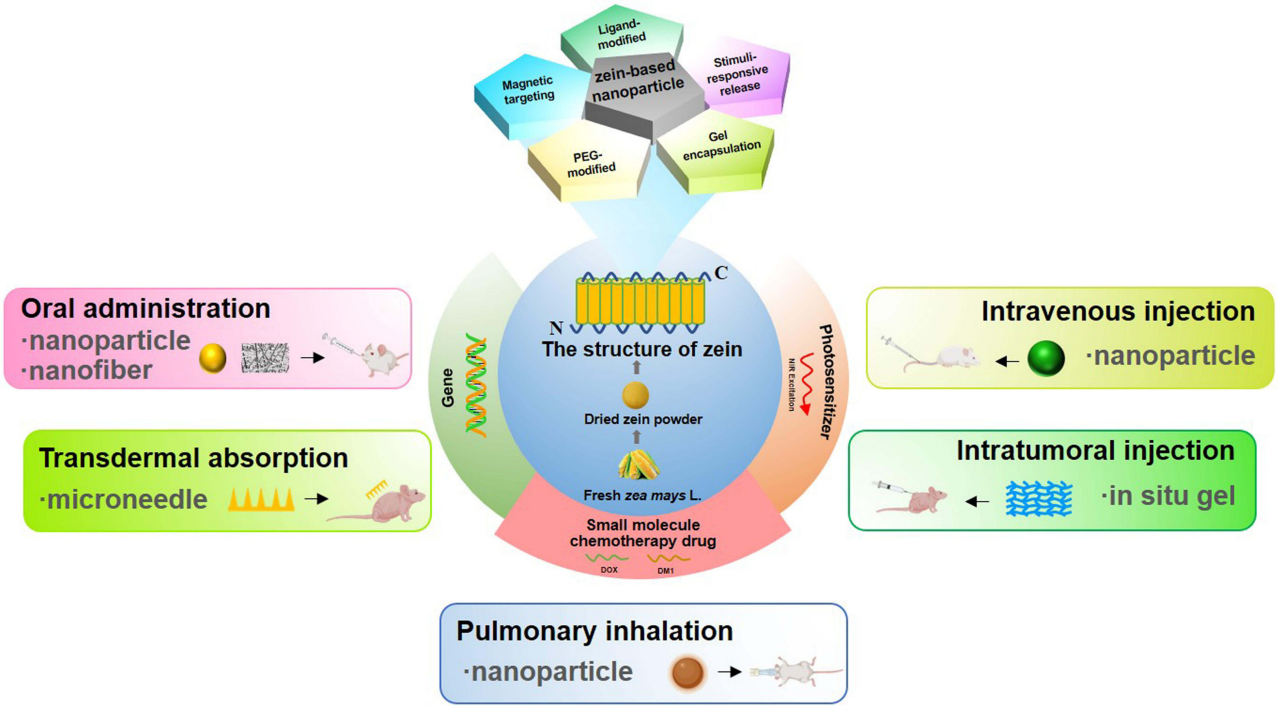


Figure 1 The origin and structure of Zein, the drug-loading zein-based carriers and appropriate routes of administration, and the functionalization of zein-based nanoparticle.

hydrophobic interactions, as well as chemical conjugation and electrostatic interaction, thanks to its free hydroxyl and amino groups that exhibit different charges under different pH conditions. Additionally, metal nanoparticles or phenolic acid/metal ion membranes can be deposited on zein-based nanoparticles to enhance drug delivery efficiency. In the following sections, we will discuss the different types of antitumor drugs that can be loaded onto zein carriers and the primary mechanisms of interaction between zein and these drugs.

Small Molecule Chemotherapy Drug

Currently, chemotherapy is one of the main methods used to treat tumors,[33] Commonly used drugs include doxorubicin,[34] paclitaxel,[35] and camptothecin,[36] etc. Although these drugs have strong antitumor activity, their clinical application is limited due to drawbacks such as cellular drug resistance, systemic toxicity, poor water solubility and short half-life period.[37–39] To address these limitations, zein, with its amphipathy, can form nanoparticles by self-assembly. The hydrophobic inner core can effectively encapsulate lipid-soluble drugs through hydrophobic interaction and other forces to improve the solubility of the drugs. Additionally, based on the enhanced permeability and retention (EPR) effect, zein-based nanoparticles can reduce the distribution of the drugs in normal tissue, minimizing the side effects of drugs. For example, Fangyuan Dong[40] prepared doxorubicin/zein nanoparticles (DOX-zein-NPs) by self-assembly of zein through hydrophobic interaction and hydrogen bonding, which effectively enhanced the water solubility of doxorubicin. Compared with the non-specific rapid release of free doxorubicin, DOX-zein-NPs slowly released DOX under normal extracellular pH conditions and rapidly released DOX under lower intracellular pH conditions. This phenomenon indicated that zein-based nanoparticles can prolong blood circulation of the drug, reduce cytotoxicity to normal cells, and enhance targeted cytotoxicity to specific tumor cells. Maytansine (DM1) is a potent inhibitor of tubulin polymerization that can effectively treat various malignancies, including breast cancer, melanoma, multiple myeloma, liver cancer, and lung cancer.[41–43] However, its clinical application is limited by strong side effects, narrow therapeutic window, and poor water solubility.[44] Xianglong Yu[45] prepared DM1-loaded ZNPs through hydrophobic interaction. In vitro release experiments showed that the ZNPs released about 20% of DM1 within the first 8 hours and about 40% after the second 24 hours, indicating a good controlled release effect. When fetal bovine serum (FBS) was used to evaluate the stability of ZNPs under physiological conditions, the results showed that only a small amount of DM1 leaked into the serum within 24 hours, and there was no obvious adsorption or precipitation in the reaction solution, indicating that the DM1-loaded ZNPs could remain stable in the serum. In vitro cytotoxicity experiments showed that free DM1 had a dose-dependent antiproliferative activity, while ZNPs loaded with DM1 had no cytotoxicity, indicating that the encapsulation of zein effectively reduced its killing effect on normal cells.

Photosensitizer

Phototherapy is a treatment for tumors that includes reactive oxygen species (ROS)-mediated photodynamic therapy (PDT) and fever-mediated photothermal therapy (PTT). This treatment has several advantages, such as less side effects, less drug resistance, and faster recovery.[46] NIR light absorbing dyes like indocyanine, napthalocyanines and porphyrins coordinated with transition metals have been used for light mediated therapeutics.[47–52] However, many photosensitizers used in phototherapy have poor selectivity to lesions, poor targeting, poor water solubility, and low bioavailability.[46] To overcome these limitations, zein, an amphiphilic nanomaterial, can self-assemble into nanoparticles through hydrophobic interaction to encapsulate photosensitizers, thereby improving their solubility and achieving tumor targeting.

For example, indocyanine green (ICG), as a fluorescent dye approved by FDA for clinical use, has been studied for photodynamic therapy and photothermal therapy of tumors.[53–56] However, its water solubility is very poor. Therefore, it is prone to aggregation and precipitation in aqueous solutions, leading to self-quenching and reduced emission intensity. Eun-Hye Lee[28] encapsulated it in zein phosphatidylcholine hybrid nanoparticles (Z/PC-NP) nanoparticles. During 10 days of storage in free ICG solution, its absorption peak at 780 nm decreased in a time-dependent manner. At the same time, a new absorption peak appeared and increased at 894 nm, indicating the formation of ICG aggregates. In contrast, Z/PC-NP (zein 3–7 mg) had no 894nm peak during the 10-day incubation period. This indicates that the ICG-zein interaction in Z/PC-NP reduces the interaction between ICG molecules and effectively inhibits the aggregation of ICG.

Cytotoxicity experiments showed that free ICG could not significantly inhibit the growth of A253 cells. In contrast, the Z/PC-NP formulation significantly inhibits cell proliferation and retains the photosensitizing activity of ICG.

Integration of diagnosis and treatment is a promising cancer treatment strategy, and gold nanoparticles (GNPs) can be used as a contrast agent and activated by increasing the local temperature to kill tumor cells.[57–59] However, low photothermal conversion efficiency and photostability, poor water solubility, and tumor-targeting ability moderate its applicability.[60,61] To address these issues, Deepak S. Chauhan et al[62] demonstrated the facile and green synthesis of gold deposited zein nanoshells (AuZNS) using environmental benign solvent ethanol (Figure 2). AuZNS was of size around 100 nm. When even given double the therapeutic dosage, AuZNS still showed high inertness and biocompatibility. They used two different cancer cell lines viz. MCF-7 (breast cancer) and C33A (cervical cancer) to evaluate its antitumor effect. The results showed almost equal therapeutic effect. The absorbance was tuned at 808 nm for imaging-guided plasmonic photothermal therapy of cancer. AuZNS also exhibited better X-ray attenuation in comparison to the commercially available iodine-based contrast agent.

Combination therapy has been proposed to improve the therapeutic efficacy, including the combination of chemotherapeutic agents, chemo-energy, chemo-gene, chemo-small molecules, and chemo-immunology.[14,63] Xianglong Yu[14] prepared an "all-in-one" and "one for all" nanoplatform, which combined "chemo−immuno−photo-thermal therapy" (Figure 3). Specifically, Docetaxel (DTX, a chemo-agent) and cynomorium songaricum polysaccharide (CSP, an

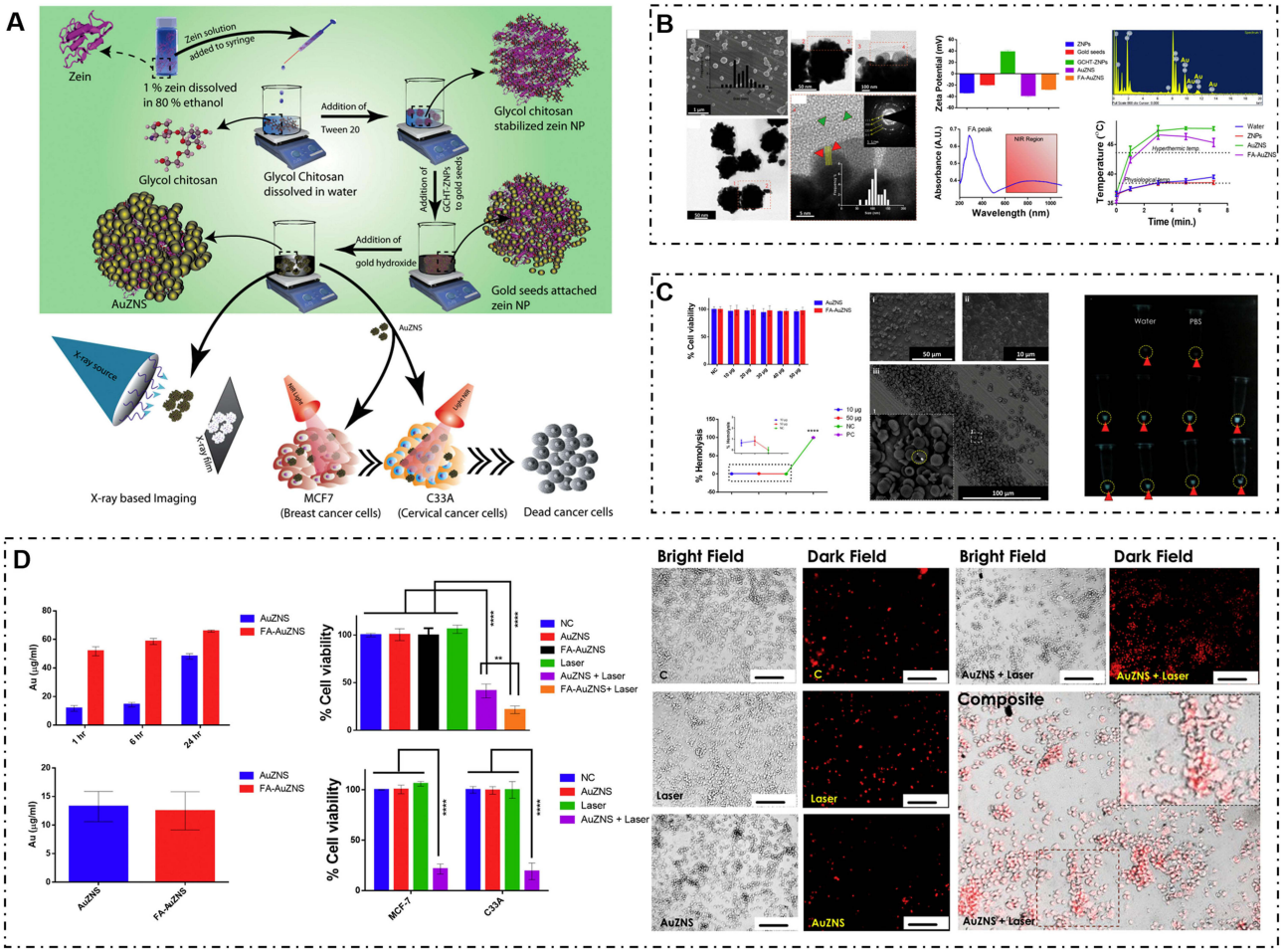


Figure 2 (**A**) Illustration showing the green synthesis and application of AuZNS for imaging-guided plasmonic photothermal therapy. (**B**) Micrographic images and size distribution; Zeta potential measurement, elemental analysis, absorbance spectrum, and photothermal transduction. (**C**) Biocompatibility and hemolysis study; X-ray of negative control (top row), AuZNS (middle row) and Omnipaque (bottom row) taken at different conc. (**D**) Qualitative analysis of non-targeted photothermal therapy on C33A cells using propidium iodide; Uptake study, targeted and non-targeted photothermal therapy. **p * 0.01, ****p * 0.0001. Reprinted with permission from Chauhan DS, Arunkumar P, Prasad R, et al. Facile synthesis of plasmonic zein nanoshells for imaging-guided photothermal cancer therapy. Mater Sci Eng C Mater Biol Appl. 2018;90:539–548. Copyright 2018. Elsevier.[62]

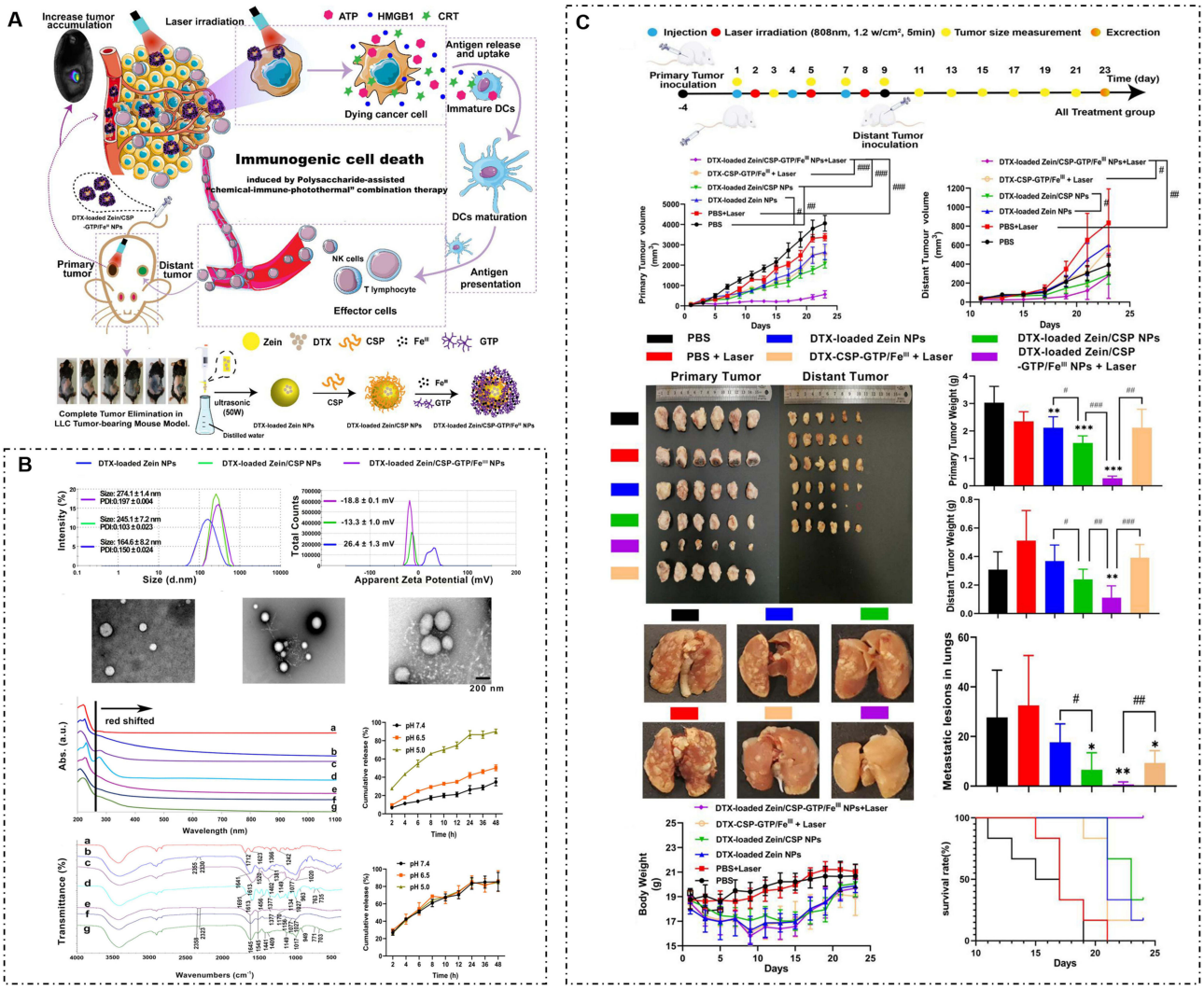


Figure 3 (**A**) Schematic diagram of the "all-in-one" and "one-for-all" nanoplatform for combined "chemo–immuno–photothermal" therapy. (**B**) Fabrication and characterization of DTX-Loaded Zein/CSP-GTP/FeIII NPs and the in vitro drug release. (**C**) In vivo therapeutic efficacy of various treatments on 4T1 tumor-bearing mice. *P < 0.05; **P < 0.01; ***P< 0.001 compared to control. #, P < 0.05; ##, P < 0.01; ###, P < 0.001. Reprinted with permission from Yu X, Han N, Dong Z, et al. Combined Chemo-Immuno-Photothermal Therapy for Effective Cancer Treatment via an All-in-One and One-for-All Nanoplatform. ACS Appl Mater Interfaces. 2022;14 (38): 42,988–43,009. Copyright 2022, American Chemical Society.[14]

immunomodulator) were loaded into zein nanoparticles coated by a green tea polyphenols/iron coordination complex (GTP/Fe^{III}, a photothermal agent). This nanoplatform was spherical in morphology with an average particle size of 274 nm, and achieved pH-responsive drug release. In the pharmacodynamic test, it can effectively destroy the tumors, remove the metastatic lesions, and prevent tumor recurrence by inducing the ICD (immunogenic cell death) effect and building long-lived antitumor immune responses.

Gene

Gene therapy is a promising new approach for cancer treatment, offering benefits such as gene delivery and gene silencing.[64–67] However, free genes can be easily degraded, have difficulty reaching the target site, and can cause toxicity to normal tissues during delivery.[68–71] Nanoparticle carriers are effective in controlling drug release, actively and passively targeting tumor sites, protecting the loaded drugs in and out of cells, as well as promoting the cellular uptake or subcellular transport.[72] Therefore, they are ideal gene delivery carriers. Zein, a protein containing hydrophobic amino acids and polar glutamine, can self-assemble to form nanoparticles due to its amphiphilicity. Zein's polar side chain can interact with negatively charged DNA to load genes through electrostatic interaction, making it an ideal gene therapy

delivery vehicle.[73,74] For instance, Fathia Zaki El Sharkawi[75] prepared zein nanoparticles loaded with plasmids encoding PTEN and TRAIL, and the morphology of the prepared DNA-loaded ZNPs was investigated by transmission electron microscopy (TEM). The results show that the prepared ZNPs have solid dense structure, round uniform shape and rough surface, which may be due to the surface-adsorbed DNA on the ZNPs. Compared with the normal control group, the expression level of p53 in the liver cancer-induced animals was significantly decreased (P value < 0.01). However, p53 expression was induced in the PTEN and TRAIL gene-loaded nanoparticles group compared with the untreated group ($p<0.0001$, $p<0.01$). VEGF was highly expressed in HCC-induced animals. On the other hand, an anti-angiogenic effect of the genes was observed in the nanoparticle group, with significantly lower expression levels of VEGF compared to untreated animals. In addition, PTEN and TRAIL in ZNPs significantly inhibited liver metastasis, reducing MMP-2 expression in liver homogenates of treated animals compared to untreated animals.

Loading Loading Efficiency and Their Limitations

Table 1 summarized the different loading methods and their different applications. As shown in Table 1, almost all the zein-based nanoparticles have a content encapsulation and loading efficiency. There are even individual nanoparticles with a loading efficiency of over 80%. However, when the encapsulation and loading efficiency is high enough, we can find that the particle size of most of zein-based nanoparticles has been over 150 nm. To reduce systematic clearance and enhanced the penetration and internalization of tumors, the nanoparticles with diameter of 12–50 nm are most appropriate.[5] Additionally, monocytes and reticuloendothelial systems can readily remove drug carriers with diameters larger than 200 nm.[76] Therefore, in theory, the therapeutic efficacy of these zein-based nanoparticles still needs to be improved. As for the surface charge, while almost all zein-based nanoparticles have a surface charge with an absolute value exceeding 30 mV, making them relatively stable and resistant to aggregation, the charge can also impact their circulation and recognition by the immune system.[77,78] Additionally, negatively charged endothelial cells can also cause retention of cationic nanoparticles, thereby reducing their circulation.[79,80] Conversely, negatively charged nanoparticles are repelled from the cell-free layer, limiting their extravasation.[5] Cationic nanoparticles are more conducive to transvascular transport and tumor penetration.[5] Therefore, to optimize the therapeutic efficacy of zein-based nanoparticles, their surface charge needs to be improved to balance their circulation, transvascular transport and tumor penetration, while avoiding recognition and clearance by the immune system.

Route of Administration

Currently, the main routes of administration of anticancer drugs include intravenous injection, oral administration, transdermal absorption, intratumoral injection and pulmonary inhalation. However, each of these routes has its own advantages and disadvantages.[90] To overcome some of these limitations, zein, with its many excellent characteristics (Figure 4), has been proposed as a drug carrier for various routes of administration. In the following sections, we will discuss the potential applications of zein as a drug carrier in each of these five routes of administration.

Intravenous Injection

Intravenous drug administration is a popular and effective route of administration due to its high bioavailability, minimal irritation to other body parts, and ability to bypass the gastrointestinal environment. However, there are also some disadvantages associated with intravenous injection. For instance, the drug system needs to be highly pure to prevent infections, and the dosage is limited, requiring multiple injections that can decrease patient compliance. Additionally, most anticancer drugs have low water solubility, making them prone to aggregation in the bloodstream or clearance by macrophages and the liver, ultimately reducing their efficacy. Fortunately, these issues can be addressed to some extent by encapsulating the drugs in nanoparticles.

Zein is a natural, biodegradable, and low immunogenicity amphiphilic substance that can be used as a drug carrier material. It is a vegetable protein, which makes it safer and reduces the risk of zoonotic diseases. Zein can assemble into nanoparticles through hydrophobic interaction with drugs, increasing the water solubility of the drugs and reducing their clearance by macrophages or the liver. Zein-based nanoparticles can also effectively control the drug release, prolong the drug's residence time in the blood circulation, and reduce the number of administrations. So zein is an ideal carrier

Table 1 Different Loading Methods and Their Applications About Zein-Based Nanoparticles

Loading Methods	Anti-Tumor Drug	Formulation	Particle Size (nm)	PDI	Zeta Potential (mV)	Encapsulation Efficiency (%)	Loading Efficiency (%)	Ref.
Hydrophobic action	Doxorubicin	Sodium caseinate, zein	204.53 ± 1.53	0.17 ± 0.02	−45.20 ± 0.21	90.06 ± 0.26	15.01 ± 0.14	[40]
	Maytansine	Zein	112.3 ± 6.16	0.213 ± 0.02	37.0 ± 1.14	82.97 ± 0.80	3.32 ± 0.03	[45]
	Thymoquinone	Polyvinyl alcohol, zein	175 ± 8.2					[81]
	Pterostilbene	Zein	104.5 ± 6.2	0.16 ± 0.01	33.4 ± 1.8	95.1 ± 3.6		[82]
	5-fluorouracil	Zein	114.9 ± 59.4		−45 ± 0.3	60.7 ± 1.74	9.17 ± 0.11	[92]
	Lovastatin	Zein	67.2 ± 4.1		24.08 ± 3.4	86.10 ± 5.31		[83]
	Beta carotene	Zein	92.3 ± 3.6	0.187 ± 0.02	–25.91 ± 0.3	68.2 ± 2.3		[145]
	Curcumin	Pegylated Zein	124 ± 4	0.25 ± 0.03	−7 ± 1.6	95 ± 4		[196]
	Piceatannol	Sodium deoxycholate, zein	157.45 ± 1.62			93.14 ± 2.15		[105]
	Resveratrol	Low-molecular-weight sodium hyaluronate, zein	152	0.102		62.52		[84]
	Broccoli extract	Zein				86.9 to 99.3		[85]
	VORINOSTAT, bortezomib	Lecithin, zein	Approximately 160	Approximately 0.20	Approximately −25	Both approximately 60	Both approximately 2.8	[86]
	Exemestane, resveratrol	Glutaraldehyde, zein	141.4 ± 2.2		Approximately −34	96.0 (exemestane), 95.4 (resveratrol)		[101]
	Resveratrol	Zein, sodium caseinate, chitosan	295 ± 38	0.20 ± 0.04	29.7 ± 1.2	51 ± 2		[21]
	Curcumin, piperine	Zein, chitosan	550			92 (curcumin), 87 (piperine)		[102]
	Tretinoin, genistein	Glyceryl monostearate, stearyl amine, zein	154.5 ± 1.5	0.18 ± 0.007	32.5	90.2 (genistein), 97 (tretinoin)		[87]
	Luteolin	Sodium caseinate, zein	215.4 ± 8.11	0.258 ± 0.07	−14.72 ± 3.22	Approximately 92	Approximately 7.5	[100]

	Carvacrol	Lecithin, zein	249.73 ± 9.97		−14.96 ± 0.36	78.11 ± 1.50	13.01 ± 0.25	[104]
	Indole-3-carbinol	Carboxymethyl chitosan, zein	113.5 ± 2.7	0.19 ± 0.01	19.53 ± 2.26	77.79 ± 3.79		[103]
	3,30-diindolylmethane	Carboxymethyl chitosan, zein	89.1 ± 4.3	0.19 ± 0.00	19.80 ± 0.80	78.08 ± 0.69		
	Paclitaxel	Sodium carboxymethyl cellulose, zein	189.1 ± 0.5	0.27 ± 0.01	54.4 ± 1.36	95.5 ± 0.20		[88]
	Honokiol	Hyaluronic acid, zein	Approximately 210.4		−34.54 ± 2.8	88.1 to 93.6		[12]
	Dasatinib	Oleic acid, Fe_3O_4, lactoferrin, zein	232.4 ± 20.33	0.259 ± 0.02	41.93 ± 3.34	83.01 ± 4.98	7.42 ± 0.47	[191]
	Etoposide, all-trans retinoic acid	Zein, chondroitin sulfate, $CaCl_2$	222.7 ± 3.29	0.307	−13.6 ± 2.5	66 (etoposide), 84.67 (all-trans retinoic acid)	2.26 (etoposide), 2.57 (all-trans retinoic acid)	[138]
	Docetaxel	Chondroitin sulfate, zein	157.8 ± 3.6	0.20 ± 0.01	−26.8 ± 0.4	64.2 ± 1.9		[139]
	Curcumin	Hyaluronic acid, zein	Approximately 250	Approximately 0.20	Approximately −60	94.15	9.41	[137]
	Genistein, all-trans retinoic acid	Glyceryl monostearate, stearyl amine, zein	206.0 ± 2.5	0.2	+37.0 ± 1.8	87.5 ± 1.4 (genistein), 98.7 ± 2.8 (all-trans retinoic acid)		[135]
	Rapamycin, wogonin	Lactoferrin, glutaraldehyde, zein	212.2 ± 13.4	0.206 ± 0.02	25.9 ± 4.26	98.00 ± 4.90 (wogonin), 61.90 ± 3.10 (rapamycin)	4.67±0.23 (wogonin), 5.83±0.29 (rapamycin)	[146]
	10-hydroxycamptothecin	Folic acid, zein	404.0 ± 70.15		−32.8 ± 4.90	91.97 ± 2.45	5.75 ± 0.15	[140]

(*Continued*)

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Table 1 (Continued).

Loading Methods	Anti-Tumor Drug	Formulation	Particle Size (nm)	PDI	Zeta Potential (mV)	Encapsulation Efficiency (%)	Loading Efficiency (%)	Ref.
	Gefitinib	Folic acid, SPIONs, zein	292		−46.8 ± 1.2	80.95 ± 1.47	6.97 ± 0.13	[190]
	Doxorubicin	Folic acid, zein	Approximately 210		Approximately 15	88.56	1.434	[141]
	Exemestane, luteolin	Lactoferrin, zein	272.4 ± 3.7	0.28 ± 0.03	+16.3	57 (exemestane), 50 (luteolin)		[89]
	Exemestane, luteolin	Polyethylene glycol, zein	207.0 ± 0.7	0.34 ± 0.01	−27.4	78.3 (exemestane), 90.3 (luteolin)		
	Doxorubicin	Tannic acid, Cu^{II}, zein	178.9 ± 2.6	0.20 ± 0.04	−46.1 ± 0.67	92.7 ± 3.6		[161]
	Doxorubicin	Tannic acid, Fe^{III}, zein	188.5 ± 1.4	0.23 ± 0.03	−41.1 ± 0.35	95.6 ± 2.9		
	Nobiletin	Tannic acid, Fe^{III}, zein	143.3 ± 4.5	0.29 ± 0.06	−34.6 ± 1.2	97.4 ± 0.6	80.7 ± 0.5	[162]
	Nobiletin	Tannic acid, Al^{III}, zein	165.1 ± 3.8	0.33 ± 0.04	−35.7 ± 1.1	96.8 ± 0.8	80.9 ± 0.7	
	Gambogenic acid	Polydopamine, zein	312.2 ± 3.6	0.212 ± 0.027	−40.8 ± 0.9	82.18 ± 4.5	26.65 ± 2.9	[167]
	Doxorubicin hydrochloride	Hydroxyapatite, zein	207.2 ± 7.23	0.150 ± 0.021	−27.7 ± 2.45	44.75 ± 0.27		[170]
	Ellipticine	Sodium caseinate, zein	166.7 ± 3.8	0.081 ± 0.020	−23.5 ± 0.4			[76]
	Ellipticine	Poly ethylene imine, zein	137.6 ± 3.9	0.060 ± 0.015	+24.9 ± 2.5			
	Hydroxycamptothecin	AuNPs, polydopamine, folic acid, zein	189.7 ± 9.5	0.142	−40.6 ± 5.5	93.62	8.76	[174]
	Indocyanine green	Phosphatidylcholine, zein	216 ± 33	0.210 ± 0.029	−47.9 ± 1.4			[28]
	Curcumin	Gum arabic, zein	188.8	0.13	−30.0	92.94 ± 3.15	3.57 ± 0.12	[107]
	Curcumin	Hyaluronic acid, zein	Approximately 300	0.33	−56.9	97.24 ± 2.23	7.48 ± 0.17	
	Curcumin	Pectin, zein	346.4	0.25	−34.4	95.05 ± 1.74	6.79 ± 0.12	

Electrostatic interaction	PTEN or TRAIL	Zein	132.5 ± 39.95		−37.1 ± 8.63	40.79 ± 16.72		[75]
Chemical conjugation	Paclitaxel	Bis(2-hydroxyethyl) disulfide, zein	229.9 ± 0.3	0.2 ± 0.011	−40.5 ± 0.4		0.696	[13]
Deposition	AuNPs	Chitosan, zein	Approximately 115		−38.27 ± 2.36			[62]
Hydrophobic action and chemical conjugation	Celastrol, sulfasalazine	Chondroitin sulphate, SPIONs, oleic acid, zein	154.4 ± 2.8	0.202	−34.8 ± 0.25	86.7 ± 0.32 (celastrol)	12.84 ± 0.12 (celastrol), 2.01 ± 0.10 (sulfasalazine)	[189]
Hydrophobic action, electrostatic interaction and deposition	Cynomorium songaricum polysaccharide, docetaxel, green tea polyphenols	Zein	274.1 ± 1.4	0.198	−18.8 ± 0.1	44.96 (docetaxel), 74.9 ± 3.6 (cynomorium songaricum polysaccharide), 96.5 ± 0.3 (green tea polyphenols)	1.22±0.07 (docetaxel), 67.07 ±1.05 (cynomorium songaricum polysaccharide), 3.96 ± 0.01 (green tea polyphenols)	[14]

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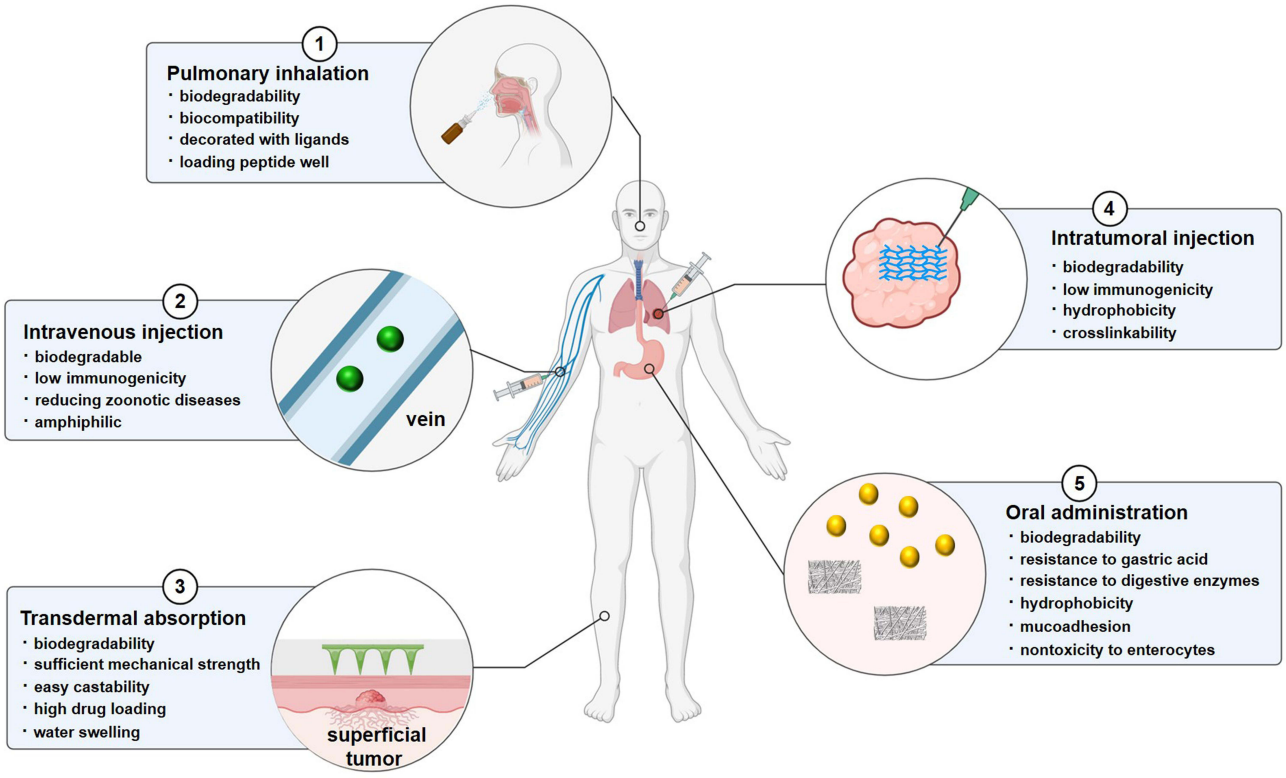

Figure 4 Routes of administration of zein carriers and the main properties of zein used in each route of administration.

material for intravenous injection of drugs. 5-Fluorouracil (5-FU) has a long history of use as a chemotherapeutic agent. But less than 20% of an injected dose undergoes enzymatic activation.[91] The oral bioavailability of 5-FU is unpredictable due to high variability in enzymatic degradation. In addition, 5-FU has a relatively short half-life period and toxicity to the bone marrow and the gastrointestinal tract.[91] To solve above problems, Lai[92] prepared zein nanoparticles (ZP) loaded with 5-fluorouracil (5-FU). The optimized ZPs have an average size of 114.9 nm, which can target liver tumors through the EPR effect. Moreover, the ZPs also showed sustained release properties in vitro and effectively prolonged the drug's residence time (7.2-fold increase) in blood circulation.

Oral Administration

Due to the simple administration method, no direct damage to skin or mucous membrane and relatively low cost of production, oral administration has high patient compliance and is the most common and convenient route of administration.[93,94] However, the harsh gastrointestinal environment poses challenges for achieving satisfactory levels of bioavailability through oral administration. Zein, with its resistance to gastric acid and digestive enzymes, hydrophobicity, biodegradability, mucoadhesion, and nontoxicity to enterocytes, offers several advantages that can compensate for these deficiencies.[93,95,96] Therefore, encapsulating the drugs into zein-based carrier can effectively increase the bioavailability of the drugs and improve their therapeutic effect. Currently, zein-based oral drug delivery system include oral nanoparticles and oral nanofibers. Details will be given below.

Oral Nanoparticle

Zein's amphipathic nature allows it to self-assemble into nanoparticles that can be loaded with drugs. Because of its inherent hydrophobicity and resistance to gastrointestinal conditions,[93,95,97–99] zein-based nanoparticles can protect drugs and achieve controlled release, without damaging the gastrointestinal tract. This makes zein-based nanoparticles a promising nanocarrier for oral drug delivery. For instance, Shinde[100] prepared zein nanoparticles loaded with luteolin, which successfully increased the solubility of luteolin, improved its oral availability, and achieved sustained release of

the drug in the intestine. To further improve the stability, penetration, and colon targeting ability of antineoplastic drugs in the gastrointestinal tract, in some cases, zein-based nanoparticle has been proposed to be prepared in combination with other excipients, such as glutaraldehyde,[101] chitosan,[21,102] carboxymethyl chitosan,[103] sodium caseinate,[100] soybean lecithin[104] and bile salts,[105] etc. Phuong H.L. Tran[106] has discussed this in detail in his recently published article, so they will not be repeated here. But recently, there was a very interesting study. Lu Liu[107] prepared CUR-encapsulated nanoparticles, which were fabricated with zein alone (Zein-CUR) and with zein and a polysaccharide (PS) such as gum Arabic (GA), hyaluronic acid (HA) and pectin (PC), respectively (PS-Zein-CUR) (Figure 5). The results showed the three PS-Zein-CUR formulations had significantly higher (17–22%) CUR encapsulation efficiency (EE) than Zein-CUR. They also effectively inhibited cell viability and colony formation.

Oral Nanofiber

Drug-loaded zein nanofibers is another oral antitumor drug delivery system. Nanofibers prepared by electrospinning technology can achieve efficient drug loading and slow release, and are commonly used in wound healing.[108] Moreover, nanofibers can control the drug release rate by modification of the degradation rate of the fibers.[108] Until recently, electrospun fibers were tested for oral administration of poorly soluble or unstable drugs.[109] It is well known that increasing the contact area between the drug and the solvent can effectively promote the dissolution of the drug. Nanofibers have a larger area, which means can improve the solubility of the drugs. For example, formulation of diosmin or flubendazole in electrospun nanofibers can make the drugs completely amorphizate, thereby resulting in a very rapid release and increasing the bioavailability of the drug. Zein has been widely used as a carrier for preparing

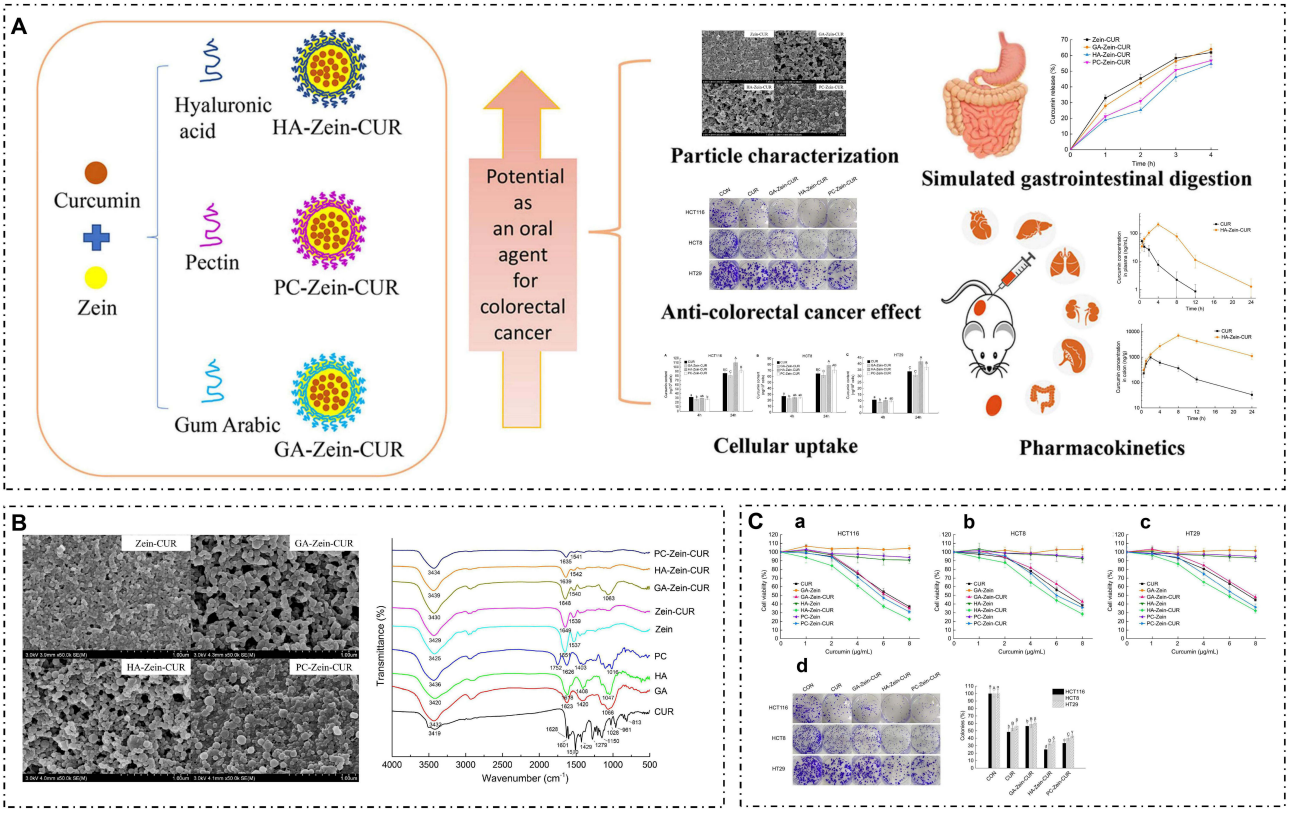

Figure 5 (**A**) Experimental scheme. The evaluation of the potential of PS-Zein-CUR an oral agent for CRC therapy: physicochemical properties, anti-CRC effects, cellular uptake, and gastrointestinal simulated digestion in vitro and pharmacokinetics and tissue distribution in vivo. (**B**) Spectroscopic analysis of composite nanoparticles. (**C**) Inhibitory effect of PS-Zein-CUR on CRC (HCT116, HCT8, and HT29) cell growth. Cells were treated with the indicated doses of CUR, PS-Zein, and PS-Zein-CUR for 48 h, respectively. (a–c) Cell viability measured by using a CCK-8 cell counting kit. (d) Left: images of colony formation assay; Right: quantitative analysis of colony formation expressed as percentage relative to vehicle control. Bars marked with different letters indicate significant difference at $P < 0.05$. Reprinted with permission from Liu L, Yang S, Chen F, et al. Polysaccharide-Zein Composite Nanoparticles for Enhancing Cellular Uptake and Oral Bioavailability of Curcumin: Characterization, Anti-colorectal Cancer Effect, and Pharmacokinetics. Front Nutr. 2022;9: 846,282. Copyright 2022, Frontiers.[107]

nanofibers,[110,111] and it is also a good carrier for oral drugs.[97,98] Therefore, the use of oral nanofibers prepared with zein has a good application prospect. Francisca Acevedo[29] prepared gallic acid (GA)-loaded polyethylene oxide/zein nanofibers by coaxial electrospinning. Under the optimal process conditions, the loading efficiency of GA was as high as 77%. The release of GA was effectively controlled in both acidic and neutral pH media. Compared with free GA, the loading by zein nanofibers effectively increased the cytotoxicity of GA against gallbladder cancer cell lines GB-d1 and NOZ, indicating that zein-based oral nanofibers are a promising drug delivery system.

Transdermal Absorption

Transdermal drug delivery (TDD) offers enormous advantages over oral, nasal, intramuscular and intravenous routes of administration, such as painless administration, good patient compliance, and self-administration.[112] Transdermal drug delivery systems (TDDSs) not only facilitate the sustained transport of therapeutic drugs through the skin, but also help drugs overcome certain barriers (such as first-pass metabolism), improving the transport of drugs with low solubility and bioavailability.[113] Microneedles, a new type of transdermal drug delivery technology, consist of a series of micron-sized arrays in a patch. Microneedle technology has good patient compliance, great permeability, and better drug efficacy compared to traditional transdermal absorption methods such as subcutaneous injection and patch.[114] Microneedle technology has been used in the local treatment of cancer models, including melanoma,[115] basal cell carcinoma,[115] breast cancer,[30] and skin cancer.[116] The materials used to prepare microneedles include silicon, metal, ceramics, glass, sugars, polymers, etc. However, these materials have limitations due to low drug loading efficiency, brittleness, easy decomposition at high temperature, and poor biocompatibility.[114] Zein, due to its advantages of sufficient mechanical strength, easy castability, high drug loading, and biodegradability, is expected to be a new material for the preparation of microneedles.[117] Shubhmita Bhatnagar[30] used micromolding technique to prepare zein microneedles co-loaded with gemcitabine and tamoxifen for local treatment of breast cancer. The maximum drug loading of tamoxifen and gemcitabine were 607 ± 21 and 1459 ± 74 μg, showing sufficient mechanical strength to be inserted into pigskin. Although zein is insoluble in aqueous media or skin tissue,[117] the experimenters observed that zein microneedles would swell after being cultured in aqueous medium for a long time, which is beneficial for the therapeutic drug to be embedded in the matrix or coated on the surface of the microneedle for transdermal administration.

Intratumoral Injection

Chemotherapy is one of the most important treatments for malignant tumors. However, malignant solid tumors have a high incidence of multiple primary malignant tumors, and their unique microenvironment reduces the clinical efficacy of conventional chemotherapy. Interstitial chemotherapy, first proposed by Brem, provides an alternative to conventional chemotherapy.[118] Recently, new formulations for interstitial chemotherapy have been developed, such as gels, microchips, nanoparticles, polymeric wafers, and in situ gels. Among them, in situ gel is gaining popularity as a vehicle for drug delivery systems.[119–123] The polymers used for in situ gel are mainly synthetic,[124] while the biocompatibility and safety of synthetic materials need to be improved. Zein, a natural plant protein from corn, is a promising alternative due to its low immunogenicity and biodegradability. Moreover, Zein has poor solubility in physiological saline or body fluids due to its hydrophobicity. Therefore, the zein solution can rapidly undergoes a phase transition to be a semi-solid, forming the three-dimensional network gel. And the drug will slowly release from the gel to achieve long-term effects.[125–128] So, zein is a good material for preparing tumor in situ hydrogels. Doxorubicin (DOX), a cytotoxic anthracycline, is the first-line treatment option for many hematological diseases and solid tumors.[129] Xiaoying Cao[31] developed Dox-loaded Zein in situ gel for interstitial chemotherapy. Under the scanning electron microscope, there was no obvious pores on the surface of the zein in situ gel at the first hour. However, at 48 h, the surface of the zein in situ gel was eroded and numerous pores were observed. On the 12th day, the zein in situ gel formed a network structure inside and connected to each other, forming a diffusion barrier for DOX. But its inner porosity was much higher than that of the outer, and the surrounding diffusion barrier area formed a buffer after contacting with water, which would help to modulate the initial burst release of water-soluble drugs. After intratumoral injection of the hydrogel, the system converted from a liquid to a semi-solid. Compared with free doxorubicin solution, due to the continuous release of

DOX-loaded Zein in situ gel, the local DOX concentration in solid tumors can be maintained above the anti-tumor threshold concentration for a long time, so as to achieve the purpose of reducing toxicity and improving efficacy.

Pulmonary Inhalation

The administration of drugs through pulmonary inhalation is an attractive drug delivery target due to its non-invasive nature, potential for higher systemic bioavailability, and availability of a large surface area. Moreover, pulmonary inhalation can also avoid the first passage through metabolism and starting therapeutic effects more quickly.[130,131] However, drugs that enter the lungs directly can be destroyed by enzymes and expelled by mucous cilia. Therefore, a method must be found to protect the drugs during lung administration. Nanoparticles have been found to encapsulate drugs, protecting them from damage in the pulmonary microenvironment and achieving a relatively uniform distribution of drug doses among the alveoli.[132–134] Therefore, the nanoparticles have the huge potential to be a drug carrier in pulmonary inhalation. Zein has amphiphilic properties and can self-assemble into nanoparticles to protect drugs from damage by the pulmonary microenvironment. It is worth noting that, as a protein, it can have a strong affinity with anti-tumor peptides or proteins, which is expected to achieve efficient drug encapsulation. In addition, the lungs have highly expressed neonatal Fc receptor (FcRn), and zein has a modifiable surface which can be linked with the ligands of it, thereby improving delivery efficiency.[9] Fatima Hameedat et al[9] used insulin as a protein model to prepare zein-based nanoparticles conjugated with ethylene glycol and FcRn-targeted peptides. The experimental results demonstrated the FcRn targeting and the increasing of the permeability of insulin, which fully meet the requirements of the pulmonary drug delivery system. In another study, Nayra M. Kamel et al[135] prepared hybrid lipid nanocore-protein shell nanoparticles (HLPNPs) coloaded with all-trans retinoic acid (ATRA) and genistein (GNS), which enabled dual tumor-targeting with biotin and ATRA. The results showed that HLPNPs enhanced the uptake of A549 lung cancer cells and the cytotoxicity of loaded drugs. To improve their deep lung deposition, they fabricated the dual-targeted drug-loaded HLPNP nanocomposites. In vivo, the inhalable nanocomposites were superior to aerosolized or i.v. nanoparticle suspension against lung carcinoma bearing mice.

Drug Delivery System Functionalization

Currently, many nano-drugs are delivered to the tumor site through the EPR effect. However, this method has some drawbacks, such as easy clearance from the bloodstream, insufficient accumulation at the target site, and unsatisfactory therapeutic effects. Zein, as a protein, contains free carboxyl and amino groups that can be modified and functionalized to improve its nanoparticle drug delivery system. Researchers have developed targeted and microenvironment-sensitive release systems that take into account the unique characteristics of tumor cells, including specific receptor expression, low pH, and high glutathione concentration at the tumor site. Additionally, the surface of zein is PEGylated to prolong the circulation time of nanoparticles, and zein nanoparticles are encapsulated in hydrogels to further enhance their drug protection and release control capabilities. In the following section, we will discuss the functionalization of the zein-based nanoparticle drug delivery system in more detail.

Ligand-Modified

At present, the EPR effect is a relatively recognized way of accumulation of nano-drugs in tumors. However, its accumulation efficiency is low and the specificity of targeting is insufficient. To improve the targeting of nanoparticles to tumor sites and their uptake by tumor cells, many ligands that can specifically bind to receptors expressed on tumor cells have been modified on the surface of nanoparticles.[136] The structure of zein contains some free carboxyl groups and amino groups, which can be linked to ligands by amide reaction or other methods. Hyaluronic acid[12,137] (Figure 6), chondroitin sulfate,[138,139] folic acid,[140,141] biotin,[135] all-trans retinoic acid,[135] etc., are commonly used ligands for zein nanoparticles. These receptors are linked to zein in various ways (such as carbodiimide reaction, electrostatic interaction, ionic hydrogen bonding) and show good tumor targeting ability. The Table 2 shows their current application to zein nanoparticles. The types, receptors, connection mechanism, loaded drugs and achieved effects of tumor-targeting ligands are summarized.

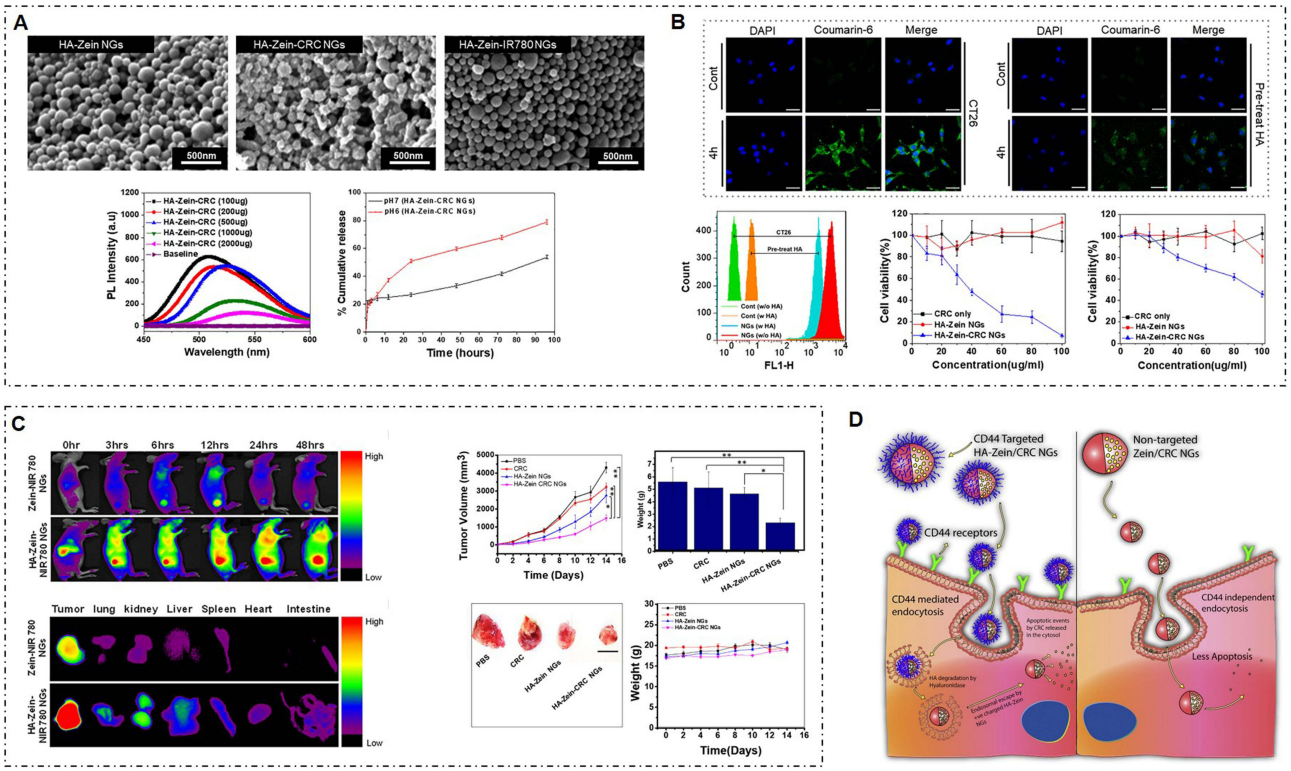

Figure 6 (**A**) SEM images of NGs, photoluminescence of the HA-Zein CRC NGs with varying CRC amounts, and cumulative release percent of CRC from the HA-Zein CRC NGs in two different pH environments. (**B**) Cellular uptake, cell viability with the HA-Zein-CRC NGs. (**C**) In vivo imaging and biodistribution analysis of nude mice with CT26 tumors after the tail vein was injected with Zein-IR780 and HA-Zein-IR780 NGs. (**D**) Schematic illustration of CRC encapsulated HA-Zein NGs for HA receptor targeting against CT26 colorectal cancer cells, and the selective uptake mechanism of HA-Zein NGs for curcumin delivery to HA receptor overexpressing cancer cells. *P < 0.05; **P < 0.01. Reprinted with permission from Seok HY, Sanoj Rejinold N, Lekshmi KM, et al. CD44 targeting biocompatible and biodegradable hyaluronic acid cross-linked zein nanogels for curcumin delivery to cancer cells: In vitro and in vivo evaluation. J Control Release. 2018;280:20–30. Copyright 2018, Elsevier.[137]

Stimuli-Responsive Release

Table 3 summarized different release methods and their applications about zein-based nanoparticles. The table shows that most of nanoparticles without stimuli-responsive release generally exhibit two-phase release, with the drug being initially released suddenly from within the shell or surface of the nanoparticles, and then gradually released from the drug wrapped inside the particles, which prolongs the blood circulation of the drug.[145,146] The isoelectric point of zein is 6.8,[92] s which results in different sustained release effects at physiological pH and tumor site pH. However, compared to stimuli-responsive nanoparticles, tumor specific release of normal zein-based nanoparticles is still insufficient. Numerous studies have shown that tumor sites have the characteristics of low pH and high GSH concentration. As shown in Table 3, nanoparticles with additional pH or GSH stimuli-response exhibit significant tumor-microenvironment release, which can reduce the toxicity and side effects of the drug and enhance the efficacy. Therefore, when designing carriers, we can make full use of the easy modification characteristics of zein to functionalize it and achieve precise drug release at tumor site.

pH Sensitive

The tumor microenvironment is characterized by acidosis and hypoxia, which distinguishes it from normal tissues.[147] Acidosis results from the production of acidic substances through the fermentation of high sugar during the growth and proliferation of tumor cells. Additionally, hypoxia at the tumor site exacerbates acidosis.[148–150] The pH of normal tissues is usually between 7.3–7.4,[151] whereas tumor tissues typically have a pH of 6.4–7.[152] At the subcellular level, the pH of lysosomes within tumor cells can drop as low as 4.5–5.5.[153,154] As a result, many studies are focusing on designing drug delivery systems that can respond specifically to the acidic environment of tumors, enabling targeted drug delivery.[155–157]

Table 2 The Ligands Used in the Zein-Based Nanoparticles and Achieved Effects

Ligand	Receptor	Connection Mechanism	Loaded Drugs	Effect	Ref.
Hyaluronic acid	CD44	Electrostatic interaction	Magnolol	In vitro: Enhanced the uptake of the drug by tumor cells and the inhibitory and pro-apoptotic effects of the drug on tumor cells, and can more effectively inhibit the migration and invasion of tumor cells. In vivo: Increased the accumulation of the drug in the tumor site of mice, which can effectively inhibit the growth of tumor cells and liver and lung metastasis.	[12]
		Hydrogen bonding and electrostatic interaction	Curcumin	In vitro: Enhanced the uptake of the drug by CT 26 cell and the anticancer efficacy on the CT26 cell line. In vivo: Improved the targeting of drugs to tumor sites and can effectively inhibit the growth of tumor.	[137]
Chondroitin Sulfate	CD44	Carbodiimide coupling reaction	Etoposide (ETP) and all-trans retinoic acid (ATRA)	In vitro: Effectively increased the uptake of ETP and ATRA by MCF-7 cells, and the toxic effects of the drugs on tumor cells. In vivo: Effectively reduced the expression of Ki-67 and enhanced the inhibitory effect of the drug on tumors.	[138]
	CD44	Electrostatic interaction	Docetaxel	In vitro: Effectively increased PC-3 cells uptake of docetaxel and inhibition of tumor growth. In vivo: Improved the targeting of drugs to tumor sites and can effectively inhibit tumor proliferation.	[139]
Biotin (vitamin B7), all-trans retinoic acid (ATRA)	Biotin receptors, retinoic acid receptors	Carbodiimide coupling reaction	All-trans retinoic acid (ATRA) and genistein (GNS)	In vitro: The coupling of biotin and ATRA to the zein shell had the highest internalization and cytotoxicity in A549 lung cancer cells compared to single biotin-targeted nanocarriers and non-targeted nanocarriers. In vivo: Reduced the toxic and side effects of drugs and the expression of Ki-67. Moreover, effectively promoted the apoptosis of tumor cells.	[135]
Folic acid	Folate receptors	Carbodiimide coupling reaction	10-Hydroxycamptothecin	In vitro: Promoted drug uptake by A549 cells and exhibited high cytotoxicity.	[140]
	Folate receptors	Ionic hydrogen bond	Doxorubicin	In vitro: Showed better cytotoxicity against HeLa, HepG2 and MCF-7 cancer cells. In vivo: Effectively inhibited the growth of tumor cells.	[141]
Lactoferrin	Lactoferrin receptor	Carbodiimide coupling reaction	Dasatinib and Fe_3O_4	In vitro: Enhanced drug cytotoxicity and tumor uptake, and effectively inhibited tumor migration.	[142]
	Lactoferrin receptor	Carbodiimide coupling reaction	Rapamycin (RAP) and wogonin (WOG)	In vitro: Promoted the uptake of nanoparticles by MCF-7 cells and effectively inhibited the proliferation of MCF-7 cells. In vivo: Effectively inhibited the growth of tumor cells and tumor vascular cells.	[143]
	Lactoferrin receptor	Electrostatic interaction	Exemestane and luteolin	In vitro: Effectively increased the cytotoxicity of both drugs against MCF-7 and 4T1 mouse cell lines, and promoted the uptake of nanoparticles by tumor cells. In vitro: Effectively enhanced the pro-apoptotic effect of the drug on tumor cells.	[144]

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Table 3 Different Releasing Methods and Their Applications About Zein-Based Nanoparticles

Releasing Methods	Anti-Tumor Drug	Formulation	Efficiency	Ref.
Diffusion or Degradation	Pterostilbene	Zein	At 36 hours, PTS diffusion showed biphasic sustained permeation manner with maximum magnitude of 89.1±2.65%.	[82]
	Doxorubicin	Sodium caseinate, zein	After the initial 6 h of rapid release, the release of DOX from DOX-zein-NPs reached a sustained release. Moreover, the release of DOX from DOX-zein-NPs also showed a pH-responsible feature.	[40]
	Maytansine	Zein	The results showed that the DM1 release from the ZNPs was biphasic characterized by initial fast release of about 20% of drug during the first 8 h followed by a second phase of slow release with about 40% of DM1 was released after 24 h.	[45]
	Thymoquinone	Zein	The TQ-ZN NPs showed a burst release of TQ was observed. After 6 hrs, around 25% of TQ was released from NPs. At the end of 24 hrs, 80% of TQ released.	[81]
	5-fluorouracil	Zein	A controlled release profile was observed from 5-FU-loaded ZPs. More 5-FU was released in pH 6.8 buffer solution than in pH 7.4 buffer solution.Oppositely, the burst release of 5-FU was less in pH 6.8 phosphate buffer solution (22.4%) than in pH 7.4 (35.7%) solution after half hour dissolution.	[92]
	Lovastatin	Zein	At 36 h, the diffusion of LVS-ZN NPs demonstrated an LVS permeation pattern, which reached a value of 96.80 ± 3.12%.	[83]
	Beta carotene	Zein	The βC-NP formulations established biphasic, namely initial rapid followed by slow and sustained release for four days (96 h).	[145]
	Curcumin	PEGylated zein	Curcumin release was sustained up to 24 h from mPEG-zein micelles	[196]
	Piceatannol	Sodium deoxycholate, zein	The results showed that the prepared BZ provided a satisfactory gradual release profile. Moreover, at 8 h, it showed about 50% of drug release (49.8% ± 6.1). By 24 h, the drug release was about 94.2 ± 5.9%.	[105]
	Resveratrol	Low-molecular-weight sodium hyaluronate, zein	RES released about 55.7% and 66.7% under the conditions of pH 7.4 and 5.0 at 24 hr. At the time point of 2 hr, RES released 48.6% at pH 5.0, but at 7.4, the release was only 29.1%.	[84]
	Vorinostat, bortezomib	Lecithin, zein	Both Vor and Bor were released in a controlled manner from ZNP/VB until the study period ended.	[62]
	Exemestane, resveratrol	Glutaraldehyde, zein	Around 58.7 and 62.2% of EXM and similarly about 30.9 and 36.1% of RES were released after 24 h in presence and absence of the digestive enzymes, respectively.	[101]
	Resveratrol	Sodium caseinate, chitosan, zein	A biphasic release profile is observed, characterized by a rapid initial release phase, where it was released about 25% of RVT in 4 h, and a sustained phase, where it was released about 39% of RVT after 120 h.	[21]

	Tretinoin, genistein	Glyceryl monostearate, stearyl amine, zein	GEN exhibited biphasic release pattern from TRE/GEN-MLPNs with an initial burst release of 60.23% in the first 8 h, followed by sustained drug release of 67% after 72 h. TRE had a very prolonged release without initial burst effect. Only 1.3% of TRE was released from MLPNs after 72 h compared with 49.6% from free TRE solution.	[87]
	Luteolin	Sodium caseinate, zein	After nanoparticles were moved to PBS with pH 6.8, luteolin appears to be released more rapidly.	[100]
	Carvacrol	Lecithin, zein	The release of CV was merely 9% within first 2 h of incubation at highly acidic pH of 1.2 (Simulated condition for stomach) and continues at increased rate from 3 h to 24 h under intestinal pH of 6.8 and reached 78% of total entrapped CV.	[104]
	Indole-3-carbinol	Carboxymethyl chitosan, zein	Both I3C and DIM-encapsulated nanoparticles demonstrated similar trends, the burst effect occurred within 0.5 h; followed by sustained release for more than 6 h. For zein nanoparticles without CMCS coating, around 45–50% of the compounds released from nanoparticles within 0.5 h, while for CMCS coated nanoparticles the burst effect of both compounds were reduced to around 40%.	[103]
	3,30-diindolylmethane	Carboxymethyl chitosan, zein		
	Paclitaxel	Sodium carboxymethyl cellulose, zein	When compared with bulk PTX, the zein–CMC NPs released PTX in a sustained manner, which provided an enduring capacity in the fight against cancer cells. After 10 h of incubation, almost 100% of the PTX was solubilized from bulk PTX, while less than 40% was obtained from the NPs. About 80% of the PTX was released from the NPs after 72 h of incubation. In addition, a significant initial burst was not observed in the release curve.	[88]
	Honokiol	Hyaluronic acid, zein	Compared to free HNK solution, Zein-HNK and HA-Zein-HNK nanoparticles exhibited slow sustained release process. Both formulations exhibited a biphasic pattern characterized with fairly rapid release during the initial period and followed by a slow release over 48 h.	[12]
	Dasatinib	Oleic acid, Fe_3O_4, lactoferrin, zein	Magnetic micelles exhibited a very slow release of DAS without initial burst at pH 7.4. Additionally, magnetic micelles exhibited more drug release rate in acidic condition compared to neutral condition.	[191]
	Etoposide, all-trans retinoic acid	Chondroitin sulfate, $CaCl_2$, zein	The CR micelles demonstrated the most powerful sustained release pattern of both, ETP and ATRA, in comparison to the UCR micelles and free drugs.	[138]
	Docetaxel	Chondroitin sulfate, zein	Both F1 and F2 exhibited sustained DTX release patterns up to 48–72 h in the biological fluid	[139]

(*Continued*)

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Table 3 (Continued).

Releasing Methods	Anti-Tumor Drug	Formulation	Efficiency	Ref.
	Curcumin	Hyaluronic acid, zein	More CRC was released in the higher acidic pH environment compared to that in the neutral pH environment. Release profile of CRC showed burst release at initial state of lower pH environment. Seventy-nine percent of the CRC was released within 4 days	[137]
	Genistein, all-trans retinoic acid	Glyceryl monostearate, stearyl amine, zein	The dual-targeted core-shell HLPNPs depicted a biphasic pattern of GNS release demonstrated as an initial burst, characterized by fast release during the first 0.25–8 h with about 55% of drug being released after 8 h, followed by a slower sustained release up to 65% drug release after 72 h.On the other hand, the release of ATRA from dual-targeted HLPNPs was found to be very slow, reaching only 2% after 72 h.	[135]
	Celastrol, sulfasalazine	Chondroitin sulphate, SPIONs, oleic acid, zein	On the other side, both CST-loaded PMs and SPIONs/CSTloaded PMs showed very slow release of CST without initial burst effect.Besides, Regarding SFZ release, it was not detected all over the release test period.	[189]
	Rapamycin, wogonin	Lactoferrin, glutaraldehyde, zein	The WOG release from the micelles was biphasic characterized by initial fast release of about 64% of drug during the first 6 h followed by a second phase of very slow release with about 67.59% of WOG was released after 24 h.	[146]
	10-hydroxycamptothecin	Folic acid, zein	Less than 40% HCPT is released from HCPT NC/F40 NPs in the first 10 h, then the release becomes steady and reaches 95.5% in 92 h.	[140]
	Gefitinib	Folic acid, SPIONs, zein	The release curves of the GEF-FSZs at different pH values could be similarly divided into two phases: the fast release stage within 8 h and the stable release stage after 8 h. As the free one, GEF was released rapidly from GEF-FSZs within an initial 8 h through passive diffusions. Then, the release of GEF was delayed. Furthermore, the release of GEF was accelerated as the pH value decreased from pH 7.4 to 5.0	[190]
	Doxorubicin	Folic acid, zein	The DOX release from both NP-DOX and FA-NP-DOX followed a biphase pattern: an initial fast release followed by a sustained release. And FA-NP-DOX has the best controlled release property among the three in the test.	[141]
	Nobiletin	Tannic acid, Fe^{III}, zein	From the result, almost all free NOB was released rapidly within 5 h, while NZT-Fe NPs and NZT-Al NPs exhibited slower release profiles and released NOB completely within 25 h.	[3]
	Nobiletin	Tannic acid, Al^{III}, zein		

Stimuli-responsive release	Doxorubicin	Tannic acid, Cu^{II}, zein	DOX-loaded zein NPs showed a relative slower release profile in the release media and about 80% of the DOX was released from NPs after 8 h incubation. Compared with DOX-loaded zein NPs, DOX/zein-TA/CuII showed inefficient and slow release of DOX at pH 7.4 and 6.2. Approximately 10% DOX was released within 8h, and little further release was detected for 36h. On contrary, release of DOX was readily accelerated under acidic conditions; about 25% and 55% of DOX released within 8 h at pH 5.0 and pH 4.0, respectively. As shown in Figure 4i, when FeIII was chosen as the coordinating center, the responsive pH onset was dramatically reduced. No significant release of DOX was observed even at pH 4.0.	[161]
	Doxorubicin	Tannic acid, Fe^{III}, zein		
	Gambogenic acid	Polydopamine, zein	Compared with quick and complete release of GNA solution within 24 h, cumulative 57% and 72% of GNA were released from GNA@ZeinPDA NPs and GNA@Zein NPs after 72 h at pH 7.4. Furthermore, GNA@Zein-PDA NPs showed pH-responsive release with faster GNA release (about 97%) at pH 6.86 than that of at pH 7.4, which neither GNA solution nor GNA@Zein NPs achieved	[167]
	Doxorubicin hydrochloride	Hydroxyapatite, zein	For HA/Zein-DOX NPs, the release was highly pH-dependent, with 91.8% release at pH 6.86 but only 27.2% release at pH 7.4 after 2 h. The cumulative release of Zein-DOX NPs reached 92.2% and 59.6% after 2 h, respectively. Compared with Zein-DOX NPs at 24 h (about 61.8%), HA/Zein-DOX NPs showed lower release at pH 7.4 (about 28.1%).	[170]
	Ellipticine	Sodium caseinate, zein	The release of EPT from both zein/SC and zein/PEI nanoparticles was significantly influenced by the environmental pH conditions. EPT was released faster at acidic pH values (pH 5.5), compared to the neutral pH value (pH 7.4).	[76]
	Ellipticine	Poly ethylene imine, zein		
	Hydroxycamptothecin	AuNPs, polydopamine, folic acid, zein	It was seen clearly that 95.4± 4.1% and 73.2± 4.2% of drug released from HCPT nanocrystals after 24h at physiological pH and endosomal/lysosomal pH, respectively. By comparison, the percentage of HCPT release from HCPT@AuNPs-Zein-PFA NCs was found to be 58.4 ± 3.0% after 24h at endosomal/lysosomal pH (~ pH 5.0), which was 3.42-fold higher than the drug released in PBS buffer at physiological pH (pH 7.4). Obviously, there was an accelerated HCPT release from NCs, reaching more than 80% within 24 h in the presence of enzymes.	[174]
	Paclitaxel	Bis(2-hydroxyethyl) disulfide, zein	There was no release of PTX from zein-S-S-PTX_NP when no GSH was added. With GSH, the PTX showed an initial burst release of 80–90% in the first 5 min. From 5 to 20 min, the cumulative release amount of PTX vibrated between 80–90% because of the unsteady distribution of the PTX in the dialysis bag in such a short time. After 30 min, the cumulative release amount of PTX was stabilized at 90% and gradually increased to 98.2% after 120 min.	[13]
	Cynomorium songaricum polysaccharide, docetaxel, green tea polyphenols	Zein	Drug release kinetics were examined at different pH values (pH 7.4, 6.5, and 5.0). Compared to physiological conditions (pH 7.4), the DTX-loaded Zein/CSP-GTP/FeIII NPs exhibited a more rapid and extensive DTX release under acidic conditions (pH 6.5 and 5.0). And the cumulative releases at 48 h in the different mediums (pH 7.4, 6.5, and 5.0) were 34.9 ± 4.2%, 51.7 ± 3.2%, and 90.2 ± 2.4%, respectively.	[14]

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The formation of metal-ligand coordination bonds is well known to be sensitive to external pH conditions.[158] Tannic acid (TA), a polyphenol polymer, has a strong metal chelating ability and an affinity for support materials, making it an excellent material for preparing metal-ligand complexes.[159,160] Hongshan Liang[161] used zein as a carrier to prepare nanoparticles coated with an acid-sensitive membrane formed by the coordination of tannic acid (TA) and metal ions, which simultaneously loaded DOX (Figure 7). The in vitro drug release curve showed that the nanoparticles only with zein as a carrier material released about 80% of DOX within about 8 h at pH 7.4, 6.2, 5.0, and 4.0, while DOX/zein-TA/Cu^{II} NPs exhibited inefficient and slow DOX release at pH 7.4 and 6.2. Only about 10% DOX was released within 8h and little further was released within 36h. In contrast, under the acidic conditions of pH 5.0 and pH 4.0, the release of DOX within 8 h was 25% and 55%, respectively. The results showed that the system was responsive to pH stimulation, and the carrier could effectively prevent the release of the loaded drug at pH simulating normal human tissues, but slowly

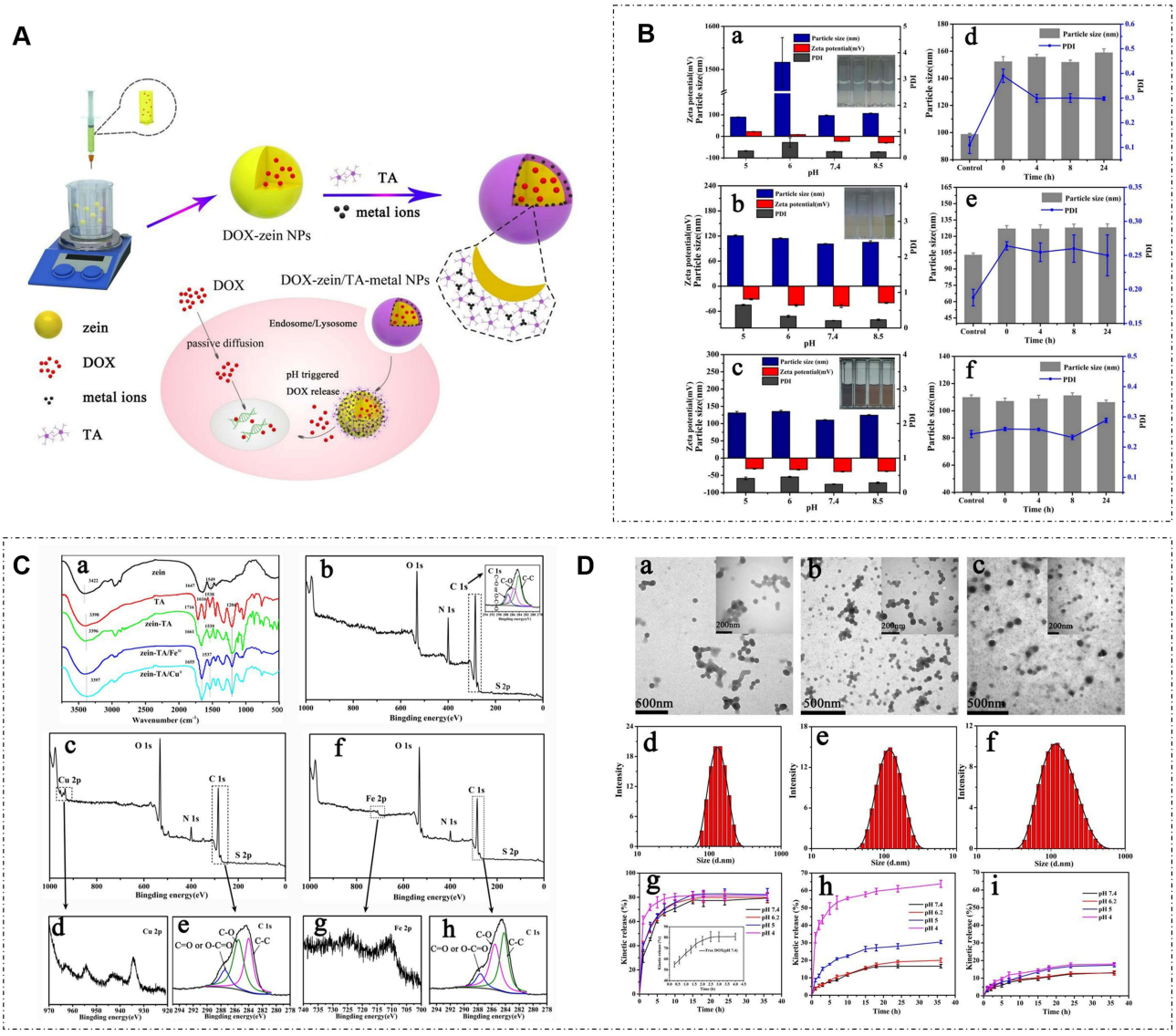

Figure 7 (**A**) Scheme: The preparation of DOX-loaded zein NPs coated by metal-TA films and the proposed model for pH-dependent drug release in tumor cells. (**B**) Influence of the pH on Particle size, PDI and zeta potential of pre-formed zein NPs (a), zein-TA/Cu^{II} NPs (b) and zein-TA/Fe^{III} NPs (c). Particle size and PDI in culture media as a function of time: zein NPs(d), zein-TA/Cu^{II} NPs(e) and zein-TA/Fe^{III} NPs (f). (**C**) Fourier transform infrared spectroscopy (FTIR) spectra of different samples (a). XPS survey spectra of zein(b); zein-TA/Cu^{II} NPs (c), (d) and (e); zein-TA/Fe^{III} NPs (f), (g) and (h). (**D**) TEM image and size distribution of zein NPs(a) and (d), zein-TA/Cu^{II} NPs(b) and (e) and zein-TA/Fe^{III} NPs (c) and (f). In vitro release profiles of DOX-loaded zein NPs (g), zein-TA/Cu^{II} NPs (h) and zein-TA/Fe^{III} NPs (i) in PBS under different pH conditions. Reprinted with permission from Liang H, Zhou B, Li J, et al. Supramolecular design of coordination bonding architecture on zein nanoparticles for pH-responsive anticancer drug delivery. Colloids Surf B Biointerfaces. 2015;136:1224–1233. Copyright 2015, Elsevier.[161]

release at the acidic pH simulating the tumor microenvironment. At the same time, the author also used an acid-sensitive film formed by the coordination of tannic acid (TA) and metal ions to coat Nobiletin (NOB)-loaded zein nanoparticles in another paper.[162] The nano system also exhibited a lower drug release rate at pH 7.4.

Polydopamine (PDA) is a hydrophilic biopolymer,[163,164] and its monomeric dopamine hydrochloride can also spontaneously oxidize on various substrate surfaces, indicating good material adhesion. Meanwhile, PDA has good stability in a neutral environment, but is decomposed by protonation in the slightly acidic environment of the tumor site, indicating it has a good pH-responsive function.[165,166] Liqiong Zha[167] prepared PDA-coated gambogic acid-loaded zein nanoparticles, namely GNA@Zein-PDA NPs. In the in vitro pH-sensitive experiment, the free drug was completely released within 24 h, while both GNA@Zein-PDA NPs and GNA@Zein NPs showed good drug sustained-release properties at pH 7.4, respectively releasing within 72 h. 57% and 72%. However, at pH 6.86, GNA@Zein-PDA NPs showed better drug release ability than GNA@Zein NPs, about 97%, showing a certain pH-sensitive release property.

Hydroxyapatite, which has a composition similar to that of human bone, is a promising bone substitute that is degradable and pH-sensitive.[168,169] Liqiong Zha[170] prepared hydroxyapatite-coated DOX-loaded zein nanoparticles, namely HA/Zein-DOX NPs, by biomimetic mineralization. In the in vitro release experiments, the drug release rates of free DOX in the first 2 h were both about 92% at pH 7.4 and pH 6.86. The drug release from HA/Zein-DOX NPs showed a high pH dependence. At pH 7.4, only 27.2% of DOX were released from HA/Zein-DOX NPs within 2 h. While at pH 6.86, the release rate of HA/Zein-DOX NPs was as high as 91.8% within 2 h. Although Zein-DOX NPs also showed some acid-sensitive properties at pH 6.86, the drug release reached 59.2% at pH 7.4 within 2 h, indicating that its blocking effect on drugs is not as good as HA/Zein-DOX NPs in normal tissue pH environment. In tissue distribution experiments, HA/Zein-DOX NPs also effectively reduced the distribution of DOX in cardiac tissue and reduced the cardiotoxicity of DOX.

Furthermore, some researchers have designed a drug delivery system that achieves pH sensitivity and receptor targeting due to the highly expressed specific receptors on the surface of tumor cells and the slightly acidic environment of tumor tissues.[171–173] Hongdi Wang[174] used folic acid as the target and polydopamine as the acid-sensitive material to prepare zein nanoparticles loaded with gold nanoparticles and hydroxycamptothecin, namely HCPT@AuNPs-Zein-PFA. In the cell uptake experiment, the FA-functionalized nanoparticles showed better A549 cell uptake effect. In addition, the tumor targeting experiment and tissue distribution experiment also showed that the nanocomposite had good targeting. In the in vitro release experiments, under the conditions of physiological pH and endosome/lysosome pH, free HCPT nanocrystals released 95.4 ± 4.1% and 73.2 ± 4.2% of the drug within 24 h, respectively. By comparison, the percentage of HCPT release from HCPT@AuNPs-Zein-PFA NCs was found to be 58.4 ± 3.0% after 24h at endosomal/lysosomal pH (~ pH 5.0), which was 3.42-fold higher than the drug released in PBS buffer at physiological pH (pH 7.4). Compared with free HCPT and its non-targeted equivalent, HCPT@AuNPs-Zein-PFA exhibited better tumor-inhibiting ability and lower side effects both in vitro and in vivo.

GSH Sensitive

In addition to having a lower pH than normal tissues,[175] the tumor microenvironment contains 100–1000-fold higher levels of reduced GSH than normal body fluids (including extracellular fluid and blood) in the cytoplasm and nucleus.[176–178] Furthermore, due to its hypoxic nature, the GSH concentration in tumor tissue is at least 4 times higher than that of normal tissue.[179] Therefore, researchers have developed nano-drug delivery systems that can effectively control drug release and respond to stimuli in the tumor microenvironment based on the characteristics of high reduced GSH concentration.[180,181] One such system involves the use of disulfide bonds, which can be broken by redox reaction with GSH, to link drugs or carrier materials containing -NH2, -OH, or -COOH via amide or esterification reaction. These materials can form GSH-sensitive self-assembled nanomaterials particles.[182,183] Zein, an amphiphilic protein with good chemical modification ability, can self-assemble into nanoparticles that prolong the circulation time of drugs in vivo by 7.2 times.[92,184–186] Therefore, zein has great potential to prepare GSH-responsive prodrug nanoparticles. Heting Hou[13] prepared a drug delivery system like that. The disulfide bonds were linked to zein and the lipid-soluble anticancer drug paclitaxel by esterification, respectively, and GSH-responsive prodrug nanoparticles (zein-S-S-PTX_NP) were formed by self-assembly (Figure 8). In the in vitro release experiments, zein-S-S-PTX_NP showed almost no release at pH 7.4 without GSH. On the

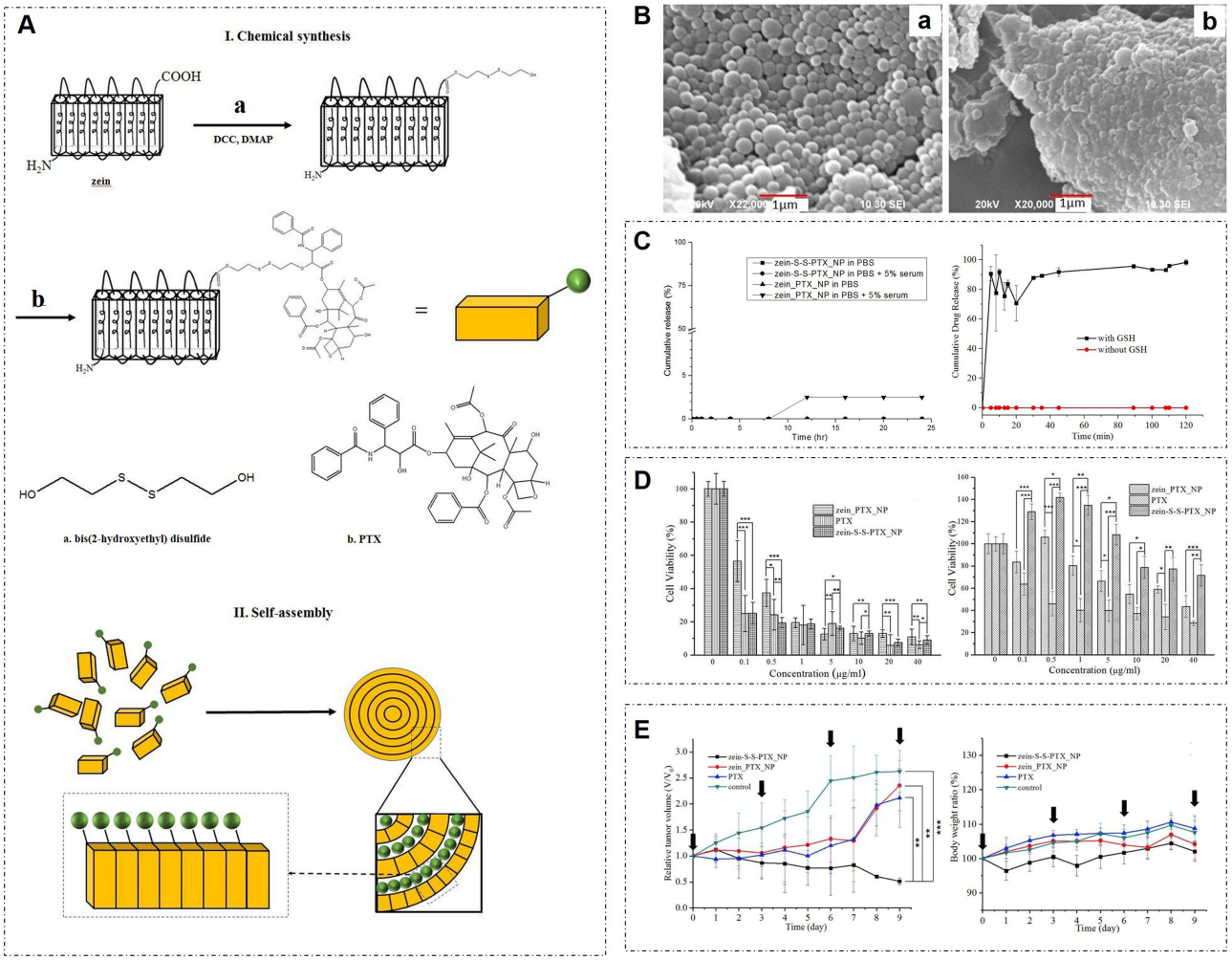

Figure 8 (**A**) Schematic diagram of: I. chemical synthesis and II. self-assembly using zein, disulfide, and PTX to form the zein-S-S-PTX_NP. (**B**) SEM images of (a) zein-S-S-PTX_NP and (b) zein_PTX_NP. (**C**) In vitro release of PTX from zein-S-S-PTX_NP and zein_PTX_NP (**D**) MTS assay of HeLa cell and NIH 3T3 cell (**E**) Relative tumor volume and body weight ratio: in vivo antitumor activity. The drugs were administrated on days 0, 3, 6, and 9 as indicated by the arrows. *P < 0.05; **P < 0.01; ***P< 0.001. Reprinted with permission from Hou H, Zhang D, Lin J, et al. Zein-Paclitaxel Prodrug Nanoparticles for Redox-Triggered Drug Delivery and Enhanced Therapeutic Efficiency. J Agric Food Chem. 2018;66(44):11,812–11,822. Copyright 2018, American Chemical Society.[13]

contrary, when 10 mM GSH was added to the buffer, the nanoparticles released 80–90% PTX within 5 min and the release rate reached 95% after 2 h. The results showed that the nanoparticles can be released almost zero under normal body fluid conditions, but can be quickly released under the condition of tumor microenvironment, showing a good controlled release effect. In vitro cell experiments, zein-S-S-PTX_NP showed the same antitumor effect as pure PTX, and showed zero toxicity to NIH/3T3 fibroblasts. In vivo antitumor experiments, the tumor volumes of zein-S-S-PTX_NP, the control, pure PTX, and zein_PTX_NP groups were 50%, 262.6%, 211.7%, and 235.5% of the original size, respectively. The above results showed that zein-S-S-PTX_NP had obvious synergistic and detoxification effect.

Magnetic Targeting

Another approach to achieve targeted therapy for tumors is through magnetic targeting. This involves adding magnetic nanoparticles to drug-loaded nanoparticles to direct their accumulation at tumor sites using an external magnetic field.[187] Superparamagnetic iron oxide nanoparticles (SPIONs) are commonly used for this purpose as they are non-toxic and can be synthesized with well-defined dimensions. They are also the only magnetic nanomaterials approved by the US FDA for use in biomedicine.[188] However, magnetic targeting alone may not be enough to improve drug uptake by tumor cells. Therefore, researchers often add aptamers to the surface of the carrier material to enhance drug uptake by tumor cells.

For example, Kholod A. Elhasany[189] used chondroitin sulfate, sulfapyridine, and zein to synthesize amphiphilic fragments that encapsulated SPIONs and celastrol (CST). In in vitro experiments, nanoparticles with chondroitin sulfate targets achieved superior cellular uptake over free RBITC. In the in vitro anti-tumor experiment, the tumor volume of the nanoparticle group without SPIONs increased by 78.24%, while the increase in tumor volume of the nanoparticle group with SPIONs was 36.26%, achieving effective inhibition of tumor proliferation. Jiafeng Pang[190] encapsulated SPIONs and gefitinib (GEF) in folic acid-conjugated zein (Fa-zein) nanocomplexes (GEF-fszs). In vitro cell experiments showed that by adjusting the external magnetic field, the ability of the anti-proliferative and in vitro cellular uptake of GEF-fszs in A549 cells was higher than those of free GEF at the same concentration of GEF. Conjugating with FA further promoted the internalization of GEF-FSZs into A549 cells. In addition, Sally A. Sabra[191] linked lactoferrin targeting tumor cell surface lactoferrin receptor with zein to form amphiphilic fragments through carbodiimide coupling reaction, and used it to encapsulate SPIONs and dasatinib, which also achieved good tumor targeting effect and tumor cell uptake ability.

PEG-Modified

In terms of the immunogenicity of zein, Hurtado López[192] found an interesting phenomenon. Injection of zein microspheres intramuscularly into mice resulted in the production of anti-zein antibodies. Feng Li[193] stated that parenteral administration of zein particles can lead to a long-term systemic immune response. Coupled with the strong hydrophobicity of zein, it is easily cleared by the body's immune system (such as macrophages). PEGylation has been widely used to avoid macrophage uptake and immunogenicity of proteins and nanoparticles.[194,195] The PEGylation of zein provides a hydrophilic surface, thus preventing macrophage uptake of zein to generate an immune response. Satheesh Podaralla[196] found after subcutaneous injection of PEGylated zein nanoparticles, mice did not produce any anti-zein antibodies. And PEGylation reduced the clearance of zein nanoparticles by macrophages, prolonging the circulation of the drug in the body.

Gel Encapsulation

According to previous introductions, zein has the advantages of hydrophobicity, biocompatibility, and biodegradability, and the nanoparticles formed by it can better enhance the water solubility of drugs. However, objectively speaking, the water solubility of the outer layer, stability and drug release properties of the zein-based nanoparticles need to be further improved. The hydrogel is a three-dimensional soft substance in which polymer chains are cross-linked to form a network in a continuous liquid environment. Encapsulating the drug-loaded nanoparticles into the hydrogel can take advantage of the hydrophilicity of the hydrogel to reduce the possibility that the nanoparticles are cleared by macrophages in the blood circulation. At the same time, due to the porosity and swelling property of the hydrogel itself, it can further enhance the controlled release effect of nanoparticles on drugs.[197] Priyanka Kaushik[198] prepared Pectin hydrogel-encapsulated doxorubicin-loaded zein nanoparticles without the use of a cross-linking agent. The isoelectric point of zein is 6.2, and pectin is a polyanion, indicating attractive electrostatic interactions between the two biopolymers at pH=2.47. The experimental results showed that the preferential binding of pectin to DOX-loaded zein nanoparticles is indeed achieved through associative electrostatic interactions. At the same time, since the drug formulation exhibits a positive charge as a whole, while the surface of HeLa cells is negatively charged, the electrostatic interaction promotes the cellular uptake of the drug, which is beneficial to enhance the efficacy of the drug. In drug release experiments in vitro, DOX was added to ZP hydrogels, followed by diffusion and swelling controlled release. Under RT conditions, the release of DOX showed a burst first followed by a controlled release. The effectiveness of anticancer therapy depends on the release kinetics of the drug. The slow and sustained release provides prolonged and sustained anticancer therapy. Doxorubicin is an anti-cancerous drug which mediates mitochondrial-dependent apoptosis, and they observed a selective toxicity towards HeLa and not HEK293cells.

Preclinical and Clinical Tests

Based on the information obtained from "ClinicalTrials.gov", there are currently no drugs that use zein as an anticancer-drug carrier on the market. However, studies have shown that empty zein nanoparticles can lower glucose levels in

animals, and clinical trials have been conducted to evaluate the effect of zein nanoparticles on blood glucose control in early diabetes patients. As for the preclinical test, currently, the main focus is on the development of various anticancer drug-loaded zein-based carriers, only evaluating the basic physical and chemical properties, release profile, and therapeutic effects. To the best of our knowledge, a more detailed evaluation of the in vivo metabolism of zein-based carrier loaded with antitumor drugs has not been conducted. To establish a robust zein-based micro/nanomedicine, accumulation rate, release rate, drug metabolism, pharmacokinetic and pharmacodynamic evaluation, and treatment scheduling should be studied.[5] Furthermore, the immunogenicity of zein-based carriers also needs to be further studied.

Conclusion

Zein, a plant-derived protein, is a promising and environmentally friendly material for drug delivery. Zein-based carriers have been explored for anticancer drugs through various administration routes, including intravenous injection, oral administration, transdermal absorption, intratumoral injection, and pulmonary inhalation. Zein has multiple methods for drug loading, such as hydrophobic interaction, chemical conjugation, deposition, and electrostatic interaction, but hydrophobic interaction has been more commonly studied for the insoluble small molecules in current anticancer drugs. Currently, nanoparticles loaded with antitumor drugs are the main type of zein-based carriers used for intravenous injection and oral administration. With the development of micro/nanotechnology, zein-based nanofibers, microneedles, and hydrogels have been explored for controlled release and more effective local treatment. Among the various administration routes, the oral route is particularly advantageous due to zein's good hydrophobicity and resistance to the gastrointestinal environment. Moreover, functionalization of zein-based carriers is crucial in drug delivery, including enhancing targeting and stimulating responsive release. Zein has free amino and hydroxyl groups, which make it easily modifiable and have the potential to be developed as a functional drug carrier. However, clinical transformation of drug-loaded zein-based carriers is still challenging due to the lack of basic research.

Current Challenges and Suggestions

Firstly, the stability. The hydrophobicity of zein not only enhances the solubility of hydrophobic drugs but also decreases the stability of the preparation. Therefore, it is necessary to add stabilizers to form stable zein-based carriers. There are several methods to improve the stability of zein-based carriers. (I) Electrostatic interaction: we can cross-link anionic materials such as anionic polysaccharides with zein by electrostatic interaction to improve its stability. The effects of alginate and hyaluronic acid have been demonstrated.[22,199] (II) Surfactants: surfactants like pluronic, tween, and lecithin can be attached to the surface of zein nanoparticles or cross-linked with zein to increase water solubility and stability. (III) Steric repulsions. McClements et al[200] developed a series of caseinate-dextran Maillard conjugates to coat resveratrol-loaded ZNs. Caseinate could endow the resultant conjugates with adsorption capacity onto the surface of ZNs whereas the dextran part provided strong steric repulsion to reduce the aggregation of zein-based nanoparticles and make them more stable.

Secondly, the clinical application. Zein is an alcohol-soluble protein, which means its production process involves the use of alcohol. The removal of organic reagents is difficult, and their excessive residue can also be harmful to the human body. Therefore, it is important to strictly control the time and temperature of ethanol volatilization during the preparation of zein-based carriers to ensure their safety. In addition, compared with human serum albumin, which has been used in Abraxane, (I) Due to the poor water solubility of zein, it is easier to clear in the body, thus reducing the cycle time; (II) Zein is a plant protein, which means its xenogenicity may lead to unpredictable immunogenicity. For the former, encapsulating it into the water-soluble gel and the modification of PEG and various polysaccharide are expected to show better solubilization. Meanwhile, we can also prepare zein-based in situ gel or microneedle, which can also reduce its contact with macrophages. For the latter, in general, from literature, the most frequently reported side effect after injection of a nanotherapeutic agent seems to be immune-mediated side effect.[19] So, it's necessary to make the immunogenicity of zein-based carrier clear. However, at present, the research on immunogenicity of zein-based carrier is mostly confined to mice. We should expand the scope and depth of research to ensure clinical safety.

Lastly, manufacturing issues. Manufacturing of micro/nanomedicine products for commercialization is technically challenging. In large-scale production, due to the polydispersity of micro/nano materials, there will be quality differences

between batches.[16] In addition, their features (ie, size, shape, cargo loading level, surface property, etc.) also play vital roles in affecting affect traffic journey and bio-distribution of particles and particle-cell interactions.[201] Therefore, for the further application of zein-based carrier, we should study how to improve the quality and efficiency of production. Compared with traditional manufacturing methods, we can utilize computer or mechanical-aided systems and resources, or automated material handling systems, to further improve above features. At present, photolithography, soft lithography, nano-imprint lithography, mechanical stretching, and microfluidic fabrication are available.

Data Sharing Statement

Data will be made available on request.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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