Hydroxyethyl starch and its derivatives as nanocarriers for delivery of diagnostic and therapeutic agents towards cancers

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Key Words:
anticancer treatment; chemical modification; diagnostic and therapeutic agents; hydroxyethyl starch; nanocarriers; prodrug

From the Contents

ABSTRACT

Many types of drugs and agents used for cancer diagnosis and therapy often have low bioavailability and insufficient efficacy, as well as causing various side effects due to their nonspecific delivery. Nanocarriers with purposely-designed compositions and structures have shown varying degrees of abilities to deliver these compounds towards cancers in passive or active manners. Despite the availability of a variety of materials for the construction of nanocarriers, natural polymers with good biocompatibility and biodegradability are preferable for such usage because of their high in vivo safety as well as easy removal of degradation products. Among the natural polymers intended for building nanocarriers, hydroxyethyl starch and its derivatives have gained tremendous attention in the field of drug delivery in the form of nanomedicines over the last decade. There is growing optimism that ever more hydroxyethyl starch-based nanomedicines will be a significant addition to the armoury currently used for cancer diagnosis and therapy.

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Introduction

Cancer is one of the most destructive diseases with high incidences and death rates. Cancer occurrence is correlated with quite a variety of factors such as culture, social intercourse, life-style, and living environment as well as hormonal and genetic aspects.[1] The growing global prevalence of cancer has made cancer diagnosis and therapy one of the most investigated aspects over the last decade.[2] Despite the varied types of therapeutic modalities available for cancer treatments so far, each of them has some associated side effects or certain limitations.[3] Chemotherapy based on cytotoxic drugs is one of the principal strategies applied to malignancies to date. However, direct administration of chemotherapeutic drugs often causes severe adverse effects on normal tissues whether it is used alone or in combination with surgery, radiation therapy, or biological therapy.[1-3] Since the administration of small molecule anticancer drugs in a free formulation has some drawbacks, chiefly including short half-life in blood circulation, nonspecific delivery and systemic toxicity, a wide variety of drug delivery systems, as an alternative to direct administration of these drugs, have now been developed to reduce their side effects while improving their bioavailability.[3, 4]

Drug delivery systems in the form of nanocarriers can deliver encapsulated drugs with several distinct benefits, such as improved solubility, prolonged plasma half-life, and reduced side effects. The desirable nanocarrier should have certain characteristics: (I) well-defined structures for stably and efficiently entrapping drugs; (II) the ability for endosomal escape; and (III) ability to release the encapsulated drugs at the target sites in response to intracellular environmental changes.[5] To meet these requirements, a large number of nanocarriers with specifically-designed structures and functions have been constructed so far for delivering different drugs, aiming at prolonging their circulation in the bloodstream, improving their cancer targeting and reducing their systemic toxicity.[2-9] In the case of polymeric nanocarriers, the utilised excipients need to be biodegradable because the accumulation of nondegradable excipients inside human tissues or organs could result in long-term toxicity.[10, 11] Among varied types of excipient materials,

polysaccharides have mainly been used to fabricate nanocarriers for delivering diagnostic and therapeutic agents owing to their unique biocompatible and biodegradable properties.[12, 13]

Hydroxyethyl starch (HES) is a semisynthetic polysaccharide obtained by hydroxylethylation of amylopectin, and it has been used as a plasma expander for years.[14] HES has several meritorious properties, such as biocompatibility, biodegradability, non-toxicity, excellent water solubility, and very low hypersensitivity.[15, 16] Additionally, HES contains a lot of functional groups, allowing convenient chemical modification to construct nanocarriers with favoured structures and multi-functionalities.[17, 18] In recent years, many investigations on HES-based nanomedicines have been conducted, and growing evidence supports the hypothesis that HES and its derivatives have superior potential in acting as promising nanocarriers for delivering diagnostic and therapeutic agents towards cancers.[15-20]

Starch and its Hydroxyethyl Derivatives

Starch

As a type of carbohydrate, starch is produced by many different living plants via photosynthesis. The innate starch has two kinds of chain structures, linear and branched, commonly referred to as amylose and amylopectin (**Figure 1A** and **B**).[21] Amylose is composed of glucosyl units that are linearly linked by α-(1-4)-linkages in approximately 99% of bonds and α-(1-6)-linkages in around 1%, and has a molecular weight in a range between 1×10^5 and 1×10^6 Da. These structural characteristics make amylose poorly soluble in water.[18, 21] Amylopectin contains approximately 95% α-(1-4)-linkages and 5% α-(1-6)-linkages and has its molecular weight ranging between 1×10^7 and 1×10^9 Da.[18, 21] Despite its multiple uses, the poor solubility in cooled water and fast degradation rate of native starch have limited its utilization in nanomedicines.[18, 19, 21, 22] To circumvent the limitations of native starch, many efforts have been directed toward modifying starch via grafting, oxidation, and esterification to achieve desirable starch derivatives and expand their applications.[14, 15, 17-20]

Hydroxyethyl starch (HES)

HES is a starch derivative prepared by reacting amylopectin with ethylene oxide in alkaline media.[14] Amylopectin is structurally similar to glycogen, a branched glucose-storage human polysaccharide, which may be the reason for the non-immunogenicity of HES.[14, 20] As far as a single glucosyl unit is concerned, hydroxyethyl modification could occur on its C-2, C-3, and C-6 sites, as illustrated in **Figure 1C**. In the case of HES, the hydroxyethyl substitution predominantly takes place at C-2 sites of the glucosyl units, as hydrolysis at the C-2 sites is notably slower than that which occurs at the C-6 sites.[23] The hydroxyl groups at the C-3 sites of the glucosyl units have much lower activity compared to that at C-2 or C-6 sites,[23] and thus, hydroxyethyl modification mainly takes place at the C-2 and C-6 sites of the glucosyl units in HES, as shown in **Figure 1D**. Hydroxyethyl modification of amylopectin plays key roles in regulating the solubility of the amylopectin and its degradation rate,[24] and accordingly, causes HES to become water-soluble at ambient temperature and its α-amylase-mediated degradation is also markedly reduced in comparison to the unmodified amylopectin. HES is often categorised into different classes basing on a few typical parameters, such as molecular weight, mole substitution, and substitution pattern (C-2/C-6 substitution ratio) of hydroxyethyl groups, and these parameters are closely correlated to the pharmacokinetics of HES.[24] Various types of HES products have been produced since the first generation of HES became commercially available.[20] Nowadays, HES is widely used as a pharmaceutical excipient for regular drugs or as a designed carrier for nanomedicines in the form of prodrugs, particles, micelles, and vesicles.[14, 18-20] The major reason why HES has become a favourite carrier material is mainly attributed to its following advantages:[14, 15] (1) good water solubility and protein repellent nature against certain opsonins such as immunoglobulins, fibrinogen, and complement proteins, allowing HES nanocarriers to evade rapid clearance from

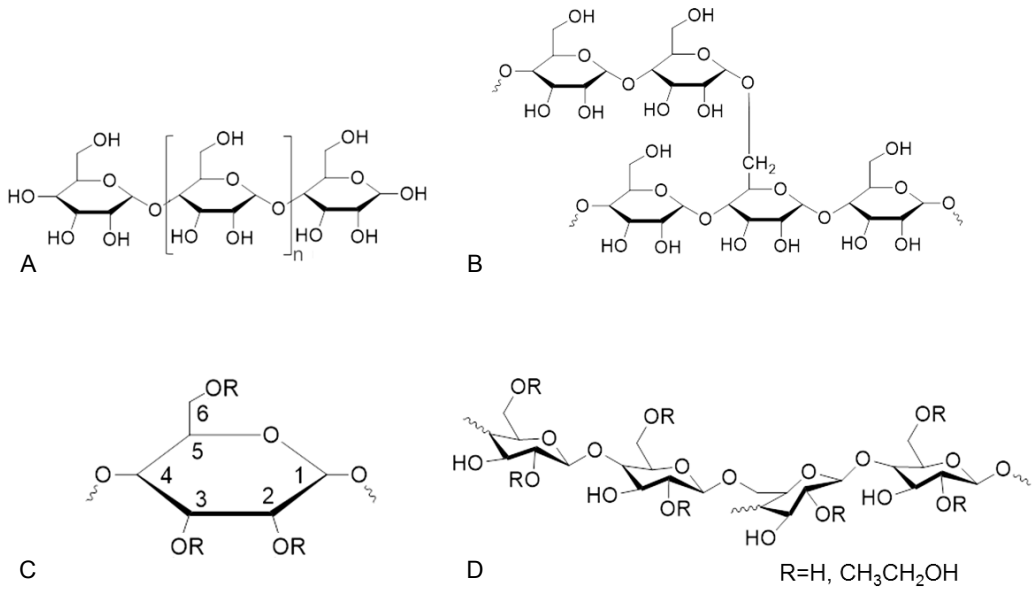

Figure 1. (A, B) Chemical structures of amylose (A) and amylopectin (B). (C) Possible substitution patterns of hydroxyethyl modification in the glucosyl unit of starch. (D) Chemical structures of HES.

the bloodstream; (2) very low hypersensitivity; (3) significantly prolonged retention time in the blood plasma and certain organs as compared to the native starch; and (4) largely improved stability of HES due to the degradation resistance of hydroxyethyl groups.

Investigations into the pharmaceutical application of HES began many years ago. A landmark event was a clinical study on the intravascular volume expansion properties of HES in the 1970s.[25] With the rapid development of nanomedicines in the last decade, HES has attracted a lot of clinical attention.[16, 19] HES contains a large number of free hydroxyl groups, which enables HES to be chemically modified in designed manners. Among diverse HES derivatives, many of them have shown great potential in acting as nanocarriers for the delivery of diagnostic and therapeutic agents toward cancers.[16, 17, 26-35]

Polymeric Prodrugs

Characteristics of polymeric prodrugs

Polymeric prodrugs are a type of polymer–drug conjugate that is commonly built by covalently binding small molecule drugs onto a polymer backbone. Prodrugs were primarily proposed through the Ringsdorf model that hypothetically consists of three major components:[36] (1) a biocompatible polymer backbone with a highly hydrophilic nature to achieve well-defined dispersivity and stability of the conjugated drugs in the aqueous medium; (2) a drug bound to the polymeric backbone with a covalent linkage; and (3) a specific moiety with designated functions to potentially interact with particular molecules or cells. Based on this model, prodrugs can offer several explicit advantages over traditional small molecule drugs. The aqueous solubility changes of many drugs when administered as prodrugs significantly improve their efficacy since 40–60% of regular drugs in development have low aqueous solubility, and in turn, exhibit poor bioavailability.[37, 38] Prodrugs also offer the opportunity to moderate the release of the conjugated drug by tailoring the structure and properties of the prodrug main chain. By doing so, the rate and duration of drug delivery might be regulated in custom-designed ways while avoiding undesired side effects which could arise from large fluctuations in concentration unavoidable with periodic drug administration.[39, 40] Prodrugs can also modulate drug pharmacokinetics, which would be beneficial for certain drugs having a short plasma half-life or showing off-target toxicities.[41, 42] In addition to the mentioned benefits, prodrugs could be capable of delivering the loaded drug towards the site of pharmacological action given that they carry targeting moieties.[43-46]

It is known that many anticancer drugs have poor water solubility and metabolic instability, and their usage in the clinic is very often limited due to their low efficacy and dose-dependent toxicity. In clinical cancer therapy, a general goal for administering an anticancer drug is to deliver it at a dose high enough to attain high cytotoxicity against cancer cells. Nevertheless, the actual applied drug dose has to be limited to minimise the toxicity to normal tissues and organs. A previous study on solid tumours pointed out that in comparison to small molecule drugs, uptake of macromolecular drugs, usually in the form of nanoparticles (NPs), would be increased due to the enhanced permeability and retention (EPR) effect arising from a combination of poor lymphatic drainage and increased vascular permeability in the tumour microenvironment.[47] Accordingly, polymeric prodrugs usually exhibit a much greater capability to accumulate at the tumour site through the EPR effect when compared to their small-molecule drug counterparts, making them attractive in cancer diagnosis and therapy.[36, 39] Besides the EPR effect, an alternative route associated with tumour uptake of nanomedicine has recently been proposed based on the transcytosis effect observed from a kind of γ-glutamyl transpeptidase-responsive camptothecin-polymer conjugate.[48] When the conjugate comes into contact with the tumour blood vessels or extravasates into the tumour interstitium, its γ-glutamyl moieties are cleaved by the overexpressed γ-glutamyl transpeptidase on the cell membrane, and accordingly, the conjugate becomes positively charged, which is quite conducive to the endocytosis of the conjugate due to its cationic character. The resulting conjugate is thus able to efficiently penetrate into the tumour via caveolae-mediated endocytosis and transcytosis. This bioresponsive strategy has potential for the development of therapeutic polymers to treat different diseases based on physiological signals.[48]

Hydroxyethyl starch-based prodrugs for anticancer treatment

HES is particularly attractive for prodrug development because it has full water solubility, tunable degradation without lengthy accumulation in the body, and good systemic tolerability. In addition, HES can be administered at a high daily dose and contains numerous functional groups,[14, 16, 19, 24, 25, 40] making it an excellent candidate for prodrug construction. Many efforts have now been made to develop HES-based prodrugs for anticancer treatments. Several first-line anticancer chemotherapeutic drugs, as shown in **Figure 2**, have been used together with HES to build different prodrugs, and the resulting prodrugs show greatly improved anticancer efficacy compared to free drugs with varying degrees of potency in clinical translation.[15, 17, 29, 31, 40, 45, 46, 49-52]

A doxorubicin (DOX) prodrug (HES-SS-DOX) was constructed by conjugating DOX onto HES through a redox-sensitive disulphide bond linkage for delivering DOX and reducing its side effects.[30] The disulphide bond linkage was designed in response to glutathione (GSH) considering that the intracellular GSH level of tumour cells can be several times higher than that of normal cells.[53] The *in vivo* examination of HES-SS-DOX prodrug demonstrated that the GSH-mediated antitumor performance was much better than free DOX whilst the free DOX-associated cardiotoxicity was greatly reduced. A similar method with some modifications in chemical reaction routes was also used to construct a type of HES-SS-paclitaxel (PTX) prodrug by replacing DOX in HES-SS-DOX with PTX, and so creating the HES-SS-PTX prodrug which was further self-assembled into stable NPs with a monodispersed characteristic.[29] It was found that after intravenous administration, the HES shell of HES-SS-PTX NPs was degraded to varying degrees by α-amylase in the bloodstream. As a result, the size of HES-SS-PTX NPs became smaller with increased circulation time, facilitating extravasation of HES-SS-PTX NPs out from blood vessels and their penetration deep into the tumour interstitium. Results obtained from *in vivo* experiments revealed that HES-SS-PTX NPs showed several advantages over Taxol, a commercially-available anticancer drug, and had potential for further clinical development. It is worth mentioning that the structure, property

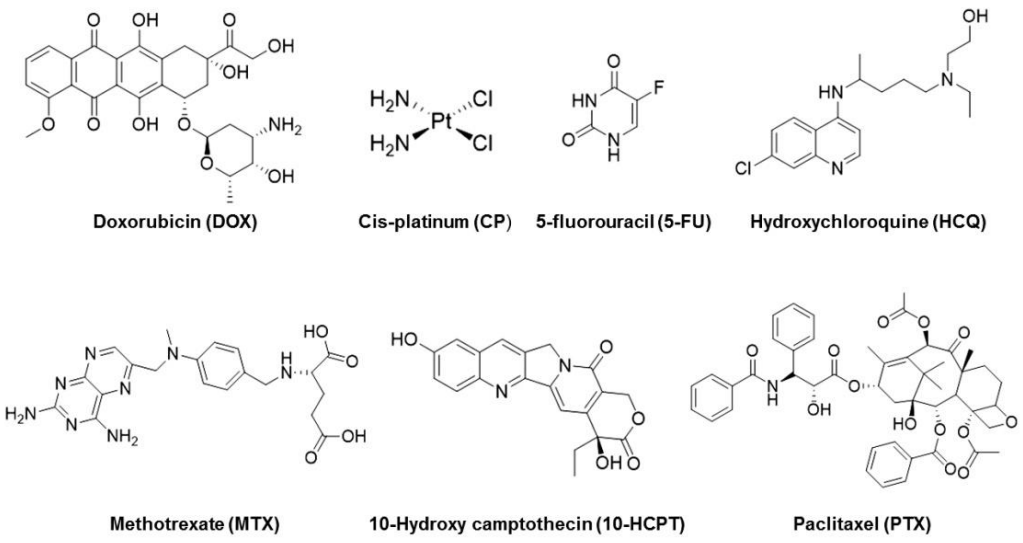

Figure 2. Chemical structures of several kinds of chemotherapeutic anticancer drugs used for constructing HES-based prodrugs. HES: hydroxyethyl starch.

and performance of the disulphide-linked prodrugs are affected to varying degrees by disulphide-bearing linkers. Some studies on disulphide-linked prodrugs indicated that the linker length played an important role in the self-assembling process of the prodrugs; and moreover, the linkage sites and the type of linkers also exerted strong influences on the performance of the prodrugs.[7, 9] These findings are meaningful for effectively designing disulphide-involved prodrugs.

5-Fluorouracil (5-FU) is commonly used in chemotherapeutic treatment for different malignant tumours. To reduce its side effects, a 5-FU derivative, 5-fluorouracil-1-acetic acid (FUAC), was conjugated onto HES to prepare a HES-FUAC prodrug through an esterification reaction between the hydroxyl groups in HES and the carboxyl groups in FUAC.[40] The *in vitro* experimental results revealed that by exposing the HES-FUAC prodrug to human plasma or rat plasma, only FUAC release was detected and there were no significant differences measured from the FUAC release profiles. However, when exposed to rat liver homogenate, the HES-FUAC prodrug would release both FUAC and 5-FU, but the release rate of FUAC was seen to be much faster than that of 5-FU. Under *in vivo* administration conditions, only FUAC was released from the HES-FUAC prodrug. The *in vivo* performance evaluation indicated that the group administered the HES-FUAC prodrug exhibited a much higher peak FUAC plasma concentration and a greatly prolonged FUAC plasma half-life when compared to the group administered free FUAC, suggesting that the pharmacokinetics of FUAC were greatly improved by use of the HES-FUAC prodrug.

10-Hydroxy camptothecin (10-HCPT) is one of the camptothecin analogues, and shows a wide variety of anticancer activities against different solid tumours. Nevertheless, several drawbacks of 10-HCPT, including low aqueous solubility, short plasma half-life, and dose-dependent toxicity, hamper its clinical application.[48-50] In an attempt to overcome these, 10-HCPT was conjugated onto HES via a covalent linkage between the carboxyl groups in the succinic anhydride-modified HES and the amino groups in the glycine spacer on the modified 10-HCPT to form a 10-HCPT-HES prodrug.[49] Testing *in vivo* revealed that the 10-HCPT-HES prodrug was able to overcome the disadvantages of 10-HCPT and had greatly enhanced anticancer efficacy in a Hep-3B-tumor-bearing nude mouse model.

Methotrexate (MTX) is an antifolate drug and is commonly used for the treatment of certain cancers, rheumatoid arthritis, and other diseases.[17] Like many other chemotherapeutic drugs, one of the major concerns for the clinical use of MTX is its dose-dependent toxicity to vital organs, especially the liver.[6] MTX was therefore connected to HES to create a HES-MTX prodrug via an esterification reaction between the hydroxyl groups in glucosyl units of HES and the activated carboxyl groups of MTX through the pre-constructed carbodiimide adducts.[17] The HES-MTX prodrug looked like a type of NP with a negatively-charged surface that is somewhat similar to the surface of the vascular endothelium in terms of the charging property. The small size and the negative surface charge of HES-MTX prodrugs may result in their longer half-life in plasma, and consequently, their increased tumour accumulation via the EPR effect. The study on the HES-MTX prodrug once again demonstrates that HES is an excellent candidate for constructing polymeric prodrugs. In order to reduce the liver toxicity associated with MTX chemotherapy, an effort was also made to build methoxy poly(ethylene glycol) (PEG)/oleanolic acid prodrug micelles with physical encapsulation of MTX.[6] Such designed nanomedicines were found to exhibit superior anti-tumour efficacy without inducing adverse effects in liver owing to the co-delivery of a hepatoprotective prodrug and MTX.

Hydroxychloroquine (HCQ) is a 4-aminoquinoline derivative, and was previously used as a common antimalarial agent. HCQ has recently been conjugated onto HES via a carbonyldiimidazole coupling route to prepare a chloroquine-modified HES prodrug (CQ-HES) with a developed ability to inhibit the invasion of pancreatic cancer cells.[52] CQ-HES prodrugs displayed the propensity to assemble into NPs in a pH-dependent manner, and

the resulting CQ-HES NPs were demonstrated to have a greatly enhanced ability to inhibit the migration and invasion of pancreatic cancer cells when compared to free HCQ. No significant HCQ release was detected from CQ-HES prodrugs, suggesting that the activity against pancreatic cancer cells was explicitly attributed to the action of CQ-HES instead of HCQ. Considering the promising ability to block cancer cell invasion and the ability to form NPs, this CQ-HES prodrug has potential for future clinical application.

Recently, a large variety of polymeric prodrugs have been developed using regular covalent linkages. In addition, some sensitive linkages with responsiveness to various stimuli such as pH, light, heat, magnetism and enzymes have also been introduced into prodrugs to imbue them with improved capabilities. Several HES-based prodrugs constructed with environmentally-responsive linkages or with active targeting functionalities have been developed to achieve improved anticancer efficiency as well as reduced side effects.

A type of DOX-bound prodrug was created by linking DOX to HES via a hydrazine (Hyd) bond to attain pH-sensitivity (HES-Hyd-DOX).[51] The HES-Hyd-DOX was synthesised via a multi-step reaction route. HES was first modified with nitrophenyl chloroformate and then with Hyd monohydrate to produce an intermediate (HES-$NHNH_2$). DOX was further reacted with HES-$NHNH_2$ to obtain HES-Hyd-DOX prodrugs, followed by the formation of HES-Hyd-DOX NPs via self-assembly. The intracellular acid-trigged disassociation of HES-Hyd-DOX NPs was detected, and the conjugated DOX was released from HES-Hyd-DOX NPs in a controllable manner with improved anticancer efficacy when compared to free DOX.

Another type of DOX-conjugated HES prodrug with pH-sensitivity was synthesised through a one-step synthesis route using oxidised HES, DOX, and a cyclopeptide (cRGD) (denoted as HES=DOX/cRGD).[45] This HES=DOX/cRGD prodrug contained Schiff base linkages between DOX and HES whilst carrying cRGD moieties. HES=DOX/cRGD prodrugs showed confirmed ability to self-assemble into NPs with the cRGD moieties protruding outward from their surface. The optimally-fabricated HES=DOX/cRGD NPs released the conjugated DOX in a pH-sensitive way and showed an ability to deliver DOX to tumours through the interaction between cRGD and its receptor $\alpha_v\beta_3$ integrin overexpressed on the membrane of certain tumour cells.

In addition to HES-Hyd-DOX and HES=DOX/cRGD prodrugs, HES has also been modified with luteinizing hormone-releasing hormone (LHRH) while carrying DOX to generate HES-DOX/LHRH prodrugs with active targeting features.[46] It is known that LHRH membrane receptors are overexpressed in many types of cancer cells associated with prostate, breast, ovarian and endometrial tumours.[46, 54] Besides, LHRH receptors are also found in both metastatic lymph nodes and lesions of prostate cancer. Importantly, LHRH receptor expression is scarce in normal tissues.[46, 54] Thus, HES-DOX/LHRH would be capable of delivering DOX targeted at cancer cells by way of LHRH-receptor mediated active targeting with improved anti-tumour efficacy. Indeed, HES-DOX/LHRH prodrugs have been demonstrated to have higher levels of anti-tumour and anti-metastasis activities based on the RM-1-xenografted mouse model while having lower systemic toxicity in comparison to free DOX and non-targeted HES-DOX, suggesting their potential for clinical translation.

Cis-platinum (CP) is a widely-used chemotherapeutic drug which has been used to treat a range of cancers since it was approved by the FDA in 1978. A CP-conjugated HES prodrug functionalised with lactobionic acid (LA; a galactose (Gal) moiety) was fabricated into NPs (LA-HES-Pt) for the actively-targeted delivery of CP.[32] The asialoglycoprotein receptor is known to be usually overexpressed on certain hepatic carcinoma cells such as HepG-2 or H22 cells.[55] Since Gal can bind specifically to the asialoglycoprotein receptor, LA-HES-Pt NPs can thus deliver CP toward hepatic carcinoma cells. *In vitro* experiments verified that LA-HES-Pt NPs can efficiently target HepG-2 cancer cells and promote cellular endocytosis while exerting much stronger effects on cancer cells compared to free CP.

Based on the above observations, it can be seen that these HES-based anticancer prodrugs differ markedly in their composition, structure, property and performance. To facilitate the identification of the main differences between these prodrugs, several of their characteristics are summarised in **Table 1**.

Hydroxyethyl starch-based Nanoparticles and Their Applications

Main methods for preparing HES-based NPs

Besides functioning as the polymeric backbone of HES-based prodrugs, HES is also utilised for preparing different types of NPs. There are three major ways to build HES-based NPs. The first method is to synthesise the required HES prodrugs, and then to assemble them into NPs via self-assembly, as mentioned earlier.[6, 7, 9, 17, 29, 30, 32, 45, 46, 51, 52] Another method is to combine certain HES prodrugs with specific agents to assemble HES-based NPs through the collaborative constraints involved in electrostatic, π–π stacking, and hydrophobic interactions.[56]

Some small molecules with near-infrared (NIR)-responsive characteristics have been used in image-guided photothermal therapy of cancers. Nevertheless, the NIR molecules that have a suitable NIR excitation window, meet *in vivo* safety requirements, and show high photothermal conversion efficiency are still very few.[57] The molecule 1,1-dioctadecyl-3,3,3,3-tetramethyl indotricarbocyanine iodide (DiR) is a type of lipophilic NIR fluorescent molecule with negligible cytotoxicity when applied at a safe concentration *in vivo*.[28, 56, 57] DiR has thus been combined with HES-SS-PTX prodrug to create NPs, as shown in **Figure 3**, to realise GSH-responsive dual-modal chemo-photothermal combination anticancer therapy.[28] It has been demonstrated that such combination therapy showed notably improved anticancer efficacy compared to single modal therapy or free PTX.[28]

Another NIR fluorescent molecule, indocyanine green (ICG), has also been used together with HES-SS-DOX prodrugs for constructing HES-SS-DOX@ICG NPs.[57] The optimally engineered HES-SS-DOX@ICG NPs had good physical and photothermal stability in aqueous media, and showed high photothermal efficiency *in vivo*. They were able to rapidly release the loaded DOX in response to a redox stimulus, and to laser irradiation. Based on the H22-tumor-bearing mouse model, these NPs were found to preferentially accumulate inside tumours in comparison to other major organs. HES-SS-DOX@ICG NPs together with dose-

Table 1. HES-based polymeric prodrugs and their characteristics

Name of prodrug	Name of drug	Responsiveness	Linkage	Strength	Reference
HES-SS-DOX	DOX	Redox	Disulphide bond	Responsive release; Improved efficiency; Reduced side effects	Hu et al.[30]
HES-SS-PTX	PTX	Enzyme/Redox	Disulphide bond	Responsive release; Enhanced penetration; Improved efficiency; Reduced side effects	Li et al.[29]
HES-FUAC	5-FU	–	Ester bond	Improved efficiency; Reduced side effects	Luo et al.[40]
10-HCPT-HES	10-HCPT	–	Amide bond	Improved efficiency; Reduced side effects	Li et al.[49]
HES-MTX	MTX	–	Ester bond	Improved efficiency; Reduced side effects	Goszczyński et al.[17]
CQ-HES	HCQ	–	Ester bond	Improved efficiency; Reduced side effects	Sleightholm et al.[52]
HES-Hyd-DOX	DOX	pH	Hydrazine bond	Responsive release	Zhu et al.[51]
HES=DOX	DOX	pH	Imine bond	Targeting; Improved efficiency; Reduced side effects	Li et al.[15]
HES=DOX/cRGD	DOX	pH	Imine bond	Targeting; Improved efficiency; Reduced side effects	Li et al.[45]
HES-DOX/LHRH	DOX	pH	Imine bond	Targeting; Improved efficiency; Reduced side effects	Zhao et al.[46]
LA-HES-Pt	Pt	–	Ester bond	Improved efficiency; Reduced side effects	Xiao et al.[32]

Note: 10-HCPT: 10-hydroxy camptothecin; 5-FU: 5-fluorouracil; CQ: chloroquine; cRGD: cyclopeptide; DOX: doxorubicin; HCQ: hydroxychloroquine; HES: hydroxyethyl starch; Hyd: hydrazine; LHRH: luteinizing hormone-releasing hormone; MTX: methotrexate; PTX: paclitaxel.

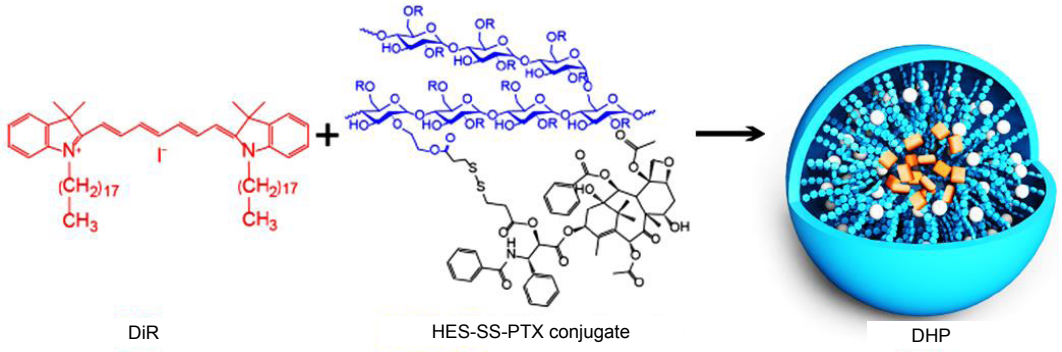

Figure 3. Schematic illustration for the construction of DiR/HES-SS-PTX NPs (DHP). DiR: 1,1-dioctadecyl-3,3,3,3-tetramethyl indotricarbocyanine iodide; HES: hydroxyethyl starch; NPs: nanoparticles; PTX: paclitaxel.

designated laser irradiation were able to fully eradicate tumours with only one injection and one single subsequent laser irradiation on the tumour site during a 14-day treatment period. In addition, they showed almost no impairment to the body.

The third method for the preparation of HES-based NPs is to graft some hydrophobic polymer side chains onto HES, and the obtained amphipathic HES grafting copolymers are further fabricated into NPs.[27, 35, 58]

Nanoparticles fabricated with amphipathic Hydroxyethyl starch copolymers

In general, the size of NPs has a critical impact on their performance. Large NPs with sizes of approximately 300 nm or larger would be likely to be detained by the reticuloendothelial system (RES) in liver and spleen, while small NPs with sizes less than 200 nm are more capable of accumulating in a tumour via the EPR effect.[3] A partial and temporary RES blockade strategy was proposed, using HES-grafted-polylactide (HES-g-PLA) copolymer NPs to enhance DOX delivery toward tumors.[27] In this strategy, large empty HES-g-PLA NPs (mean size: approximately 700 nm) were used to temporarily block up RES in tumour-bearing mice for a certain period of time before the administration of small DOX-loaded HES-g-PLA NPs (mean size: approximately 130 nm), as illustrated in **Figure 4**. Based on this sequential administration mode, the DOX-loaded HES-g-PLA NPs were able to effectively deliver DOX specifically toward tumours.

In the case of chemotherapy for solid tumours, heterogeneous

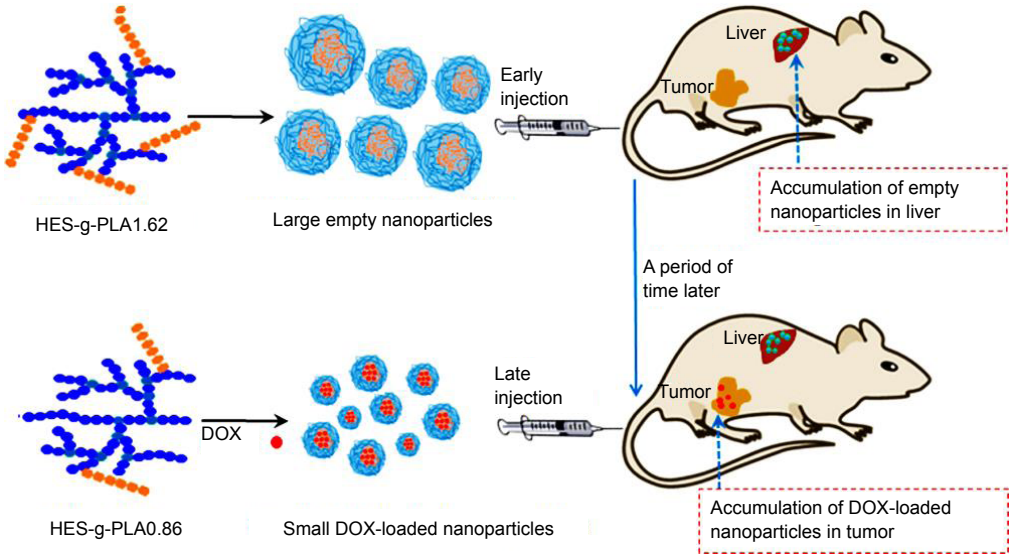

Figure 4. Schematic illustration showing the delivery of DOX toward tumours using HES-g-PLA partner nanocarriers. DOX: doxorubicin; HES-g-PLA: hydroxyethyl starch-grafted-polylactide.

distribution of a drug inside the tumour is ubiquitous because tumours usually create certain pathological barriers to prevent drugs from approaching tumour cells. Hence, tumour cells in regions of low or sublethal concentration of therapeutics would be hardly eradicated.[35, 54] Besides the possibly of causing neoplasm relapse, such sublethal or insufficient chemotherapy could also result in tumour metastasis via an epithelial–mesenchymal transition (EMT) mechanism.[35, 55] Considering the fact that transforming growth factor-β (TGF-β) plays a vital role in the EMT via interactions between TGF-β and its receptor, LY2157299, a TGF-β receptor inhibitor was co-delivered together with DOX using HES-g-PLA NPs as a vehicle, as shown schematically in **Figure 5**, to suppress the inadequate chemotherapy-promoted metastasis. The results demonstrate that the co-delivery of DOX and LY2157299 is an effective strategy to achieve this goal.[35] An *in vivo* study on mice bearing subcutaneous 4T1 tumours revealed that the co-delivery of DOX and LY2157299 simultaneously suppressed primary tumour, with a tumour inhibition rate of 80.7%, and distant metastasis.

HES has also been grafted with polycaprolactone (PCL) to create an amphiphilic copolymer (HES-PCL). The achieved HES-PCL was further functionalised with Gal to fabricate DOX/ICG-loaded nanocolloidosomes (NCs), as illustrated in **Figure 6**.[58] Such fabricated NCs thus obtained Gal-mediated targeting capability via interaction between Gal and asialoglycoprotein receptors. The functionalised DOX/ICG@Gal-HES-PCL NCs were found to have a densely-packed structure, and their shell-like surface was composed of arranged hydrophilic HES NPs. *In vivo* results indicated that DOX/ICG@Gal-HES-PCL NCs had tumour-targeting ability and were able to fully eradicate tumours through chemo-photothermal combination therapy.

Hydroxyethyl starch-involved nanocarriers

PEG is a water-soluble, biocompatible and non-immunogenic polymer which is widely used as the hydrophilic segment for modifying different hydrophobic polymers. Nowadays, PEGylation serves as an important technique to prolong the circulation time of certain NPs and to control their dosing interval.[59] However, this technique has raised several concerns. It has been reported that high intracellular PEG accumulation can alter organelle density, and concomitantly, give rise to variations in the activity of lysosomal enzymes and transporters as well as membrane glycoproteins due to the nondegradable nature of PEG.[59, 60] Furthermore, the increased stability of NPs as a result of PEGylation could impede the escape of drugs from endosomes in tumour cells, resulting in reduced efficacy.

In an attempt to circumvent the "PEG dilemma", HES has been explored as a substitute for modification of NPs.[61-64] HESylation was compared with PEGylation on the same base using polydopamine (PDA) NPs as the core material and DOX as the

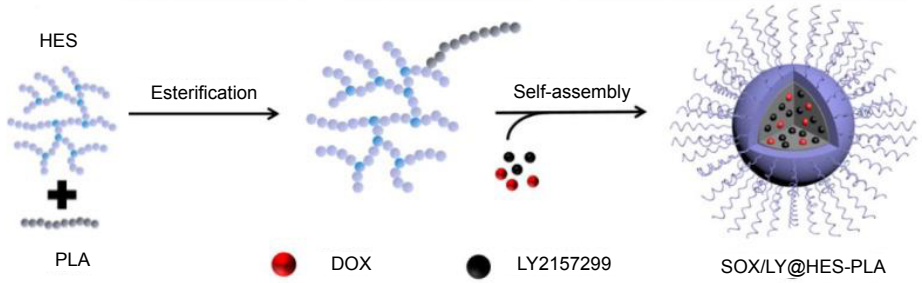

Figure 5. Schematic representation showing co-loading of DOX and LY2157299 into HES-g-PLA NPs. DOX: doxorubicin; HES-g-PLA: hydroxyethyl starch-grafted-polylactide; LY2157299: a transforming growth factor-β receptor inhibitor.

model drug since PDA is biodegradable with many advantageous properties.[65] PDA NPs were first prepared and they were then modified with thiolated HES and aminated PEG to create HES-PDA NPs and PEG-PDA NPs.[33] These NPs were loaded with DOX to finally obtain DOX@HES-PDA NPs and DOX@PEG-PDA NPs, respectively, as schematised in **Figure** 7. *In vivo* experiments revealed that HESylated PDA NPs were similar to PEGylated PDA NPs, with characteristics including good stability, high drug loading efficiency, favourable lyophilization stability, biocompatibility, and tumour inhibition rate.[59]

In some cases, HES was modified with certain hydrophobic small molecules, and was further fabricated into nanocarriers to enhance the delivery of diagnostic and therapeutic agents. One of such modified HES derivatives was synthesised by conjugating 1-octadecanethiol (C18) onto the backbone of HES via a redox-sensitive disulphide bond linkage, and the achieved HES-SS-C18 was subsequently connected to an iRGD peptide as branches.[31] This specifically synthesised iRGD HES-SS-C18 was self-assembled into nanoclusters with reduction-responsive disintegratable features for delivering DOX, as illustrated in **Figure 8**. DOX@iRGD-HES-SS-C18 nanoclusters were demonstrated to have an ability to deliver DOX towards tumours through iRGD-mediated blood vessel targeting while showing enhanced tumour penetration.[31]

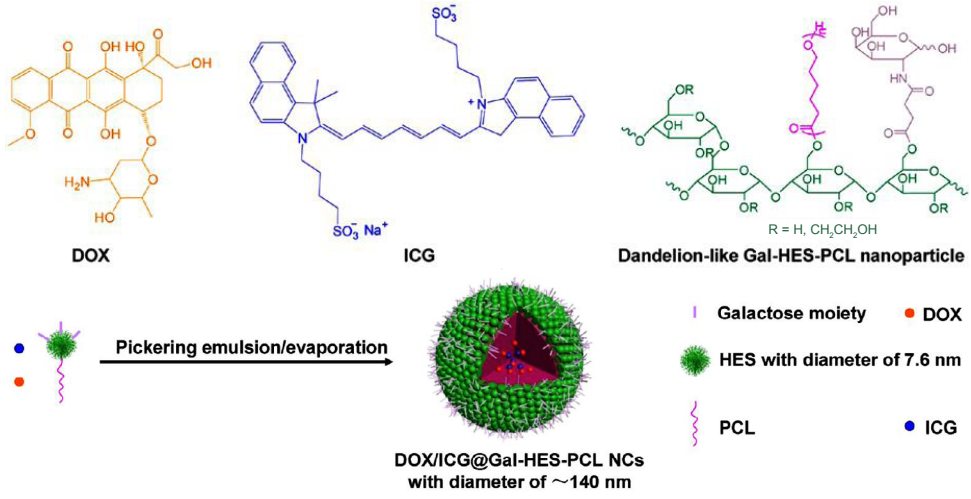

Figure 6. Schematic illustration of the structure of DOX/ICG-loaded Gal-HES-PCL nanocolloidosomes and their pickering emulsion formation. DOX: doxorubicin; Gal: galactose; HES: hydroxyethyl starch; ICG: indocyanine green; PCL: polycaprolactone.

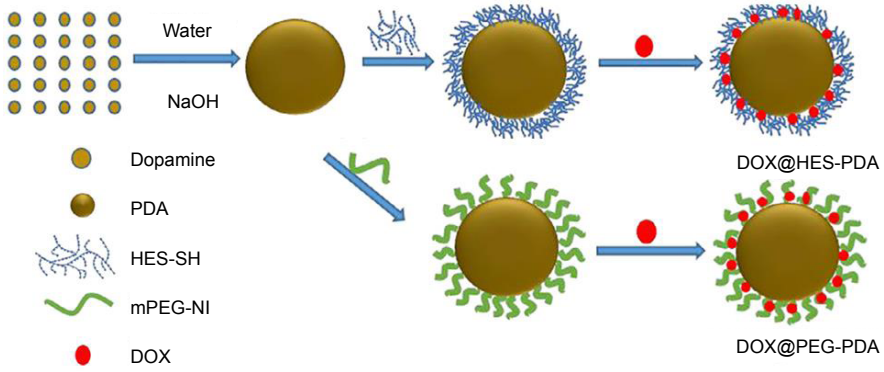

Figure 7. Schematic illustration showing the preparation of DOX@HES-PDA NPs and DOX@PEG-PDA NPs. DOX: doxorubicin; HES: hydroxyethyl starch; mPEG: methoxy poly(ethylene glycol); NPs: nanoparticles; PDA: polydopamine; PEG: poly(ethylene glycol).

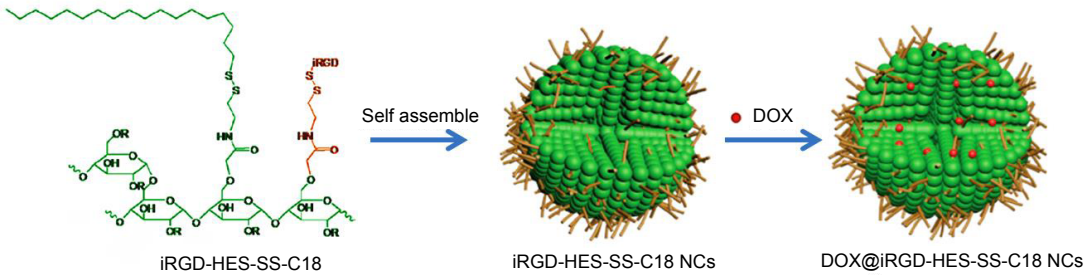

Figure 8. Schematic illustration of the fabrication of DOX@iRGD-HES-SS-C18 NCs. C18: 1-octadecanethiol; DOX: doxorubicin; HES: hydroxyethyl starch; iRGD: 9-amino acid cyclic peptide; NC: nanoclusters.

Another hydrophobically-modified HES derivative was developed by conjugating oleic acid (OA) onto the glucosyl units of HES, and the synthesised HES derivative (HES-OA) was self-assembled into ICG-loaded NPs (ICG@HES-OA) to achieve improved photodynamic therapy.[66] By co-delivering ICG@HES-OA NPs and β-phenylethyl isothiocyanate, a compound which depletes GSH, it was found that ICG@HES-OA NPs exhibited efficient singlet oxygen generation under laser irradiation, promoted cellular uptake, and enhanced tumour accumulation, whilst β-phenylethyl isothiocyanate showed a significant intracellular GSH depletion effect, suggesting that such combination therapy holds potential for clinical translation due to their synergistic antitumor effects.[66]

A type of HES-involved micelle was fabricated using a hydrophobically-modified HES derivative. HES was first modified with propynyl glycidylether to produce an intermediate with hydrophobic branches (PyHES), and PyHES was further connected with N-acetyl-cysteine (NAC) through a thiol-yne click reaction to achieve a PyHES-NAC derivative with pH-responsive features.[67] PyHES-NAC was able to self-assemble into micelles in response to changes in pH values. PyHES-NAC micelles were found to have the ability to protect the drug under acidic conditions while rapidly releasing the drug under neutral conditions.[67]

HES was first esterified with lauric, palmitic, and stearic acids to obtain different amphiphilic HES derivatives, and the derivatives were further self-assembled into micelles and vesicles, respectively.[26] It was found that only lauric acid-modified HES (HES-L) having percentage molar substitution between 8.7% and 10.3% was able to form stable nanodispersive micelles or vesicles. HES-L was selected to modify the surface of poly(lactic-co-glycolic acid) nanospheres to prevent them from adsorbing human serum albumin and fibrinogen, and meanwhile, such surface modification was compared between HES-L and Pluronic modifications.[68]

Another type of HES-involved micelle was built for delivering curcumin (CUR).[69] CUR has been widely used in the biomedical field but it shows poor water solubility and low bioavailability.[70] CUR was thus conjugated onto HES via an acid-labile ester bond and the resulting HES-CUR derivative was further assembled into micelles.[69] These HES-CUR micelles were demonstrated to have significantly enhanced antioxidant and anticancer activity compared to free CUR owing to the improved solubility and stability of CUR.

TG100-115 is an exclusive phosphatidylinositol 3-kinase-γ inhibitor, which plays an important role in the progression of different tumours by reversing the phenotype of tumour-associated macrophages.[71] TG100-115 was conjugated onto HES, followed by connection with CDM-PEG to finally construct a type of sorafenib-loaded micelle.[72] By co-delivering TG100-115 and sorafenib, a first-line drug for the treatment of advanced liver cancer, the micelles exhibited much better antitumor activity in a Hep-3B-bearing nude mouse model compared to the single-drug treatment.

In addition to the above-mentioned HES-based NPs or micelles, some HES nanocapsules have also been fabricated. HES nanocapsules with surface PEGylation were generated via interface polyaddition and attachment of PEG chains.[73] These HES nanocapsules showed potential for functioning as a platform to deliver diagnostic and therapeutic agents. Another project created folic acid-conjugated HES nanocapsules by first fabricating HES nanocapsules with carboxymethylation using an inverse miniemulsion method, and then conjugating them with NH_2-terminated folic acid.[62] These HES nanocapsules showed confirmative receptor-mediated targeting capability towards HeLa cells. In view of the many differences observed from these HES-based NPs, their main characteristics are summarised in **Table 2** to facilitate their identification.

Future Perspectives of Hydroxyethyl Starch-Based Nanocarriers

Based on the above observations, it can be concluded that in recent years, HES has evoked a great deal of research interest in nanocarriers that are intended for delivery of diagnostic and therapeutic agents for anticancer treatments. The reason why

Table 2. HES-based nanoparticles and their characteristics.

Name of nanoparticles	Name of drug	Responsiveness	Strength	Reference
DiR/HES-SS-PTX	PTX	Redox/Radiation	Combination therapy; Imaging	Li et al.[28]
HES-SS-DOX@ICG	DOX	Redox/Radiation	Combination therapy; Imaging	Yu et al.[57]
DOX@HES-g-PLA	DOX	–	RES blockade	Yu et al.[27]
DOX/LY@HES-g-PLA	DOX	–	Overcoming metastasis	Zhou et al.[35]
DOX/ICG@Gal-HES-PCL	DOX	Radiation	Targeting; Combination therapy; Imaging	Hu et al.[58]
DOX@HES-PDA	DOX	–	HESylation comparison	Wu et al.[33]
DOX@iRGD-HES-SS-C18	DOX	Redox	Targeting	Hu et al.[31]
ICG@HES-OA	PEITC	Radiation	Photodynamic therapy;Combination therapy	Hu et al.[66]
PyHES-NAC	DOX	pH	Oral delivery	Jong et al.[67]
HES-CUR	CUR	–	Improved efficiency	Chen et al.[69]
HES-TG100-115-CDM-PEG	Sorafenib	–	Combination therapy	Li and Zhao[72]

Note: C18: 1-octadecanethiol; CUR: curcumin; DiR: 1,1-dioctadecyl-3,3,3,3-tetramethyl indotricarbocyanine iodide; DOX: doxorubicin; Gal: galactose; HES: hydroxyethyl starch; ICG: indocyanine green; iRGD: 9-amino acid cyclic peptide; NAC: N-acetyl-cysteine; OA: oleic acid; PCL: polycaprolactone; PDA: polydopamine; PEG: poly(ethylene glycol); PEITC: β-phenylethyl isothiocyanate; PLA: polylactide; PTX: paclitaxel; PyHES: HES intermediate with hydrophobic branches; TG100-115: an exclusive phosphatidylinositol 3-kinase-γ inhibitor; RES: reticuloendothelial system.

HES is so valued and welcomed is due to its specific advantages, mainly high water solubility, excellent *in vivo* safety, adjustable degradability and chemically-modifiable versatility.[14, 15, 19, 20] It can be seen that the studies on HES-based nanocarriers described above mainly focus on polymeric prodrugs, HES modifications associated with hydrophobic small molecules and self-assembly of prodrugs or modified HES derivatives, while there is obviously less research on HES grafting copolymers and their assembly. HES molecules themselves behave like microspheres with a size of more than ten nanometres in their hydrated state, depending on their molecular weight, mole substitution and substitution pattern.[14, 16, 30] It has been found that HES grafting copolymers can only be assembled into larger NPs so that they are unsuitable as a carrier for nanomedicines, given that the hydrophobic branches on the HES molecules are long or have a high degree of substitution.[27, 58] Hence, it remains a challenge to regulate the length of hydrophobic side chains on the HES molecules and to control the substitution degree of side chains if there are plans to explore more HES grafting copolymers. It is known that HES has a large daily maximum-tolerated dose when used as a plasma substitute.[40] Although HES nanocarriers can be used to increase the dosage of anticancer drugs to some extent due to the high HES tolerance, the drug loading for these nanocarriers still needs to be increased to reduce the cost and to improve the bioavailability of nanomedicines. The above-presented studies reveal that HES nanocarriers have promising potential in delivering diagnostic and therapeutic agents towards cancers. Consequently the development of more HES-associated nanomedicines against cancers, and their active translation into clinical applications, should be encouraged.

Author contributions

RT wrote the draft of manuscript; YW and XY revised and edited the manuscript. All authors approved the final version of this manuscript.

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Conflicts of interest statement

The authors declare no competing financial interest.

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