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Recent advances in the design of novel polymer nanoagents for cancer theranostics

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Table of contents

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Editorial: Recent advances in the design of novel polymer nanoagents for cancer theranostics

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Editorial on the Research Topic
Recent advances in the design of novel polymer nanoagents for cancer theranostics

The significance of early and precise cancer theranostics in reducing patient mortality has been well established (Tringale et al., 2018; Crosby et al., 2022). In recent years, nanoagents have emerged as a promising tool for cancer theranostics, owing to their passive and active targeting effects on cancerous tissues (Torchilin, 2011). Among the various types of nanoagents, polymer nanoagents have gained increasing attention due to their excellent biocompatibility, ease of processability, and flexible surface functionalization (Ou et al., 2019). In this Research Topic, 9 published articles focus on the design of novel polymer nanoagents for cancer theranostics, providing valuable insights into the development of effective and precise cancer therapies using polymer nanoagents.

One review in this Research Topic, Zhang et al. summarized the recent progress of chemiluminescent polymer nanoagents based on various chromophore substrates, including luminol, peroxyoxalates, 1,2-dioxetanes and their derivatives, for cancer detection. The authors emphasized the design strategies, mechanisms, and diagnostic applications of representative chemiluminescent polymer nanoagents. Huang et al. reviewed the general advances of polymer nanoagents and presented their applications in cancer diagnosis, treatment, and theranostics, respectively. The authors also proposed the challenges and prospects of polymer nanoagents in the cancer theranostics field. As the human tongue can extend out of the mouth and is approximately 1 cm thick, the utilization of polymer nanoagents in oral cancer theranostics is less restricted by tissue depth and is easier to apply in clinical practice. Zhang et al. focused on the application of polymer nanoagents in the diagnosis and treatment of oral cancer. As one of the most frequently used clinically

approved biodegradable polymers, poly (lactic-co-glycolic acid) (PLGA)-based nanoagents have been extensively applied in the field of cancer theranostics, including emerging cancer immunotherapy (Koerner et al., 2021). Gao et al. reviewed the advances of PLGA-based nanoagents in cancer immunotherapy, focusing on cancer vaccines and tumor microenvironment modulation.

In recent years, peptide-based nanoagents have gained extensive attention from researchers due to their high specificity and low systemic toxicity (Habibi et al., 2016). Focusing on peptide-based nanoagents, Zeng et al. summarized the applications of peptide-based nanoagents in the diagnosis and treatment of bladder cancer. As a class of lipid membrane-bound vesicles released by various cells, extracellular vesicles (EVs) with high physiochemical stability and biocompatibility have emerged as a new kind of drug delivery system for cancer theranostics (Bose et al., 2018). Nan et al. reviewed the direct modification of EVs and their application for cancer theranostics. The authors highlighted the prevailing approaches for direct modification of EVs, including cargo loading strategy for EVs modification and modification approaches of the EVs membrane. For conventional chemotherapy drugs, their poor tumor targeting effect is a key factor limiting their effectiveness in cancer treatment (Zeng et al., 2021). To address this issue, Rana et al. outlined the design of Smart Drug Delivery Systems (SDDSs) and their application in the cancer theranostics field. SDDSs that respond to redox, enzyme, light, ultrasound, and magnetic stimuli are highlighted. The authors proposed that SDDSs could become the future of Translational Medicine.

In an original research article in this Research Topic, Sun et al. prepared ROS-responsive Amplex® Red (ADHP) nanoprobes and studied their application in image-guided tumor resection, which has not been explored much. ADHP probes can rapidly oxidize in response to ROS in the tumor microenvironment to form resorufin, which significantly reduces the background fluorescence signal compared to the single resorufin probe. With the fluorescence guidance of ADHP nanoprobes, the authors successfully carried out image-guided surgery of 4T1 abdominal tumors. In addition, in response to the severe toxicity and gastrointestinal side effects of colchicine that limit its application in cancer treatment, Li et al. designed and synthesized a novel colchicine-magnolol hybrid (CMH) by splicing colchicine and magnolol, a multifunctional polyphenol showing favorable gastrointestinal protection. The antitumor results showed that CMH inhibited the growth of Lewis lung carcinoma (LLC) cells 100 times more potently than cisplatin, while the cytotoxicity of CMH was 10-fold lower than that of colchicine in normal human lung cells. Western blot results revealed that CMH dose-dependently suppressed the protein expression of phosphorylated ERK.

In conclusion, the rational design of polymer nanoagents, including inner probes/small molecule drugs and the outer polymer matrix (synthetic polymers, peptides, exosomes), plays a significant role in the cancer theranostics effect of polymer nanoagents. To further improve the effectiveness of polymer nanoagents in cancer theranostics, follow-up research could focus on developing smart probes/small molecule drugs with higher sensitivity and tumor responsiveness, and polymeric matrices with enhanced biodegradability and tumor targeting effects. In addition, the biosafety and ease of preparation of polymer nanoagents also need to be considered to make them clinically applicable, which needs to be balanced with the intelligence of polymer nanoagents. We expect that this Research Topic will inspire the design and synthesis of more advanced polymer nanoagents and promote their widespread application in cancer diagnosis and treatment.

Author contributions

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MINI REVIEW
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Direct Modification of Extracellular Vesicles and Its Applications for Cancer Therapy: A Mini-Review

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Extracellular vesicles (EVs) are a class of lipid membrane-bound vesicles released by various cells and mediate cell-to-cell communication. By reason of their high physiochemical stability and biocompatibility, EVs are considered as novel drug delivery system. An increasing number of studies have indicated that EVs can be modified to enhance their loading efficiency, targeting ability and therapeutic capabilities for cancer therapy. Compared with the tedious process of gene engineering approaches, direct modification of EVs is easier, faster and versatile. This mini review will summarize the prevailing approaches for direct modification of EVs. Additionally, the potential applications of modified EVs in cancer therapy are also discussed, which will help readers gain a better understanding of the technologies and applications in this field.

Keywords: extracellular vesicles, cancer therapy, drug delivery, modification, nanomedicine

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INTRODUCTION

Aberrant proliferation and aggressive metastatic capacity are the hallmarks of malignant tumors (Hanahan and Weinberg, 2011). The conventional oncological treatment options such as surgery, radiation, and chemotherapy are associated with several disadvantages, including poor targeting and severe side effects (DeVita and Chu, 2008). On the other hand, treating metastatic cancers with a high dose of drugs may result in drug resistance of cancer cells (Aleksakhina et al., 2019). Therefore, novel therapeutic approaches need to be developed to improve the efficiency, specificity and safety of cancer therapy.

Extracellular Vesicles (EVs) are nano membrane vesicles released from various cells. EVs can carry complex cargos such as proteins, lipids and nucleic acids, and communicate directly with recipient cells (Maas et al., 2017). In addition, EVs have some special advantages, including escaping from clearance by host immune system and passing through physiological barriers (Colao et al., 2018; Liu et al., 2019), which makes them suitable to be used as potential therapeutic agents and drug delivery vehicles (Liao et al., 2019). However, limitations remain in the use of natural EVs for cancer therapy. For example, the low targeting capacity of EVs might seriously affect the therapeutic effect, and a heterogeneous range of molecules contained in EVs brings safety concerns (Jabalee et al., 2018). At present, the EVs used in the studies for cancer treatment mainly derived from tumor cells, stem cells or immune cells. EVs derived from tumor cells exhibit relatively good targeting but with a risk (Taghikhani et al., 2020), whereas EVs derived from stem cells or immune cells have a good therapeutic effect but lack of targeting (Herrmann et al., 2021).

To circumvent these problems, modified EVs have recently emerged as a new alternative strategy for cancer therapy (Vader et al., 2016). Accumulating evidence suggests that the modification of

cargo loading or membrane components of EVs enhances their loading efficiency, targeting ability and therapeutic capabilities (Yong et al., 2020). In general, the approaches of modifying the EVs can be classified into direct modification (directly remold EVs) and indirect modification (engineer the parent cells). Compared with the tedious process of gene engineering approaches, direct modification of EVs is more simple, rapid and versatile (Nie et al., 2021). In this review, we focus on the prevailing approaches for direct modification of EVs, including cargo loading and membrane modifying of the EVs. Additionally, the latest reported progress in the applications of modified EVs for cancer therapy are summarized and discussed.

DIRECT MODIFICATION OF EVS

Cargo Loading Strategy for EV Modification

The approach to load exogenous cargo into EVs can be divided into passive and active loading methods. The passive loading method refers to therapeutic drugs directly incubated with EVs (Zhu et al., 2019) or donor cells (Guo et al., 2019; Zheng et al., 2020). Generally, the hydrophobic molecules are prone to interact with lipids exposed on EV membrane surface, making passive co-incubation the best approach for hydrophobic drugs with poor solubility. Although these methods are straightforward and do not damage the structure of EVs, the loading efficiency depends on the drug properties, incubation periods and other details. For example, curcumin and cucurbitacin-I were shown to rapidly diffuse into EVs when they were incubated at 22°C for 5 min, and the EV encapsulation could penetrate through the blood-brain barrier to exert anti-tumor effects in the glioblastoma model (Sun et al., 2010; Zhuang et al., 2011).

Active loading method refers to therapeutic drugs crossed through the EV membrane by electroporation (Zou et al., 2019), sonication (Zhupanyn et al., 2020), freeze and thaw cycles (Haney et al., 2015), extrusion (Bose et al., 2018), and so on. Based on these methods, a variety of therapeutic drugs have been loaded into EVs for the treatment of refractory tumors. The drug-loaded EVs promote the accumulation of drugs in cancer cells, enhancing blood circulation time, and consequently improving their treatment outcomes. Kim et al. compared these common methods, the results suggested that all the active loading methods attained higher loading efficiencies than the passive loading method, especially sonication and extrusion methods (Kim et al., 2016). However, these methods also have some limitations. For instance, during the electroporation and thaw cycle process, the media that contains phosphate-buffered pulse or sucrose could cause EV aggregation (Yan et al., 2020). In addition, EV membrane properties may be damaged due to the extrusion method (Fuhrmann et al., 2015). Thus, the most appropriate loading method for a target molecule depends on its physicochemical properties. For example, the passive loading method is typically suitable for small molecule and hydrophobic drugs because it can cross the hydrophobic membrane of EVs (Haney et al., 2015). For small RNA cargos, electroporation is the best loading approach because of its higher loading efficiency (Lamichhane et al., 2015), while the methods such as extrusion and sonication are more suitable for larger proteins and hydrophilic molecules (Yuan et al., 2017). The cargo loading strategies for EV modification are given in **Table 1**.

Modification of the EV Membrane

The approaches of direct modification of EV membrane are broadly divided into covalent and non-covalent modification. The covalent modification enables functional groups rapidly form covalent bonds with EVs. For example, sulfhydryl is widely presented on the EVs surface, therefore, it can be employed as the binding site for EVs labeling via the michael addition reaction between maleimide and sulfhydryl. Fan et al. utilized this method to anchor quantum dots (QDs) onto the surface of exosomes. They found that the QDs-labeled exosome complex can be swiftly engulfed by tumor cells, and the tumor cells were lighted up by the fluorescence of this complex (Fan et al., 2019). Click chemistry is a copper-catalyzed azide alkyne cyclo-addition reaction under physiological conditions (Kolb et al., 2001), and is also commonly used to enable bioactive molecules to form chemical bonds with EVs. Jia et al. conjugated the membrane of EV with neuropilin-1 targeted peptide (RGERPPR, RGE) by click chemistry to obtain glioma targeting EVs (Jia et al., 2018). However, these modification approaches might change the physicochemical properties of the EV membrane. The long-term biocompatibility, stability and safety of modified EVs still need in-depth research.

EVs can also be non-covalently modified based on their natural features. The membrane of EVs mainly consists of amphiphilic substances such as phospholipids, cholesterol and glycolipids, therefore, EVs allow hydrophobic compounds to integrate into their membrane by hydrophobic interaction. For instance, Cheng et al. integrated nuclear localization signal peptides on EVs surface by shaking the peptide and EVs in an ice bath for 4 h. The modified EVs exhibit a great enhanced therapeutic effect on the inhibition of tumor growth (Cheng et al., 2019). The EVs surface is negatively charged, as a result, positively charged components can bind to the surfaces of EVs via electrostatic interaction. Zhan et al. bind cationic endosomolytic peptides L17E to the exosome membrane through electrostatic interaction. The modified exosomes exhibit an enhanced tumor accumulation, thereby efficiently delivering encapsulated cargos to tumor cells (Zhan et al., 2020). Besides, ligand-coupled molecules can specifically bind to receptors expressed on the EV surfaces. For example, transferrin receptors (TfR) are enriched at the membranes of EV derived from reticulocyte. Yang et al. synthesized a pH-responsive superparmagnetic nanoparticles cluster (SMNC), and bind to blood TfR-positive exosomes by precisely labeled with transferrin receptor (Yang L. et al., 2019).

APPLICATION OF MODIFIED EVS IN CANCER THERAPY

Based on these methods of modifying EVs, many researchers have developed new methodologies to modify and design EVs to improve their targeting ability, loading efficiency and therapeutic

TABLE 1 | Cargo loading strategies for EV modification.

Strategies	Methods	Advantages	Disadvantages	Examples	References
Passive loading	Co-incubation with EVs	Straightforward; No damage to the structure of EVs	Poor specificity; Low loading efficiency	Hydrophobic molecules such as Curcumin and Cucurbitacin-I	Sun et al. (2010); Zhuang et al. (2011)
	Co-incubation with donor cells	Straightforward; No damage to the structure of EVs	Poor specificity; Low loading efficiency	Hydrophobic molecules such as DOX and PTX	Guo et al. (2019); Zheng et al. (2020)
Active loading	Electroporation	Simple and quick; High loading efficiency	EV aggregation	Small RNA such as siRNA, shRNA	Zou et al. (2019); Lamichhane et al. (2015)
	Sonication	Relatively high loading efficiency	EV aggregation	Protein such as catalase; Hydrophobic molecules such as DOX	Zhupanyn et al. (2020); Lee et al. (2019)
	Freeze and thaw cycles	Simple and quick	EV aggregation; low loading efficiency	Protein such as catalase	Haney et al. (2015)
	Extrusion	Relatively high loading efficiency	Damage the membrane properties	Protein such as catalase; Hydrophobic molecule such as porphyrins	Bose et al. (2018); Fuhrmann et al. (2015)

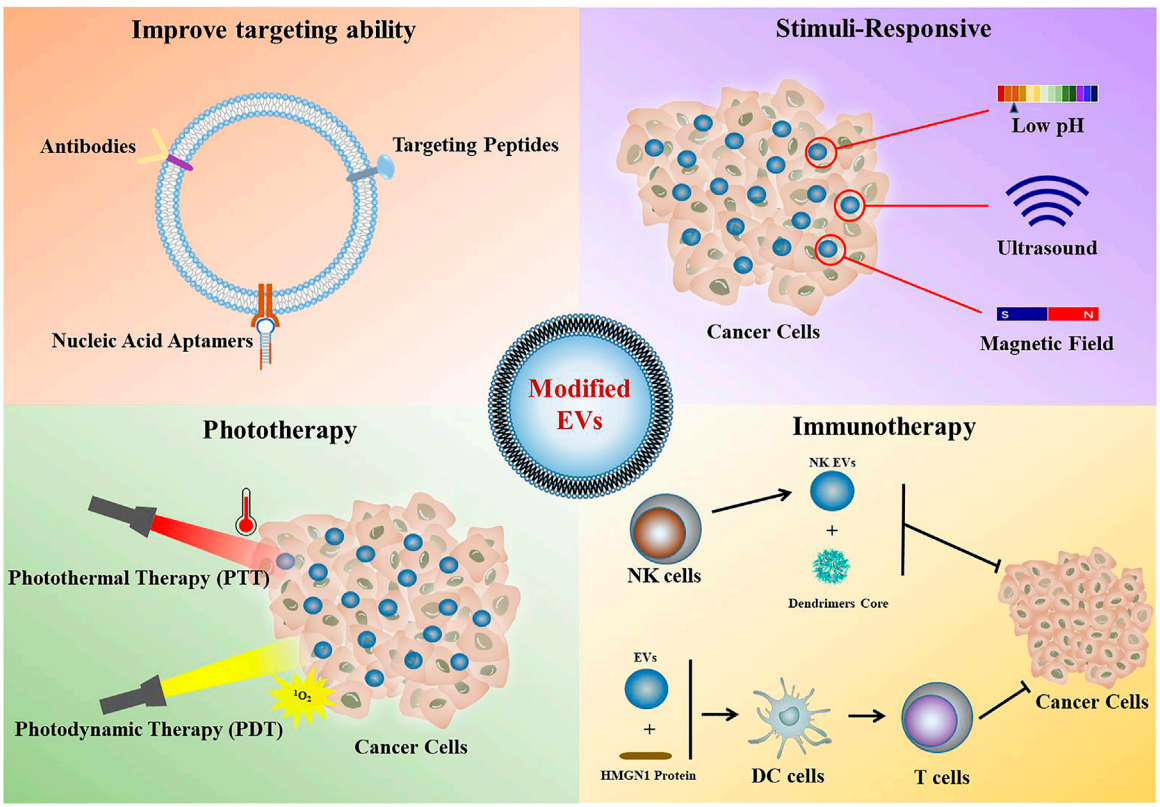

FIGURE 1 | Application of modified EVs in cancer therapy.

efficacy (**Figure 1**). In this subsection, we summarized the latest reported progress in the applications of modified EVs for cancer therapy.

Enhancement of Cell Targeting Specificity of EVs

Generally, EVs have natural targeting properties, this is mainly due to that EVs contain and transfer multiple bioactive molecules from their derived cell lineage. Wang et al. (2021) reported that neutrophil-derived EVs possess appealing blood-brain barrier penetration capability, and can aid delivery of the doxorubicin (DOX) facilely enter into brain and target to glioma via clathrin endocytosis (Wang et al., 2021). However, the intrinsic targeting capacity of most natural EVs is still unsatisfactory. The most direct strategy for improving targeting is to modify the antibodies onto the surface of the EV membrane. Nevertheless, antibodies are rarely used for targeting directly, because of their large size,

TABLE 2 | Strategies for enhancing targeting of EVs.

Strategies	Advantages	Disadvantages	Examples	Cancer Type	References
Antibody	Strong specificity	Large size; complex structure; high risk of generating an immune response	Anti-A33 antibody	Colorectal cancer	Li et al. (2018)
Antibody derivative	Simpler and more compact; Relatively low immunogenicity	The preparation process is complex and costly; There is still a risk of immunogenicity	Anti-EGFR sdAbs; Anti-HER2 scFv	Lung cancer; Breast cancer	Pham et al. (2021); Longatti et al. (2018)
Targeting peptides	Small size; easily synthesized and manipulated	Poor stability; susceptible to degradation or hydrolysis	RGD peptide; cMBP peptide	Glioblastoma; Triple negative breast cancer	Zhu et al. (2019); Li et al. (2020)
Nucleic acid aptamers	Small size; greater stability; lower immunogenicity and toxicity; simple chemical modification	The long-term biocompatibility, stability, and safety remains to be clarified	EGFR RNA aptamer; Sgc8 DNA aptamer	Breast cancer; T-cell leukaemia	Pi et al. (2018); Zou et al. (2019)

complex structure and high risk of generating an immune response (Ahmad et al., 2012). The relevant research has mainly focused on simpler fragments of antibodies such as single domain antibodies (sdAbs) (Pham et al., 2021) or single chain variable fragments (scFvs) (Longatti et al., 2018; Wang et al., 2018). Results showed that these approaches could effectively improve the tumor targeting of EVs. Compared with antibodies, targeting peptides provide several advantages including small size and lower immunogenicity (Hung and Leonard, 2015). Some peptides have been utilized to target tumor associated receptors. For example, RGD peptide can be used for glioblastoma targeted therapy via target integrin receptors (Zhu et al., 2019), and the mesenchymal-epithelial transition factor (c-Met) binding peptides can be used for triple negative breast cancer targeted therapy via target c-Met (Li et al., 2020). Besides, nucleic acid aptamers are small synthetic single stranded DNA or RNA molecules which are also capable of binding selectively to target molecules (Zhang et al., 2021). Similar to targeting peptides, nucleic acid aptamers also possess advantages such as smaller size, lower immunogenicity and simple chemical modification. For example, Zou et al. modified the EVs with diacyllipid-sgc8 aptamer which can specifically bind protein tyrosine kinase 7 (PTK7) through hydrophobic interaction, and the modified EVs can efficiently deliver molecular drugs/fluorophores to target cancer cells (Zou et al., 2019). The strategies for enhancing the targeting of EVs are given in **Table 2**.

Construction of Stimuli-Responsive EVs

Natural EVs cannot respond to exogenous stimulations, which limit their application in drug controlled release. Multiple studies have been carried out to improve the stimuli-responsive ability of EV-based nanoparticles.

Low extracellular pH is considered a key feature of tumor microenvironment (Kato et al., 2013). Modified EVs with pH-responsive materials altered their physicochemical characteristics which makes EVs respond to acidic pH of the tumor microenvironment, and further leads to sustained drug release at the tumor site. The intercalated motif (i-motif) is a pH-responsive DNA strand. Jun et al. constructed a pH-responsive delivery system by chemical modification of exosomes with biotin and ds-i-motif-bio conjugation via streptavidin on the surface of the exosomes. This system efficiently released DOX in an acidic pH responsive manner and had intact bioactivity for anti-proliferation to MCF-7 cells (Jun et al., 2018). Lee et al. designed a functional EV originated from RAW 264.7 cells by attaching a pH-responsive 3-(diethylamino) propylamine (DEAP) via sonication. The DEAP is protonated below pH 7.0, therefore, the functional EV would release drugs when its membrane disruption in response to the acidic pH of the tumor microenvironment (Lee et al., 2018). This work was further extended to target dendritic cells for anticancer vaccination, and the nanoparticle showed pH-dependent physicochemical characteristics which is consistent with the expectations (Lee et al., 2019).

Recently, there are some findings about ultrasound responsive EVs which warrant further attention. Liu et al. designed a functionalized smart nanoparticle in conjunction with an extracorporeal ultrasound device for tumor specific sonodynamic therapy. This nanoparticle was prepared by utilizing exosomes loaded with sinoporphyrin sodium (DVDMS) via a very mild incubation. Results indicated that this ultrasound-responsive natural exosome-based delivery system can non-invasively enhance homogenous tumor targeting and sonodynamic therapy toxicity (Liu et al., 2019). Sun et al. revealed that ultrasound microbubbles together with ultrasound-targeted microbubble destruction (UTMD) significantly increase the infiltration and endocytosis of EVs in these reluctant tissues such as heart and adipose tissue (Sun et al., 2019; Sun et al., 2020). These techniques may have potential applications for anti-cancer EV-based drug delivery.

Magnetic targeting is an important approach of passive targeting for tumor therapy. EVs functionalized by minute magnets could be enriched at the tumor site with the help of external magnetic fields (Xiong et al., 2021). One study from Qi et al. utilized superparamagnetic nanoparticles anchored onto EVs through Tf-Tf receptor interaction, and those modified EVs exhibited superparamagnetic behavior at room temperature. Furthermore, DOX was loaded into EVs, and these EV-based vehicles show excellent tumor targeting ability and cancer inhibition effect (Qi et al., 2016).

Modified EVs for Phototherapy

As non-invasive methods of phototherapy, photothermal therapy (PTT) and photodynamic therapy (PDT) have high clinical value

in the cancer therapy. A number of studies have loaded photothermal materials or photosensitizers into EVs so that these functionalized EVs can be used for PTT or PDT of tumors. For PTT, the photothermal conversion material could convert light energy into cytotoxic heat energy to kill cells (Wu et al., 2015). Cao et al. synthesized small fluorescent quantum dots (QDs) as the photothermal conversion material and modified with cell nucleus-target TAT (transactivator of transcription) peptides, then, packaged into RGD-EVs via electroporation to construct a versatile theranostic platform. This system mediated nucleus temperature increase under NIR-II region laser irradiation, leading to killing the breast cancer cells completely (Cao et al., 2019). Wang et al. have co-embedded Bi_2Se_3 and DOX into tumor cell derived microparticles by electroporation method, and obtained Bi_2Se_3/DOX@MPs via irradiation-induced budding. Bi_2Se_3/DOX@MPs exhibit remarkably dual-modal imaging capacity and synergistic antitumor efficacy by combining PTT with low-dose chemotherapy (Wang et al., 2020).

For PDT, the photosensitizer transfers energy from light to molecular oxygen to generate singlet oxygen, which is toxic to cancer cells (Yang R. et al., 2019). Pan et al. developed a novel nanovehicle by combining urinary EVs and Au-BSA@Ce6 nanocomposites via electroporation (Pan et al., 2020). The structures of nanovehicles collapsed under 633 nm laser irradiation, and a large number of nanoparticles were released to produce singlet oxygen in cancer cells that in turn result in suppression of tumor growth. Zhu et al. use electroporation method to prepare a hybrid nano-vesicle by loaded aggregation-induced emission (AIE) molecular onto tumor-derived EVs, this hybrid nano-vesicles could facilitate efficient tumor penetration and significantly enhance the PDT effect (Zhu et al., 2020). These findings indicate that modified EVs with rational design provide novel approaches to cancer therapy.

Modified EVs for Immunotherapy

Tumor immunotherapy has gained increased attention in recent years. The modifications of EVs derived from immune cells such as natural killer cells (NKs), dendritic cells (DCs) have been used for tumor immunotherapy. Wang et al. report a novel strategy based on NK cell derived exosomes (NKEXOs) for tumor targeted therapy. The biomimetic core-shell nanoparticles (NNs) were self-assembled with a dendrimers core loading therapeutic miRNA and a hydrophilic NKEXOs shell, the resulting NN/NKEXO showed highly efficient targeting and therapeutic miRNA delivery to neuroblastoma cells, leading to inhibit tumor growth (Wang et al., 2019). High mobility group nucleosome binding protein 1 (HMGN1) can enhance the ability of DCs to activate T cells and improve vaccine efficiency (Yang et al., 2012). Zuo et al. modified tumor-derived EVs with the functional domain of HMGN1 via an anchor peptide, and DCs pulsed by these modified EVs show long-term anti-tumor immunity and tumor inhibition effect by enhancing memory T cell response (Zuo et al., 2020). The chimeric antigen receptor T (CAR-T) cell therapy is a new strategy in adoptive antitumor treatment. CAR-T therapy can induce rapid and durable clinical responses but associated with acute toxicities. Fu et al. report that EVs derived from CAR-T cells carry CAR on their surface, and express a high level of cytotoxic molecules to induce tumor cell death. More importantly, CAR EVs do not express Programmed cell Death protein 1 (PD1), and their antitumor effect cannot be weakened by recombinant PD-L1 treatment, and that is why the administration of CAR EVs is relatively safe compared with CAR-T therapy (Fu et al., 2019). In summary, modified EVs have broad application prospects in tumor immunotherapy.

PERSPECTIVES

Despite EVs of natural origin having advantages such as good biocompatibility, low toxic side effects and good blood-brain barrier penetration, many questions remain to be answered, including insufficient loading efficiency and poor targeting. Approaches for direct modification of EVs brought new lights on resolving these problems. In recent years, an increasing number of studies have used new technologies to design and modify EVs to improve their loading efficiency and targeting ability, and these modified EVs indeed have shown exciting and encouraging results in both experimental and preclinical studies as anticancer drugs. However, the findings of some EV clinical trials did not live up to anticipated outcome, which suggest that most applications are still at an experimental stage. Because of the heterogeneity of the encapsulated and surface molecules, the use of different isolation, purification and characterization methodologies frequently results in confusion with regard to characteristics of EVs. In addition, in initial lack of standardized protocols for EV modification, resulting in contrasting results between different studies. Therefore, the standard for isolation, purification, characterization and modification of EVs need to be established. On the other hand, the functional molecules carried by EVs may bring potential biosafety problems, so that these EVs based therapeutic strategies require further preclinical research before successful clinical application. Overall, EVs based therapeutic strategies for cancer are still in their infancy, with the deepening of basic research on EVs and the development of biotechnology, the applications of modified EVs for cancer therapy are potentially broad.

AUTHOR CONTRIBUTIONS

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MINI REVIEW
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Advanced Polymeric Nanoagents for Oral Cancer Theranostics: A Mini Review

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Oral cancer is one of the most common tumours in the world threatening human life and health. The 5-years survival rate of patients with oral cancer has not been improved significantly for many years. The existing clinical diagnostic methods rarely achieve early diagnosis due to deficiencies such as lack of sensitivity. Most of the patients have progressed to the advanced stages when oral cancer is detected. Unfortunately, the traditional treatment methods are usually ineffective at this stage. Therefore, there is an urgent need for more effective and precise techniques for early diagnosis and effective treatment of oral cancer. In recent decades, nanomedicine has been a novel diagnostic and therapeutic platform for various diseases, especially cancer. The synthesis and application of various nanoagents have emerged at the right moment. Among them, polymer nanoagents have unique advantages, such as good stability, high biosafety and high drug loading, showing great potential in the early accurate diagnosis and treatment of tumours. In this review, we focus on the application of advanced polymeric nanoagents in both the diagnosis and treatment of oral cancer. Then, the future therapy strategies and trends for polymeric nanoagents applied to oral cancer are discussed, with the hope that more advanced nanomedical technology will be applied to oral cancer research and promote the development of stomatology.

Keywords: oral cancer, nanomedicine, polymer nanoagents, cancer theranostics, targeted delivery

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INTRODUCTION

Cancer is the number one killer that threatens human life and health. Head and neck tumours are the sixth most prevalent cancer type in the world, among which oral cancer is the most common, accounting for 40% (Sung et al., 2021). Despite the advances in oral cancer research over the past few decades, its 5-years survival rate has not significantly improved and still hovers around 50% (Chang et al., 2013). The main reason for the poor prognosis of oral cancer patients is delayed treatment. Only one-third of oral cancer patients are diagnosed at an early stage, with the majority being diagnosed at advanced stages because of the lack of obvious symptoms earlier (Nonaka and Wong, 2018).

The clinical diagnostic methods of oral cancer mainly include biopsy, magnetic resonance imaging (MRI), computed tomography (CT) and positron emission tomography (PET), while biopsy is still the definitive diagnostic method (Keshavarzi et al., 2017; Abati et al., 2020). The

treatment of oral cancer depends on its stage. The conventional treatment for early-stage oral cancer is surgical resection, while advanced stage treatment requires a combination of surgery, chemotherapy and/or radiotherapy (Fang et al., 2007; Naruse et al., 2016). However, many traditional chemotherapeutic agents has been limited because of their low bioavailability, inability to specifically identify tumour cells and easy clearance in the blood. Other therapies, such as immunotherapy, gene therapy, photothermal therapy (PTT), photodynamic therapy (PDT), etc., are still under ongoing research.

Nanomedicine was first proposed by scientists in 2000 (Wagner et al., 2006). The advent of nanomedicine technology has greatly changed the diagnosis and treatment of cancer. Nanomaterials are particles at the nanometre scale that have great potential in the field of medicine due to their special material properties (Lucky et al., 2016; Yu et al., 2020). In the last decade, dozens of drug products containing nanomaterials have been approved by the Food and Drug Administration (FDA) for clinical use (Bobo et al., 2016). Generally speaking, particles between 1 and 100 nm in size in any dimension are called nanoparticles (NPs). NPs usually include liposomes, dendrimers, gold NPs, magnetic NPs, quantum dots, polymeric NPs, etc., Nanomaterials serve as carriers for drug delivery. The unique structure of NPs can be used to deliver fluorescent dyes, chemotherapy drugs, photosensitisers or other biological materials, overcoming the limitations traditional diagnostic and therapeutic processes (Zheng et al., 2021). Due to the leaky vasculatures surrounding fast-growing cancer tissues, NPs carrying anticancer agents can be absorbed by tumour cells through the enhanced permeability and retention (EPR) effects, resulting in local accumulation and cytotoxicity of the tumour cells (Nakamura et al., 2016; Greish, 2010). It can also be conjugated with the corresponding antibodies, peptides, aptamers and small molecules to enhance targeting efficiency and reduce systemic toxicity (Haider et al., 2020). These are the main forms of passive and active targeting of nanoagents. To date, a variety of inorganic and organic/polymer nano-materials for oral cancer research have been reported, including NPs based on metallic and metal oxide materials, quantum dots, solid lipid NPs and polymer-based NPs (Mishra et al., 2018; Su et al., 2019; Soleymani et al., 2020). While each of these well-studied nanoagents has merits, they also have demerits. For instance, the long-term health risks of metal and metal oxide NPs in clinical application remain unknown, and solid lipid NPs are restricted by their poor drug loading capacity (Ruiz-Pulido et al., 2021). Among various kinds of nanoparticles, polymeric nanoparticles have received a lot of attention in tumor research due to their better biosafety and specific drug accumulation effect (Green et al., 2008; Sionkowska, 2011). In the past, non-biodegradable polymers (e.g., polymethyl methacrylate, polyacrylamide and polystyrene) were commonly used to fabricate nanomaterials, but such polymers are difficult to degrade and can lead to chronic inflammation. Nowadays, degradable materials are used as a good alternative to this challenge (Vijayan et al., 2013). Polymeric NPs are prepared from natural polymers (e.g., chitosan and hyaluronic acid) and synthetic polymers [e.g., poly (propylene-co-glycolide) and polyethylene glycol] in a core-shell structure, with hydrophilic blocks forming the shell and hydrophobic blocks forming the core of the nanoparticles (Elsabahy and Wooley, 2013). The size and surface characteristics of nanoparticles are turned by their preparation methods. At present, several preparation methods have been developed and can be divided into two groups, i.e., methods based on the polymerization of monomers and methods using preformed polymers. It is crucial to choose the most suitable preparation method for polymer NPs depending on the specific properties required. After the effective nanoparticles have been synthesised, they are purified by filtration, centrifugation and dialysis techniques (Crucho and Barros, 2017). According to their morphology, polymeric NPs are classified as nanocapsules and nanospheres (Guterres et al., 2007). Unlike other nanocarriers, polymeric nanoparticles can encapsulate the drug within a polymeric oily core (nanocapsules) or disperse the drug in a polymeric matrix (nanospheres) (Crucho and Barros, 2017). Their advantage is that the special core-shell structure allows specific delivery of drugs or fluorescent molecules to the focal area, **Figures 1A,B** (Zielińska et al., 2020). After the drug released, the polymer matrix is usually degraded to water and non-hazardous molecules containing hydrogen and nitrogen, and is excreted from the body (Parveen and Sahoo, 2008). Their unique properties, such as non-toxicity, water solubility and easy modification, make them promising nanomedicine candidates for a wide range of applications in oncology research, **Figure 1C** (Lim et al., 2015; Banik et al., 2016; Choudhury et al., 2019).

Although there have been many review articles summarising the development of oncology research, rather limited ones focus on the molecular/NP design and recently developed new mechanisms of the organic/polymer photothermal nanoagents. In this review, we will focus on the research progress based on polymeric nanoagents in the integration of oral cancer diagnosis, treatment and theranostic. We analyse and forecast the current trends and the future treatment strategies, in the hope that more nanotechnology will be applied to oral cancer research and will promote the development of stomatology.

POLYMERIC NANOPARTICLES FOR ORAL CANCER DIAGNOSIS

There is strong evidence that early diagnosis and treatment can lead to a reduced mortality rate in oral cancer (Petersen, 2009). The advantages of polymer fluorescent nanoprobes such as high sensitivity, non-invasiveness and good biocompatibility, make them ideal for imaging (Chan and Wu, 2015). Polymeric nanoagents function as nanocontrast agents or fluorescent probes in the early diagnosis and imaging of oral cancer. For example, Shanavas et al. produced hybrid NPs with a magnetic poly (lactide-co-glycolide) (PLGA) nanoparticle core, where the surface was modified with a folate-chitosan (fol-cht) conjugate shell, and used them as an MRI contrast agent. The hydroxyl (-OH) and amine ($-NH_2$) functional groups on the surface of chitosan are extremely reactive, allowing for facile surface modification through complex chemical processes. They

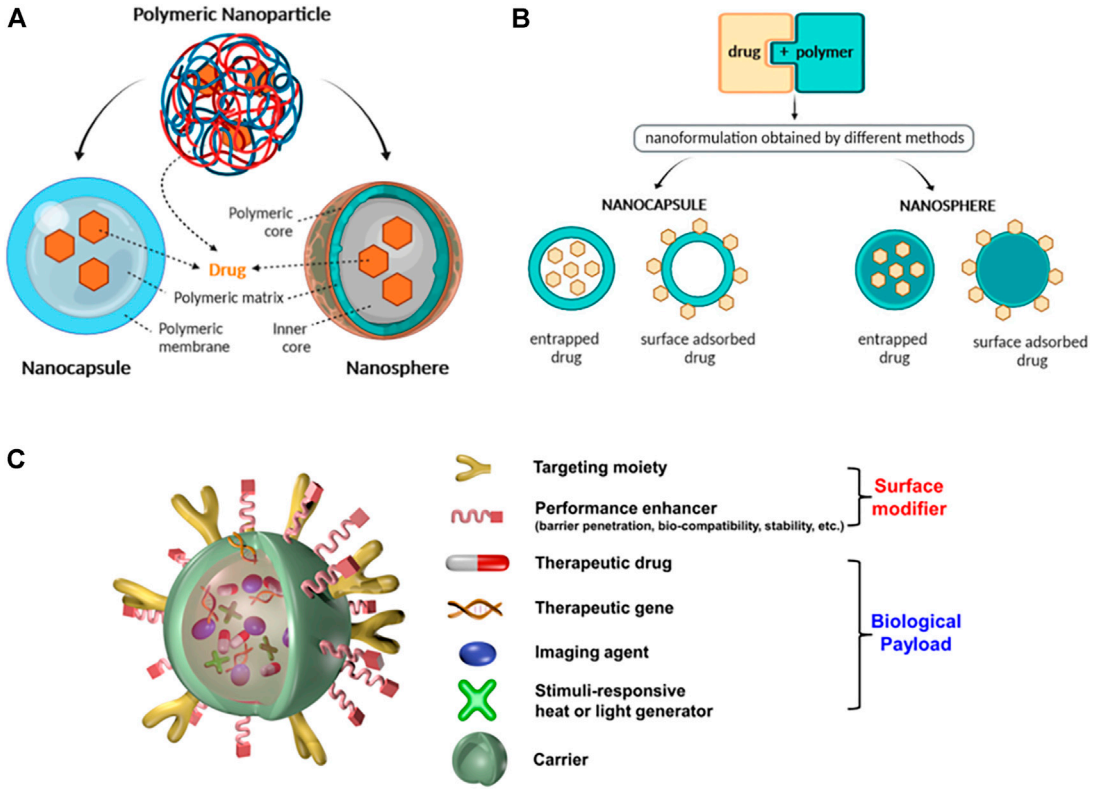

FIGURE 1 | (A) Schematic illustration of the structure of nanocapsules and nanospheres. **(B)** Different modes of association of drugs with nanospheres and nanocapsules. [**(A,B)** Reproduced from (Zielińska et al., 2020) with permission from the Multidisciplinary Digital Publishing Institute]. **(C)** Schematic illustration of a multifunctional nanocomposite. Reproduced from (Lim et al., 2015) with permission from American Chemical Society.

showed that the imaging contrast of the targeted group (folic acid receptor) was significantly better than that of the non-targeted group, facilitating the detection of early oral cancer (Shanavas et al., 2017). Inspired by polymeric drug delivery carrier systems, excellent brightness, biodegradability and low toxicity dyes such as Indocyanine green (ICG), Methylene Blue have been conjugated with polymeric nanoagents with special properties that overcome the limitations of poor stability, rapid *in vivo* clearance and low cellular uptake, achieving the early diagnosis through bioimaging of tumours. (Hill et al., 2015). ICG encapsulated in polymer nanoparticles shows significant improvement in both stability and PDT/PTT effect. Poly (styrene-co-maleic anhydride) (PSMA) is an amphiphilic polymer that can be used to encapsulate organic dyes to improve their chemical stability and biocompatibility. Chen et al. designed a kind of nanoparticles in which they encapsulated ICG with PSMA to form ICG@PSMA by self-assembly method. *In vitro* and *in vivo* studies have shown that ICG@PSMA NPs have strong NIR fluorescence, good biocompatibility, low toxicity and excellent photothermal properties. And they found that ICG@PSMA NPs have great potential in different types of cancer (Chen et al., 2021). However, achieving high brightness with dye-loaded polymer NPs requires loading large quantities of fluorescent dyes, which can cause the occurrence of aggregation-caused quenching (ACQ) and limit the brightness of dye-loaded polymer NPs. With the development of nanomaterials, the aggregation-induced emission (AIE) effect was discovered (Zhang et al., 2021). Zhang et al. synthesised an AIE material named phenylene and tetraenzenewere-dicyanomethylene-benzopyran (DPA-TPE-DCM) and applied it to the optical diagnosis of early oral cancer. The probe showed good biocompatibility and shows a high signal-to-noise ratio when applied *in vivo*. Under the guidance of fluorescence, the orthotopic tongue carcinoma in mice was successfully detected, as well as the mapping of sentinel lymph nodes smaller than 2 mm, **Figures 2A–C** (Zhang et al., 2022). Bioimaging reveals the biological processes involved in early carcinogenesis, helps detect small tumours at an early stage and aids in the assessment of resection margins during surgery. Nanotechnology provides the means for more accurate imaging of lesions and has greatly advanced the field of oncology.

POLYMERIC NANOPARTICLES FOR ORAL CANCER PREVENTION AND THERAPY

The occurrence of oral cancer is a relatively complex process, involving multiple genetic and cellular alterations (Chi et al., 2015; Li et al., 2020). In oral cancer, chemical prophylaxis is considered

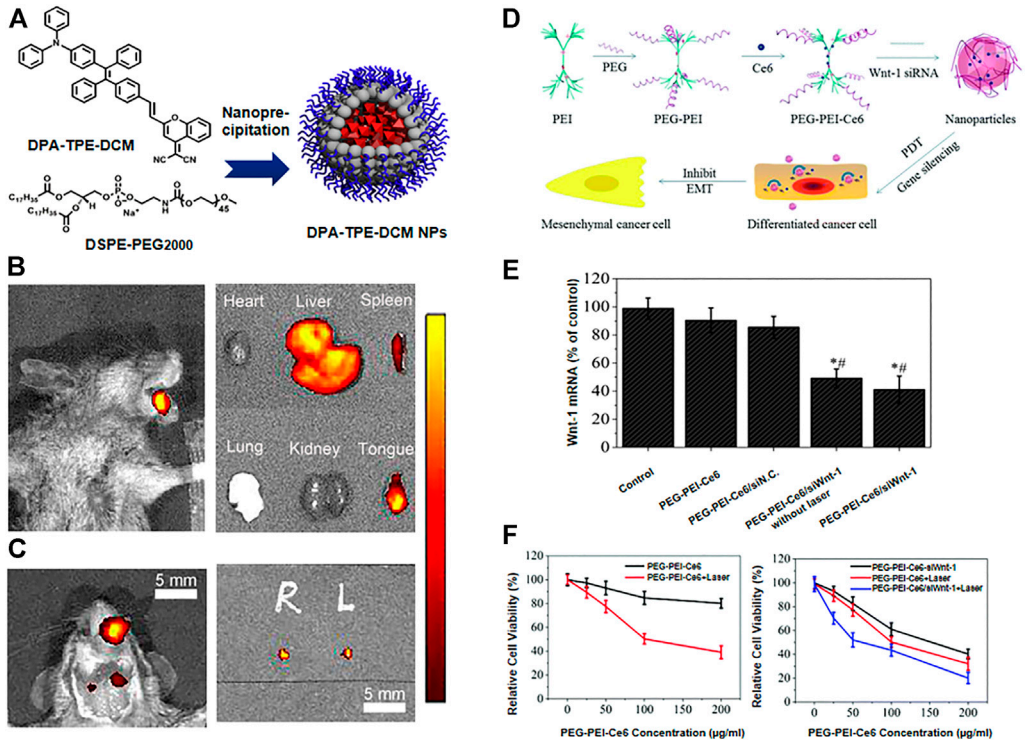

FIGURE 2 | (A) Schematic illustration of the preparation of DPA-TPE-DCM NPs. **(B)** Fluorescence imaging of tongue squamous cell carcinoma-bearing mice and *ex vivo* fluorescence images of major organs and tongue after intravenous administration of DPA-TPE-DCM NPs. **(C)** Fluorescence imaging of the mice neck and sentinel lymph nodes helped extract from the mice under the guidance of fluorescence. [**(A–C)** Reproduced from (Zhang et al., 2022) with permission from the Royal Society of Chemistry]. **(D)** Schematic illustration of PEG-PEI-Ce6/siRNA nanoparticles and the mechanism of the effects they exert. **(E)** The expression levels of Wnt-1 mRNA determined by RT qPCR. **(F)** *In vitro* cellular phototoxicity of PEG-PEI-Ce6/siWnt-1 against oral cancer cells. [**(D–F)** Reproduced from (Ma et al., 2017) with permission from the Royal Society of Chemistry].

effective in reversing, preventing or inhibiting the malignant transformation of precancerous cells. Natural plant compounds, such as flavonoids and astragalus, are of great interest due to their rich biological activity and medicinal value, making them promising chemopreventive agents (Iriti and Varoni, 2013; Glenny et al., 2010). However, the low bioavailability and poor solubility of such plant compounds lead to limited clinical application (Singh et al., 2014). To overcome this problem, the use of polymeric nanoparticle drug delivery systems for packaging biologically active plant compounds for oral cancer prevention has been clinically explored (Gohulkumar et al., 2014). Licorice is a perennial plant with well-known pharmacological properties and is considered as an adjunct to the drug treatment of many local and systemic diseases. Glycyrrhetinic acid (GA) is a kind of active compounds extracted from licorice and has significant anti-cancer properties (Roohbakhsh et al., 2016). Cacciotti et al. examined the cytotoxicity of GA on human oral squamous cancer cells (OSCC) and normal human gingival fibroblasts (HGFs) with two delivery systems: chitosan (CS) and poly (lactic-co-glycolic) acid (PLGA) based nanoparticles and polylactic acid fibers (FBs). They used a one-step osmosis based methodology to prepare CS-PLGA-based NPs (GA-NPs) loaded with GA, which were non-toxic to HGFs and had a low median toxic concentration of 200 μmol/L against human OSCCs, both of which were superior to GA-FBs (Cacciotti et al., 2018). When oral cancer progresses to an advanced stage, clinical treatments like radiotherapy and chemotherapy are mostly applied. Since most chemotherapy drugs are easily cleared by the reticuloendothelial system (RES) in blood circulation and are lowly water-soluble, poorly biocompatible, and lowly targeted, they often fail to cure tumours and instead cause serious side effects, such as vomiting, fever, allergies, and hair loss. Polymer nanoagents have been used as drug transport carriers to improve the stability of drugs, control targeted drug delivery, make the concentration of drugs in the lesion site constant and uniform. El-Hamid et al. showed that pegylated liposomal doxorubicin (Doxil) had a higher apoptotic effect on CAL-27 cells than free doxorubicin with fewer side effects (El-Hamid et al., 2019). In another study, Gupta et al. synthesised PLGA NPs encapsulating the model radiosensitising drug docetaxel, presenting higher toxicity to human oral cancer cells than free docetaxel (Gupta et al., 2018). Polymer nanoagents are inherently biocompatible and biodegradable, and have an extended residence time at the local site. Encapsulating the active drug into polymer NPs can overcome the problems of poor drug solubility and low bioavailability, enhance drug stability, thereby increasing efficacy and reducing side effects.

Optical therapy, such as PDT and PTT, is an emerging method of tumour treatment (Ou et al., 2019). PDT has been officially approved by the FDA for the treatment of localised

oesophageal cancer (Lee and Baron, 2011). The photosensitiser (PS) is activated by light in the presence of oxygen, leading to the generation of reactive oxygen species (ROS). Some researchers have used PS coupled with polymeric NPs for photodynamic therapy of oral cancer. Wang et al. designed an effective ROS-sensitive delivery carrier for chemical photodynamic therapy, named polyethylene glycol-polycarbonate-thioketal doxorubicin (PEG-PBC-TKDOX). Doxorubicin (dox) was covalently modified to self-destructive polymeric micelles for drug release *via* light-triggered ROS. The polymer system improved the efficiency of chemical photodynamic therapy and reduced off-target toxicity (Wang M. et al., 2019). In addition, nanoparticles combined with laser can be effectively used in photothermal therapy, attacking tumour cells without significant damage to other cells. In a study by Ren et al., synthesized organic compound (C3) was equipped with indocyanine green (ICG) into polyethylene glycol-polycaprolactone (PEG-PCL) to form hybrid nanoparticles (PEG-PCL-C3-ICG NPs) for combined PTT and PDT treatment of tumours under 808 nm laser irradiation. *In vitro* and *in vivo* experiments showed that PEG-PCL-C3-ICG NPs were significantly more effective than PTT or PDT separately, and had better performance and lower cytotoxicity compared to conventional PTT agents such as Au nanorods (Ren et al., 2017). Based on the special properties of nanoplatforms, some researchers have combined chemotherapy and optical therapy as a multimodal treatment approach, achieving good results in the treatment of oral cancer. Wang et al. developed a novel drug delivery system, called human serum albumin indocyanine green-cisplatin nanoparticles (HSA-ICG-DDP NPs), to ensure site-specific drug delivery/release and reduce the systemic toxicity of chemotherapy, demonstrating the synergistic effects of PDT, PTT, and chemotherapy with *in vitro* and *in vivo* experiments. As for the *in vivo* treatment, HSA-ICG-DDP NPs accumulated in tumour tissues and exhibited stronger antitumour effects compared to ICG, HSA-ICG and DDP treatments, significantly improving the therapeutic efficacy (Wang et al., 2019a). Combination therapy is a current trend in oncology treatment that improves the results and reduce side effects. Multifunctional polymeric nanocarriers are ideal materials for combination therapy.

Gene therapy has been studied in oncology treatment for several years, but the results achieved have not been significant. The advent of nanomedicine has greatly facilitated the development of gene therapy. In photodynamic therapy, activation of epithelial-to-mesenchymal transition (EMT) can lead to tumour recurrence and progression. Some investigators have enhanced the effect of PDT by inhibiting the Wnt/β-catenin signaling pathway involved in EMT progression using nanocarriers carrying corresponding small interfering RNA (siRNA). Ma et al. efficiently delivered Wnt-1 siRNA into the cytoplasm of PDT-treated oral cancer cells using polyethylene glycol-polyethyleneimine-chlorin e6 (PEG-PEI-Ce6) NPs. PEG-PEI-Ce6 nanoparticle-mediated PDT in combination with Wnt-1 siRNA significantly inhibited cell growth and enhanced the killing effect on cancer cells, **Figures 2D–F** (Ma et al., 2017). The application of nanomaterials has overcome the limitations of the traditional treatments and has provided new options for the treatment of oral cancer patients.

POLYMERIC NANOPARTICLES FOR ORAL CANCER THERANOSTICS

In traditional clinical practice, the time-phased medical model of diagnosis followed by treatment is cumbersome. Nanopolymer drug delivery platforms have been used to integrate the process of diagnosis and treatment as a new direction of tumour theranostics (Lim et al., 2015). In some investigations, highly sensitive fluorescence for diagnosis and multimodal therapy have been integrated into a single system through nanodrug delivery platforms to achieve diagnosis and treatment of oral cancer. Wang et al. designed and synthesised a multimodal near infrared (NIR)-II nanoprobe, [4,4'-((6,7-bis(4-(hexyloxy) phenyl)-[1,2,5]thiadiazolo [3,4-g]quinoxaline-4,9-diyl)bis (thiophene-5,2-diyl))bis (N,N-diphenylaniline)] TQTPA loading cis-dichlorodiammine platinum (CDDP) (HT@CDDP) by hyaluronic acid. They proved to have good stability and water solubility and exhibited biocompatibility and low systemic toxicity. *In vitro* and *in vivo* experiments demonstrated that the NPs have good imaging capabilities and are capable of drawing the outlines of orthotopic tongue tumors and metastatic lymph nodes as small as 1 mm in nude mice by IR-808 under NIR exposure. Also, the NPs can be used as a multimodal therapeutic agent combining photothermal therapy with chemotherapy to achieve combined chemotherapy-photothermal treatment (Wang et al., 2019b). The strategy of treatment with simultaneous visual diagnosis simultaneous facilitates real-time monitoring of the treatment's effects and the formulation of individualised treatment plans. Thus, it is considered a promising strategy for early clinical cancer diagnosis and treatment that deserves further investigation.

CONCLUSION

Herein, we reviewed the progress of research on polymeric NPs in oral cancer prevention, diagnosis and treatment. Polymeric NPs provide new platforms and ideas for the diagnosis and treatment of oral cancer that are worth exploring in greater depth. During the last decade, research in nanotechnology in the field of medical oncology has been in full swing. However, the polymeric NPs also have drawbacks: research indicates that some polymeric NPs are prone to hazardous degradation and toxic monomer aggregation, necessitating further research into their synthesis and chemical characteristics. Importantly, there are still some pressing issues in the study of polymeric nanoagents for oral cancer applications. Changes in the tumour microenvironment (e.g., temperature and pH) often affect the effectiveness of nanoplatform-based drug delivery systems. In this context, NPs regarding the tumor microenvironmental response are being studied extensively, still not in oral cancer. The large discrepancies between the results of *in vivo* and *in vitro* experiments have raised major

doubts concerning the effectiveness of nanosystems in humans. In addition, the targeting efficiency of polymer NPs *in vivo* has not achieved the desired effects. Due to the lack of specific markers in oral cancer, since some proteins that are overexpressed on the surface of tumour cells also exist in normal cells, the manner to further improve the efficiency of passive and active targeting remains to be elucidated.

There is no denying that nanotechnology, especially polymeric nanocarrier platforms, has the potential to be the most effective and beneficial form of treatment and diagnosis of cancer in the future. Further research is needed to translate nanotechnology concepts into practical applications. In the coming years, it will play a key role in early tumour detection, diagnosis and treatment procedures. However, polymer NPs-based diagnosis and treatment of oral cancer still has a long future.

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All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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REVIEW
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Advanced Peptide Nanomedicines for Bladder Cancer Theranostics

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Cancer is still a global public health problem. Although remarkable success has been achieved in cancer diagnosis and treatment, the high recurrence and mortality rates remain severely threatening to human lives and health. In recent years, peptide nanomedicines with precise selectivity and high biocompatibility have attracted intense attention in biomedical applications. In particular, there has been a significant increase in the exploration of peptides and their derivatives for malignant tumor therapy and diagnosis. Herein, we review the applications of peptides and their derivatives in the diagnosis and treatment of bladder cancer, providing new insights for the design and development of novel peptide nanomedicines for the treatment of bladder cancer in the future.

Keywords: peptide, bladder cancer, diagnosis, treatment, nanomedicines

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INTRODUCTION

One recent report from the World Health Organization's International Agency for Research on Cancer (IARC) released the latest global cancer burden data, showing that 4.57 million cancer cases and 3 million resultant deaths increased in 2020 in China. Among them, bladder cancer is one of the common urinary malignancies and ranks among the top ten cancers in terms of morbidity and mortality. Bladder cancer is one of the most expensive cancers to cure because of its high recurrence rate (Barani et al., 2021). Although new techniques involving radiotherapy, immunotherapy, chemotherapy, etc., are blossoming in the treatment of bladder cancer (Booth et al., 2018; Tree et al., 2018; Wołącewicz et al., 2020), their toxic side effects and high costs limit their broad applications in clinical applications. Early diagnoses, including the examination of circulating tumor cells, CT scan, magnetic resonance imaging, positron emission tomography, bone scan, chest X-ray, etc., are crucial for the diagnosis and treatment of bladder cancer (Todenhöfer et al., 2018; van der Pol et al., 2018; Wu et al., 2018), but their disadvantages, such as nonspecificity, heterogeneity, and excessive detection, still limit their potential clinical applications (Faba et al., 2019). Cystoscopic biopsy can improve the diagnostic accuracy, but it is difficult to identify superficial mucosal lesions such as carcinoma *in situ*, and it is invasive. Abscission cytology is a standard non-invasive test for the diagnosis and monitoring of bladder cancer. It has the disadvantage of being insensitive to low-grade tumors and depends on accurate diagnosis by the pathologist (DeGeorge et al., 2017).

The primary purpose of drug delivery is to send enough drug payloads to the lesion sites while minimizing their exposure to healthy tissues. To improve the specificity and pharmacokinetics of anticancer drugs and avoid the side effects of traditional therapies, two main strategies involving drug carriers and covalent modifications are widely used. Drug carriers such as nanoparticles and

hydrogels can protect drugs from the external environment before on-demand release when they reach lesion sites. Meanwhile, the physical and chemical properties of the drug carriers significantly determine their biological distributions (Fan et al., 2021; Huang et al., 2021). Covalent modifications enable temporarily masking or limiting the bioactivity of the drugs and confer them with the desired pharmacokinetics (Lin et al., 2019; Cooper et al., 2021; Yang et al., 2021). It is noteworthy that both the abovementioned strategies can alleviate the burden of drug metabolism and improve the therapeutic effects of the original drugs. Among numerous drug molecules, peptides are highly competitive candidates for the treatment of bladder cancer because of their small sizes, high specificity, low systemic toxicity, etc. In addition, the diagnosis of bladder cancer mainly depends on pathology and imaging examinations, while the detection accuracy is still low. Using specific biomarkers on bladder tumor cells, peptide nanotechnology can significantly improve the sensitivity and specificity for the diagnosis of bladder cancer. (Pan et al., 2014; Tummers et al., 2017).

PEPTIDE-INSTRUCTED TUMOR DIAGNOSIS

Magnetic resonance imaging (MRI) is a noninvasive technique for tumor diagnosis in current clinical medicine. Although it has been shown that MRI has the ability to display three-dimensional anatomical details without injury and provide high spatial resolution without invasiveness, MRI is still less sensitive than fluorescence imaging for monitoring tiny tissue damage, cellular activity, molecular activity, etc (Chandra et al., 2010; Schroeder, 2008). Therefore, the development of new contrast agents is expected to enable the improvement of the detection accuracy of MRI. Paramagnetic Gd^{3+} complexes and superparamagnetic iron oxide (SPIO) nanoparticles are two widely used contrast agents in MRI detection. Compared with the paramagnetic Gd^{3+} complex, SPIO is a better alternative to MRI contrast agents, of which the signal contrast is several orders of magnitude higher than that of the traditional Glacki contrast agent (Jun et al., 2008). In a study of human bladder tumors, the researchers reported that 1.5T magnetic resonance imaging using SPIO as the contrast agent realized *in situ* detection of malignant tumors with a small size to ~4 mm, while it was unable to effectively distinguish the depth of tumor invasion into the bladder walls (Beyersdorff et al., 2000). The main reason is that the cellular internalization levels of SPIO are limited, and less than 1% of SPIO is internalized by nonspecific endocytosis pathways (Moore et al., 2001). Due to the great promise of SPIO in MRI applications, researchers have endeavored to develop a variety of SPIO conjugates to enhance its cellular uptake ratio, in which cell-penetrating peptides (CPPs), such as R11, are considered to be one of the best transporters to improve the active internalization of nanoparticles into target cells (Hsieh et al., 2011; Ding et al., 2017). Ding et al. recently also developed an SPIO nanoparticle whose surfaces are unctionalized with bladder cancer-specific fluorescein isothiocyanate (FITC) labeled cell-penetrating peptide (CPP) -polyarginine peptide (R11) for active targeting and imaging for bladder cancer, respectively. Their study showed that SPIO-R11 nanoparticles can be internalized by T24 cells in a dose-dependent manner, and that SPIO-R11 internalized dose is higher than that of SPIO itself, since R11 is a cell-permeable peptide that enables efficient drug delivery. Transmission electron microscopy (TEM) results indicated that SPIO-R11 is mainly located in cellular vesicles and lysosomes, but no signals in the nucleus were found. Due to the cellular specificity of SPIO-R11, the uptake of nanoparticles into bladder cancer cells was significantly higher than that of immortalized bladder epithelial cells. In addition, SPIO-R11 had a lower T2 relaxation time in MRI than SPIO. These results suggested that SPIO-R11 has great potential as a targeted contrast agent for the diagnosis and treatment of bladder cancer (Ding et al., 2017).

Tumor cells are mutated from normal cells, of which the signal transduction pathways are significantly different from those of normal cells. Therefore, many signaling regulators or regulatory proteins are overexpressed in tumor cells and can be used as specific targets for tumor diagnosis (Oh and Bang, 2020; Wilson et al., 2021). Recently, targeted peptides have attracted intense attention because they can specifically bind with receptors on tumor cells. By conjugating with radioactive or fluorescent probes, scientists have prepared a variety of peptide probes to specifically orient and image tumors (Ciobanasu, 2021; Kwak et al., 2021; Lu et al., 2021; Sonju et al., 2021; Wang et al., 2021). For instance, Wei et al. recently constructed a loaded nanoscale oxygen generator (PLZ4@SED) by conjugating superparamagnetic iron oxide nanoparticles (SPIOns) with peptide motifs specific to bladder cancer cells. PLZ4@SED showed good tumor targeting and permeability to patient-derived bladder cancer cells. Meanwhile, they illustrated that the presence of PLZ4@SED can improve the contrast of MRI and promote chemotherapeutic efficacy by producing oxygen through the Fenton reaction to relieve hypoxia. It was also reported that PLZ4@SED presented great potential in the diagnosis and treatment of bladder cancer (Lin et al., 2021). Sweeney et al. (2017) demonstrated one successful application of mesoporous silica nanoparticles (MSN), which are functionalized using a bladder cancer-specific peptide CyC6, as the magnetic resonance contrast agent. Due to the effective binding of the modified MSN to tumor cells, tumor boundaries were much clearer in the T1-and T2-weighted MRI and fluorescence cystoscopic inspections compared to the traditional technique.

Cystoscopy is one gold standard for the diagnosis of bladder cancer. However, cystoscopy is an invasive and costly technique, and it is difficult to detect flat malignancies using this technique. Meanwhile, urine cytology is low-sensitivity for detecting low-grade lesions, of which the detection accuracy is highly dependent on the experience of the cytopathologists (Grossman et al., 2006; Alfred Witjes et al., 2017; Babjuk et al., 2017). Recently, several potential biomarkers have been identified that could potentially provide noninvasive and objective approaches for the detection of bladder cancers (Kluth et al., 2015). Lee et al. presented one peptide conjugate consisting of fluorescein and the peptide sequence of CSNRDARRC. They reported that the peptide conjugate can specifically bind to bladder cancer tissue using

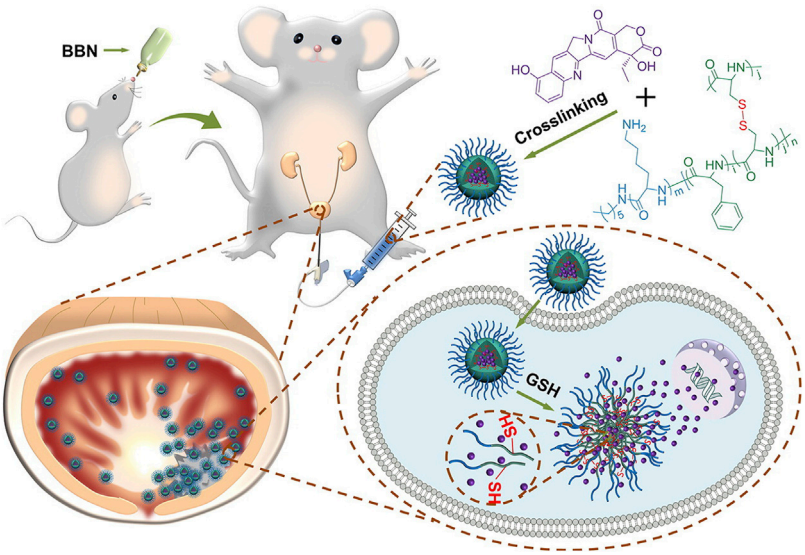

FIGURE 1 | After intravesical instillation of NG/HCPT, the selective accumulation of nanodrugs in tumor tissue finally releases HCPT triggered by GSH(Guo et al., 2017).

frozen sections. Meanwhile, the peptide conjugate could selectively bind to bladder tumor epithelial cells when it was injected into the bladder cavity using a tumor-bearing rat model. Furthermore, Lee et al. found that the peptide conjugate had the ability to indicate bladder tumor cells in urine, presenting great potential to be exploited as a real-time diagnostic probe to detect bladder cancer (Lee et al., 2007). Prothrombin activators (TSPs) can prevent angiogenesis in a variety of pathological conditions. Some structural domains and peptide derivatives of TSP-2 enable the promotion of angiogenesis in BC tissues. 4N1K (KRFYVVMWKK), derived from the C-terminal cell-binding domain of TSP-2, plays an important role in the pathology and prognosis of bladder cancer. Using the hematoxylin-eosin (H&E) staining technique to examine tumor tissues from bladder cancer patients, Nakamura et al. verified that 4N1K was significantly correlated with the tumor apoptosis index and microvascular density but negatively related to T stage, metastasis and tumor grade; promising 4N1K may be a useful biomarker and a new therapeutic target for UC-UUT patients (Nakamura et al., 2019).

PEPTIDE-INSTRUCTED LOCAL CHEMOTHERAPY

Transurethral resection combined with chemotherapeutic infusion is the standard treatment protocol for nonmuscular invasive bladder cancer. However, the low bioavailability (GuhaSarkar and Banerjee, 2010) and short retention period of the current chemotherapeutic drugs (Tyagi et al., 2006; Wirth et al., 2009) restricted their exposure time at the lesion sites. Along with the advancement of nanotechnology, nanocarrier drug delivery systems show advantages in solving these problems. Guo et al. (2017) designed and synthesized a kind of positively charged intelligent peptide nanocarrier cross-linked with disulfide bonds [i.e., PLL-P (LP-*co*-LC). They prepared one nanogel system (NG/HCPT) using this nanocarrier by artificially loading 10-hydroxycamptothecin (10-HCPT). Compared with free 10-HCPT, NG/HCPT not only has a higher drug loading rate, longer retention time, and stronger tissue penetration ability but can also accurately and rapidly release 10-HCPT into bladder cancer cells, significantly enhancing the corresponding antitumor effects and reducing the side effects (**Figure 1**). In 2020, Guo et al. further synthesized a new R_9-polyethylene glycol poly (L-phenylalanine-L-cysteine) nanogel (R_9-PEG-P (LP-*co*-LC)] based on NG/HCP, which can improve the adhesion and permeability of chemotherapeutic drugs. They prepared the R_9NG/HCPT nanogel using 10-HCPT as a model drug. The morphology of R_9NG/HCPT is similar to that of an octopus with a spear. Highly positively charged R_9 with strong membrane penetrability can help R_9NG/HCPT pass across the bladder walls and enhance its cellular adhesion interactions through nonspecific and electrostatic interactions, thus enabling prolonged exposure to chemotherapeutic drugs at the lesion sites. This system significantly improved the tumor suppression efficiencies of 10-HCPT in both *in situ* mice and rat tumor models, suggesting great potential in the local chemotherapy of bladder cancer (Guo et al., 2020).

Polymeric micelles constructed using amphiphilic block copolymers have been widely explored in recent decades due to their high drug loading efficiency, long cycle time, well-controlled release ability, and good targeting properties (Xiao et al., 2012). Recently, Zhou et al. developed an amphiphilic diblock copolymer poly (ε-caprolactone)-b-polyoxyethylene (PCL-b-PEO) containing integrin targeting motif c (RGDfK) and imaging dye FITC. The copolymer assembled into micelles and strongly interacted with bladder cancer T24 cells. After encapsulation with doxorubicin (DOX), the micelle could

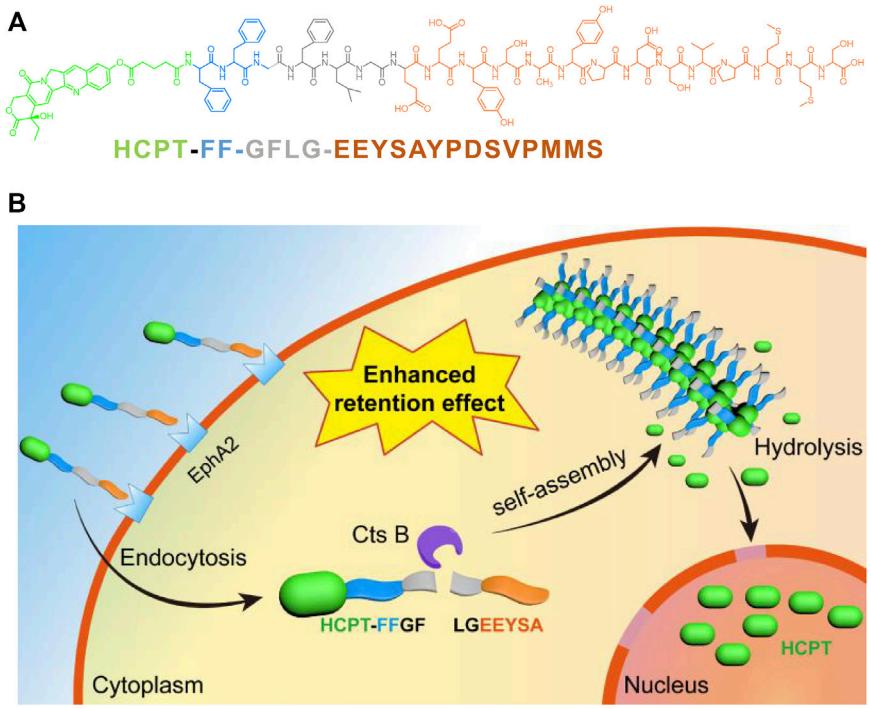

FIGURE 2 | (A) The molecular structure of HCPT-FF-GFLG-EEYSAYPDSVPMMS; **(B)** The illustration of the bladder tumor cell targeting, intracellular fibrillation and drug release of HCPT-FF-GFLG-EEYSAYPDSVPMMS(Zeng et al., 2021a).

efficiently prevent the proliferation of T24 cells and was expected to be used as a nanoscale drug delivery system for bladder perfusion chemotherapy (Zhou et al., 2013).

The combination usages of two or more drugs showed great advantages in cancer treatments involving improving therapeutic efficacy, lowering side effects, and preventing drug resistance, which are promising strategies for the treatment of refractory cancers. The positively charged adhesive chitosan-polymethacrylic acid (CM) nanocapsules loaded with DOX and cisplatin modified with peptide (Pt-Aly) presented high drug loading efficiency and sustained drug release properties. Meanwhile, CM nanocapsules can be firmly attached to the surface of the bladder cavity, prolong the retention time of the payload in the bladder, and have the effect of synergistically killing UMUC3 bladder cancer cells. In addition, CM nanocapsules have no obvious damage to the urothelium, which is expected to cooperate with intravesical chemotherapy in the treatment of non-muscle invasive bladder cancer (Lu et al., 2016). Overall, the intelligent peptide nanogel systems have much more powerful retention efficiency and permeability, providing a promising drug delivery platform for local chemotherapy of bladder cancer.

PEPTIDE-ASSISTED SYSTEMIC CHEMOTHERAPY

Systemic chemotherapy is one of the dominant techniques used to treat musculoskeletal invasive bladder cancer (Calabrò and Sternberg, 2009; Yin et al., 2016). However, nonspecific distributions of traditional chemotherapeutic drugs in human bodies have caused severe toxicity to normal tissues, including liver and kidney organs, bone marrow, gastrointestinal tract tissues, etc., and significantly limited their clinical applications. Therefore, researchers are endeavoring to develop new drug delivery systems that can transport the chemical drugs into the desired sites to improve their therapeutic effects (Cheng et al., 2019; Sonju et al., 2021). Peptide-drug conjugates are promising prodrugs for the treatment of cancer that combine one or more traditional chemical drugs with short peptides through biodegradable linkers. This prodrug strategy can uniquely and specifically employ the bioactivity and self-assembling properties of short peptides to enhance the therapeutic efficacy of traditional drugs (Cooper et al., 2021). Zeng et al. (2021b) recently developed one short-peptide prodrug, HCPT-FF-GFLG-EEYASYPDSVPMMS, consisting of 1) a self-assembling motif (i.e., -FF-); 2) an EphA2 targeting sequence on T24 cancer cells (i.e., YSAYPDSVPMMS); and 3) one short peptide linker responsive to the CtsB enzyme (i.e., GFLG). They found that this prodrug could be efficiently encapsulated by T24 cells and cleaved intracellularly by CtsB, resulting in nanofibrils in T24 cells (**Figure 2**). The formation of nanofibrils loaded with HCPT prolonged its circulation period *in vivo*. Moreover, this prodrug system could precisely deliver HCPT into T24 cancer cells, reduce its accumulation in normal tissue and lower the side effects. Pan et al. prepared one kind of nanomedicine, DC-PNM-PTX, in which one bladder targeting peptide sequence, PLZ4, one polymeric micelle, and the chemical drug paclitaxel (PTX) were involved. They reported that DC-

PNM-PTX could specifically target bladder cancer cells, prevent bladder tumor growth in a xenograft tumor model, and efficiently prolong mouse survival compared to unmodified PTX. Nanomaterials modified with multiple ligands targeting cell membrane receptors play positive roles in tumor therapy, which is beneficial for reducing the toxicity and side effects of traditional chemotherapy and improving antitumor outcomes (Pan et al., 2016).

PEPTIDE-INSTRUCTED GENE THERAPY

Gene therapy is a revolutionary technique that directly uses therapeutic genes to treat various diseases. As one alternative to traditional treatments (Dunbar et al., 2018; High and Roncarolo, 2019), the first clinical trial of gene therapy was approved in 1989, and nearly 2,600 trials have been completed or are under their ways worldwide until now (Ginn et al., 2018). However, it is still challenging to direct the genes into targeted cells without damaging other cells. It has been shown that virus-like particles (VLPs) from human JC polyomavirus (JCPyV) can package and deliver exogenous DNA into sensitive cells for gene expression (Chang et al., 1997). To improve the specificity of gene therapy, Lai et al. (2021) conjugated SPB peptides targeting bladder cancer cells onto JC polyomavirus (JCPyV) virus-like particles (VLPs) and succeeded in the delivery of the suicide gene thymidine kinase. Both *in vitro* and *in vivo* experiments illustrated that the suicide gene was only expressed in human bladder cancer cells but not in lung cancer and neuroblastoma cells that were sensitive to JCPyV VLP infection, implying the great specificity of VLP-SPBs. Meanwhile, the gene transduction efficiency of VLP-SPBs is approximately 100-fold that of the VLP itself. The binding of JCPyV VLPs with specific peptides can improve their original affinities and change the expression directions of the packed genes. Moreover, VLP-SPBs presented the ability to selectively prevent the growth of bladder tumors but had no significant inhibitory effects against lateral lung tumors. In general, gene therapy is one flourishing technique to treat various diseases, and malignancies are their main enemy. The applications of the targeted peptide delivery systems enable artificial control and regulation of gene expression at the cellular level, thus succeeding in disease treatments but not affecting normal tissues and organs.

Epidemiological data have shown that more than 50% of human malignancies, including bladder cancer, are related to mutations in the p53 gene (Hainaut et al., 1997). Mutant p53 protein enables the acceleration of tumor formation and metastasis and is associated with resistance to radiotherapy and chemotherapy, as well as poor prognosis (Al-Sukhun and Hussain, 2003). The functional restoration of p53 protein can promote the expression of downstream genes to block cell cycles or induce cell apoptosis, resulting in the suppression of tumor progression. It has been shown that one C-terminal peptide sequence (p53c) can restore the binding ability to specific DNA sequences and the transactivation function of the mutant p53 gene, leading to p53-dependent apoptosis of tumor cells (Selivanova et al., 1997). However, due to the lipophilicity of biological membranes and their roles as biological barriers to defeat exterior enemies, many synthetic compounds cannot cross cell membranes. R11 can be specifically captured by bladder and prostate tissues and is promising for use as a drug or probe carrier for the treatment and detection of upper urinary tract tumors (Hsieh et al., 2011; Ding et al., 2017). Zhang et al. showed that the synthetic peptide R11-p53c can be effectively and preferentially delivered into bladder cancer cells, resulting in the reactivation of the p53 gene and inhibition of tumor growth. More interestingly, R11-p53c also presented excellent antitumor effects in primary and metastatic tumor models, which could prolong the survival period while having no significant systemic toxicities. In addition, their study also illustrated that R11-p53c could prevent the growth of both mutant and recombinant p53c tumor cells but had no significant inhibitory effects on normal cells. It was also noted that transcriptional levels of several p53 target genes were upregulated after treatment with R11-p53c. Overall, R11-p53c has the potential to treat both primary and metastatic bladder cancer and should be a promising therapeutic agent for the treatment of upper urinary tract tumors. (Hsieh et al., 2011).

PEPTIDE-MEDIATED PHOTOTHERMAL THERAPY

Photothermal therapy (PTT) is a highly promising strategy to defeat malignancies that mainly utilizes photothermal materials to convert light energy into heat *in situ*, finally raising the local temperature to result in cell apoptosis and tumor killing (Gao et al., 2019; Chen et al., 2020; Jiang et al., 2020). By taking advantage of photothermal conversion, PTT has been widely used in a variety of tumor treatments, and some of them are under clinical trials (Timko et al., 2010; Chen et al., 2014). One crucial issue for PTT applications is to develop carrier materials with good selectivity to tumor cells. Tao et al. (Tao et al., 2019) loaded folate-modified vincristine into polydopamine-coated Fe_3O_4 (Fe_3O_4@PDA-VCR-FA SPs) and applied them for the treatment of bladder cancer. PDA shells can not only improve colloid stability and biocompatibility but also enhance photothermal effects and prolong the blood circulation period. The half-life period in blood and the tumor retention rate of Fe_3O_4@PDA-VCR-FA SPs are 2.83 h and 5.96% ID g^{-1}, respectively, which are significantly improved compared with those before folic acid modification. The superparamagnetism of Fe_3O_4 and the loading of vincristine enable arming Fe_3O_4@PDA-VCR-FA SPs with nuclear magnetic resonance imaging (NMRI) and chemotherapy abilities. With the further help of near-infrared laser-triggered photothermal therapy, Fe_3O_4@PDA-VCR-FA SPs can completely remove bladder cancer and prevent its recurrence. Moreover, no obvious toxicity to the liver, kidney or other organs was detected through biochemical and pathological tests, suggesting the good biocompatibility of Fe_3O_4@PDA-VCR-FA SPs. Zeng et al. (2021b) recently reported a novel RGD-mediated photosensitive drug-peptide conjugate (BBTD + GA/PEG-RGD) for the treatment of musculoskeletal invasive bladder

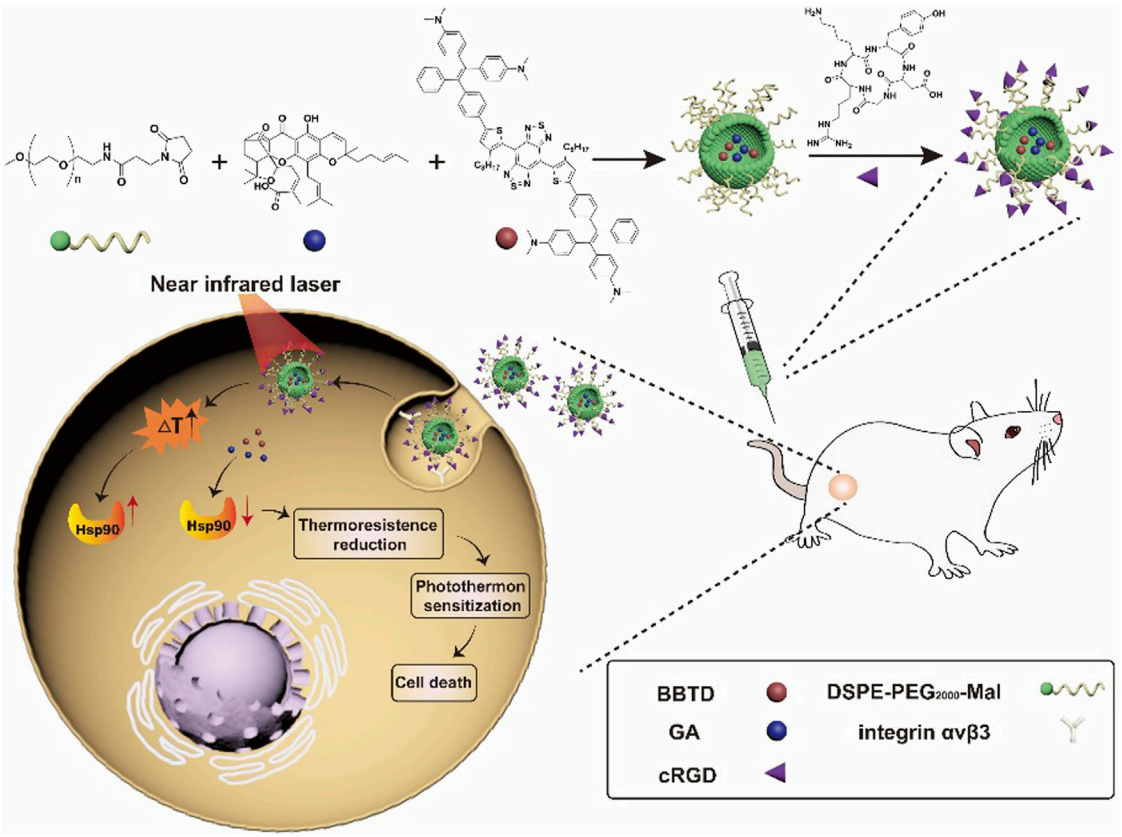

FIGURE 3 | Schematic illustration of synthesizing BBTD + GA/PEG-cRGD nanoparticles for photohyperthermia therapy of MIBC(Zeng et al., 2021a).

cancer. This system can specifically target integrin $\alpha_v\beta_3$ outside the membrane of bladder cancer. Meanwhile, the system can prevent the overexpression of heat shock protein 90 and reduce the resistance of cancer cells to heat stress, finally succeeding in low-temperature PTT with great antitumor properties (**Figure 3**). Furthermore, the results of animal experiments showed that this system had advantages involving 1) good tumor targeting ability and stability; 2) less thermal damage to normal tissue; 3) great therapeutic effects against musculoskeletal invasive bladder cancer; and 4) a longer survival period compared to the control groups. Low-temperature PTT is highly effective in preventing tumor growth without damaging normal tissues, promising great clinical applications for optical tumor therapy in the future.

THERAPEUTIC PEPTIDES

Mitochondria play an important role in apoptotic death (Bock and Tait, 2020), and some anticancer agents can destroy mitochondrial functions and induce tumor cell apoptosis (Vasan et al., 2020). One typical example is the cationic amphiphilic peptide KLAKLAKKLAKLAK (i.e., KLA). KLA is a natural antibacterial peptide that can bind and damage negatively charged bacterial membranes. Normally, KLA does not damage eukaryotic membranes and has no toxicity to eukaryotic cells. However, internalized KLA can rupture the mitochondrial membrane, resulting in cytochrome C release and cell apoptosis (Huang et al., 2017). KLA is always conjugated with transmembrane peptides (CPPs) to promote its internalization efficiency by tumor cells; however, the conjugated KLA-CPPs also have high cytotoxicity to normal cells because of their nonspecific interactions (Wang et al., 2016). To overcome the potential nonspecific interactions, Jung et al. designed and synthesized a mixed peptide (Bld-1-KLA) consisting of 1) a targeting peptide to bladder cancer cells CSNRDARRC (Bld-1) and 2) an effector peptide D-KLAKLAKKLAKLAK (KLA) that can destroy the mitochondrial membrane and induce apoptosis. Bld-1-KLA can selectively bind and internalize into bladder cancer cells to induce cell apoptosis without significant toxicity to other tumor cells and normal cells. After intravenous administration of Bld-1-KLA in the HT1376 tumor-bearing mouse model, it was shown that Bld-1-KLA had a higher tumor homing and inhibition ability than the control groups (**Figure 4**). Together, these results suggest that Bld-1-KLA is a promising targeted therapeutic against bladder cancer (Jung et al., 2016).

Fibroblast cytokine 9 (FGF9) is overexpressed in many cancer cells (Ren et al., 2016; Mizukami et al., 2017), and its targeted receptor FGFR3c is an important driver of bladder cancer

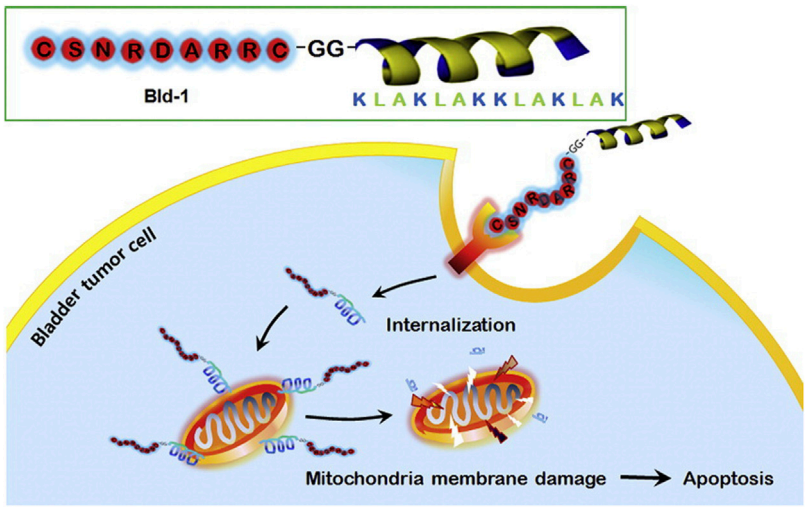

FIGURE 4 | Bld-1-KLA destroys the mitochondrial membrane and induces apoptosis.

progression (Iyer and Milowsky, 2013; Wang et al., 2020). The important role of FGFR3c makes it an important therapeutic target for the treatment of bladder cancer. Wang et al. (2020) reported one FGF9 binding peptide, P4, using the phage display technique. Meanwhile, they found that P4 is highly homologous to the immunoglobulin-like domain II-III (D2-D3) of FGFR3c using sequence comparison. Functional analysis showed that P4 had the ability to prevent the FGF9-induced aggressive phenotypes, including cell proliferation, migration, and invasion, and inhibit tumor progression by downregulating the MAPK and Akt cascade pathways. More importantly, FGF9 was found to be a potential driver of drug resistance in gastric and bladder cancer cells, in which the presence of P4 can increase the sensitivity of chemical drugs. In conclusion, Wang's study identified a novel FGF9-binding peptide that may serve as a potential agent to treat malignancies with abnormally upregulated FGF9.

CONCLUSION

In recent decades, significant success has been achieved in the diagnosis, treatment, and prevention of bladder cancer. However, bladder cancer is characterized by polycentricity, multiple occurrences, and recurrence, suggesting great challenges for its clinical treatment. With the development of modern biosynthesis technology, peptide nano drugs have become one of the hot spots in drug research. Compared with monoclonal antibody drugs, recombinant protein drugs and small molecule drugs, peptide nano drugs have the characteristics of simple spatial structure, significant curative effect and high safety, and have been widely used in the diagnosis and treatment of tumors. With the continuous progress of related technologies, the clinical application of peptide nano drugs is more and more in-depth, and the development space is broad. Peptides are promising for intracellular delivery of chemical drugs, DNA, siRNA, fluorescent molecules and nanoparticles. Compared with other chemical entities, peptides have the advantages of low molecular weight, low cost and good stability. At the same time, polypeptides can be easily modified to attach and enter tumor cells, and finally transport the goods to the desired and desired places. In general, peptide nanodrugs can improve tumor targeting and permeability, reduce systemic toxicity, reduce and prevent recurrence, shorten treatment time and reduce treatment cost. They are of great value for the clinical application of bladder cancer. Peptide drugs have outstanding advantages. With the continuous progress of biotechnology and peptide synthesis technology, peptide drugs have broad market development space and are expected to become one of the main drugs for cancer diagnosis and treatment (Lin et al., 2021).

AUTHOR CONTRIBUTIONS

SZ and XF wrote the manuscript. SX, ZX, and ZM collected references. QL supervised the whole work. All the authors approved this manuscript.

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Poly lactic-co-glycolic acid-based nanoparticles as delivery systems for enhanced cancer immunotherapy

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Cancer has emerged as one of the most severe diseases in modern times, various therapies have advanced remarkably in recent decades. Unlike the direct therapeutic targeting tumor cells, immunotherapy is a promising strategy that stimulate the immune system. In cancer immunotherapy, polymeric-based nanoparticles can serve as deliver systems for antigens and immunostimulatory molecules, and they have attracted increasing attention and revolutionized cancer therapy. Poly (lactic-co-glycolic acid) (PLGA) is the most frequently used clinically approved biodegradable polymer and has a broad scope of modification of its inherent properties. Recent advances in PLGA based drug delivery systems in cancer immunotherapy have been described in this mini review, with special emphasis on cancer vaccines and tumor microenvironment modulation.

KEYWORDS

PLGA, immunotherapy, adjuvants, antigens, cancer, nanoparticles, drug delivery

Introduction

Cancer immunotherapy has received extensive attentions in the past decades, and it has been the fourth most important cancer therapy, after surgery, radiation therapy, and chemotherapy. The rational combination of cancer immunotherapy with other therapeutic modalities has gradually become an emerging therapeutic strategy (Chen et al., 2021). However, mainly due to the lack of effective vectors, many pre-clinical trials have failed to progress to the clinical stage. Nanotechnology offers an opportunity to overcome these limitations. Compared to its bulk structures, nanoparticles have remarkable properties such as smaller size (1–100 nm in diameter), greater surface area to volume ratio, higher cell penetration ability, and enhanced physicochemical properties (Javad et al., 2015). Due to these unique properties, nanoparticles hold great interests in various biomedical applications, and they have also been extensively used as carriers in cancer immunotherapy. As the most widely used immunostimulatory nanoparticles, polymeric nanoparticles are highly appreciated for their preeminent biocompatibility, aqueous solubility, high payload capacity, backbone stability and

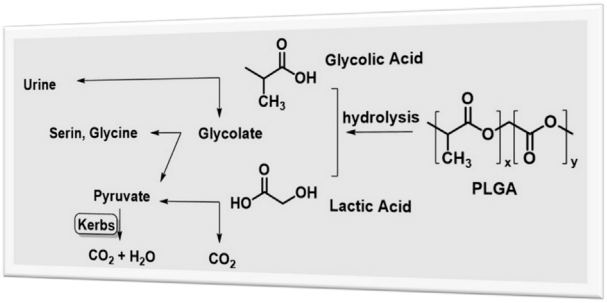

FIGURE 1
Hydrolysis of PLGA and metabolization of the two monomers.

feasibility of modification to increase targeting ability or responsiveness (Thakur et al., 2020).

The widely developed polymeric nanoparticle-based delivery systems for cancer immunotherapy usually have a core–shell (also known as membrane-core) structure wherein the hydrophobic or charged polymers form the inner core, while the shell-forming polymers have neutral, hydrophilic and flexible properties for stealth nanoparticles. Based on the amphiphilic property, a variety of formulation strategies such as the oil-in-water (O/W) single emulsion process (for hydrophobic cargos) and the water-in-oil-in-water (W/O/W) double emulsion method (for hydrophilic cargos), have been used. Stimuli-responsiveness (temperature, pH, enzymatic, reductive or oxidative, etc.) can be imparted into the core, shell and/or the linkages.

PLGA (poly-D,L-lactide-co-glycolide) is one of the most successfully used biodegradable polymers for the development of nanomedicines (Acharya and Sahoo, 2011; Rezvantalab et al., 2018; Roointan et al., 2018). Since the body effectively deals with the two biodegradable monomers of PLGA (lactic acid and glycolic acid, which can then be metabolized *via* the Krebs cycle yielding nontoxic byproducts H_2O and CO_2, see Figure 1), it shows very minimal systemic toxicity for drug delivery. A major advantage of PLGA over other polymers is that PLGA has been approved by the U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMA) for pharmaceutical applications *via* parenteral and mucosal routes, leading PLGA-based nanoparticles in a good position for clinical trials (Vasir and Labhasetwar, 2007; Rocha et al., 2022).

Because vaccines can be easy to deploy and have historically represented an approach that has brought enormous medical benefit, therapeutic vaccines against cancer have been explored since the early discovery of tumor-specific antigens (Zhang et al., 2018). In the cancer-immunity cycle, cancer vaccines can primarily promote the cancer antigen presentation step (Chen and Mellman, 2013) (Figure 2) to accelerating and expanding the production of T cell immunity. Although vaccine strategies for the generation of tumor-specific immunological responses continue to have great promise, they were limited on two aspects. First, a general lack of understanding of how to identify proper cancer vaccine delivery system to achieve potent cytotoxic T cell responses. Second, immunosuppressive environment within the tumor resulted in poor therapeutic outcome in clinic. Thus, in

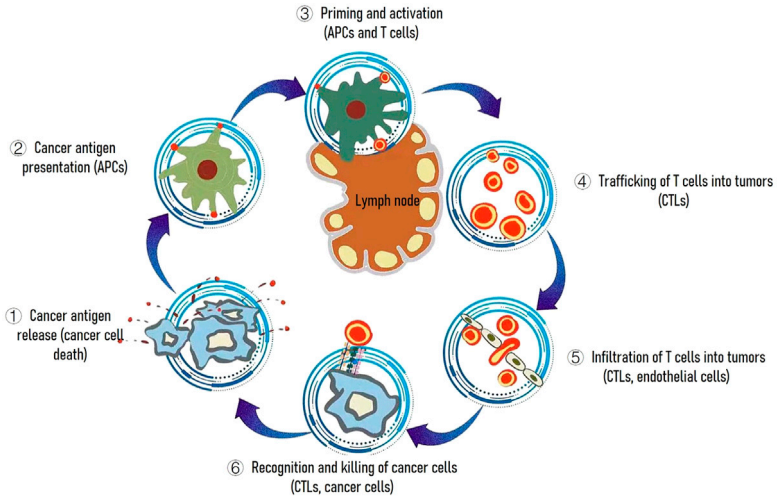

FIGURE 2
The cancer-immunity cycle. The goal of cancer immunotherapy is to initiate or reinitiate a self-sustaining cycle of cancer immunity. Abbreviations are as follows: APCs, antigen presenting cells; CTLs, cytotoxic T lymphocytes.

this review, we have focused on both cancer vaccines and tumor microenvironment modulation aimed at resulting in an effective systemic antitumor immunity.

Cancer vaccines

Tumor-associated antigens (TAAs), the adjuvants, and the delivery system are three essential components of therapeutic vaccine (Li et al., 2018). In cancer immunotherapy, PLGA nanoparticles have been tested as delivery systems to ameliorate the efficiencies of therapeutic vaccines. Tumor-associated antigens (TAAs), adjuvants as toll-like-receptor (TLR) agonists, and also tumor lysates have been encapsulated in PLGA nanoparticles (Kohnepoushi et al., 2019). In a study reported by Chen et al. (2016), photothermal agent Indocyanine green (ICG), and Toll-like-receptor-7 agonist imiquimod (R837), were co-encapsulated into PLGA nanoparticles by oil-in-water (o/w) emulsion method. When the multifunctional nanoparticles were used for photothermal ablation of primary tumors, they can generate TAAs, which induce vaccine-like immune responses with R837 as the adjuvant. In combination with the checkpoint-blockade therapy using anti-cytotoxic T-lymphocyte antigen-4 (CTLA4), the generated immunological responses are able to attack the remaining tumor cells in mice, provide inhibition in metastasis and offer a strong immunological memory effect. This strategy showed that combining tumor-specific vaccines that stimulate cytotoxic T lymphocytes (CTL) responses with immune checkpoint blockade therapy is an attractive tool. Recently, Koerner et al. (2021) developed a vaccine based on PLGA particles possess a relatively broad size distribution ranging from nanosized up to 1.5 μm sized particles. Ovalbumin (OVA) and double-stranded (ds) RNA adjuvant Riboxxim were co-encapsulated into the PLGA particles. PLGA particles induced the supreme adjuvant effect of Riboxxim, potently activated murine and human dendritic cells, and elevated tumor-specific $CD8^+$ T cell responses. This PLGA particle vaccine delays tumor progression, suppresses tumor metastasis, and provides prolonged survival of immunized mice, and its advantageous therapeutic potency was further enhanced by immune checkpoint blockade that resulted in reinvigoration of cytotoxic T lymphocyte responses and tumor ablation.

Fast recognition and non-specific clearance of nanoparticles by innate immune system, clinical applications of nanoparticles are usually hampered. To further improve the potency, deliver more antigens to the desired site, some modifications of PLGA nanoparticle-based delivery systems were studied. To enhance antigen processing and presentation, the endosomal membrane disrupting agent hydroxychloroquine (HCQ) was encapsulated into PLGA nanoparticles with ovalbumin (OVA) (Liu et al., 2018). *In vitro* experiments showed the nanoparticles enhanced OVA escape from the lysosome into the cytoplasm and also improved cross-presentation of antigen. *In vivo* studies concluded that this co-delivery nanovaccine can provide strong $CD8^+$ T cell immune responses that induced tumor cell apoptosis and long-lasting antigen-specific memory immune responses in vaccinated mice. Zhang et al. (2020) have developed a PLGA nanoparticle vaccine in which PLGA nanoparticle as delivery system encapsulated the antigenic peptide HPV16 $E7_{44-62}$ and adenosine triphosphate (ATP) as adjuvant component. Peptides were encapsulated into PLGA nanoparticles using a two-stage emulsification method, ATP was introduced by simple mixing 10 μl ATP with 90 μl of $E7_{44-62}$-loaded nanoparticles. Employing PLGA nanoparticles increased lymph node accumulation, and dendritic cell (DC) uptake of the E7 peptide. ATP adjuvant further increased the migration, nanoparticle uptake, and maturation of DCs. ATP-adjuvanted nanoparticles stimulated cell-mediated immune responses, completely abolished the growth of TC-1 tumors, produced long-lasting immunity and significantly delayed tumor progression in vaccinated mice.

Because of the "proton sponge" effect-induced antigen escape, cationic polymers can facilitate antigen adsorption and uptake by APCs such as DCs (Chen et al., 2014). To endow the PLGA nanoparticles with positive surface charge, a relatively safe cationic surfactant dimethyl-dioctadecyl-ammonium bromide (DDAB) is chosen, and the DDAB/PLGA nanoparticles were prepared using nanoprecipitation (Han et al., 2021). The positively charged surface of the DDAB/PLGA nanoparticles enabled the negatively charged antigen of the model antigen ovalbumin ($OVA_{257-264}$) to be easily absorbed to the surface *via* electrostatic interaction to obtain an OVA@DDAB/PLGA nanovaccine. Experiments performed *in vitro* revealed that the nanovaccine induced antigen escape from lysosome into cytoplasm with 10 times increased cross-presentation activity than naked OVA. The nanovaccine showed excellent draining lymph nodes (LNs) transportation ability by passive lymphatic drainage and active DC transport. After immunization, the OVA@DDAB/PLGA nanovaccine can stimulate both humoral and cell-mediated immune responses and offer a strong immunological memory effect.

Another strategy that exhibits great potential in the disease diagnosis and therapeutics is membrane coating nanotechnology (Liu et al., 2022). It has revolutionized the design of cancer vaccine by endowing targeting, antigen presentation and immune stimulation. Diverse membrane coating platforms have been developed for cancer vaccine design. In a study by Yang et al. (2018), TLR7 agonist imiquimod (R837) loaded PLGA nanoparticles were coated with B16-OVA cancer cell membranes, whose surface proteins could act as tumor specific antigens. The obtained nanoparticles were further modified with mannose by a lipid-anchoring method. This PLGA complex was efficiently targeted and internalized by

antigen presenting cells (APCs) such as DCs, which triggered potent antitumor immunotherapeutic efficacy. Yang et al. (2019) designed a lipid-coated PLGA hybrid particles for the co-delivery of mRNA and TLR7 adjuvant. In this carrier system, the PLGA core enabled the efficient loading of the hydrophobic gardiquimod, and the lipid shell loaded the mRNA *via* electrostatic interaction. The hybrid nanovaccine led to the effective antigen expression and DC maturation *in vitro*, also a stronger antigen-specific immune response was obtained. The spatial/temporal overlap of the antigen and adjuvant *via* the core-shell nanoparticles are found to be beneficial for tumor growth inhibition. Zhou et al. (2020) designed a nanovaccine composing of a PLGA core to encapsulate TLR7 agonist imiquimod (R837), a phospholipid membrane to load antigen peptide (αOVA) and apolipoprotein E3 (ApoE3). Incorporation of ApoE3 facilitate nanovaccines uptake through the micropinocytosis pathway, and significantly promoted DCs maturation and antigen presentation. More *in vivo* studies showed that these nanoparticles migrated to the lymph nodes, leading to strong T cell immune responses. The nanovaccine also provided inhibition in metastasis in lung, and exerted superior therapeutic efficiency on B16-OVA tumor-bearing mice when in combination with αPD-1 therapy. Nowadays the membrane-coated technology with membranes from different types of cells offers promising opportunities for cancer immunotherapy, cell membranes employed have gradually shifted from natural to engineered (Chen et al., 2020; Liu et al., 2020). In the nanovaccine developed by Gou et al. (2021), peptide CBP-12 expressed biomimetic cancer cell membrane coating strategy was adopted to specifically target Clec9a$^+$ DCs. The membrane coated PLGA drug-delivery system efficiently delivers tumor antigen and STING agonist (cGAMP) to Clec9a$^+$ DCs, significantly enhanced IFN-stimulated expression of genes and antigen cross-presentation of Clec9a$^+$ DCs, eliciting strong antitumor effects in anti-PD-1-resistant tumor models without obvious cytotoxicity. Moreover, combination of the nanovaccine with radiotherapy remarkably enhances the cancer immunotherapy effects.

Tumor microenvironment modulation

The immunosuppressive tumor microenvironment consists of cells, soluble factors, signaling molecules, extracellular matrix, and mechanical cues (Swartz et al., 2012), it is created by the tumor and dominated by tumor-induced interactions (Whiteside, 2008). Modulating the tumor microenvironment with efficient modulator will significantly promote the immune responses inside a tumor. Using nanoparticles for remodeling the tumor microenvironment is a promising immunotherapeutic strategy to overcome "immune escape" and resulting in an effective systemic antitumor immunity.

Efficient capture and presentation of tumor antigens by APCs, especially dendritic cells (DCs), are crucial for anti-tumor immunity. However, APCs are immunosuppressed in the tumor microenvironment. Kim et al. (Kim et al., 2018a) showed that incorporating TLR 7/8 agonists into PLGA nanoparticles could significantly increase co-stimulatory molecule expression and antigen presentation in DCs compared to free agonists. *In vivo* studies showed that these nanoparticles migrated to the lymph nodes, triggering DC activation and expansion, leading to enhanced cytotoxic T lymphocytes (CTL) responses, and in turn, improving prophylactic and therapeutic efficacy in melanoma, bladder, and renal cell carcinoma tumor models. Later, to overcome fast clearance from the injection site, pH responsiveness is incorporated into the TLR7/8 agonist delivery platform (Kim et al., 2018b). Bicarbonate salt was adopted, the salt generates carbon dioxide at acidic pH, which can disrupt the polymer shell to rapidly release the payload. The acid-responsive formulation was characterized by higher drug encapsulation and DC activation leading to the expansion of activated natural killer (NK) cells and antigen-specific CD8$^+$ T cells. Da Silva et al. (Da Silva et al., 2019) used PLGA nanoparticles as delivery vehicles for the co-delivery of three immune adjuvants [the TLR3 agonist Poly (I:C;pIC), TLR7/8 agonist Resiquimod (R848) and the chemokine Macrophage Inflammatory Protein-3 alpha (MIP3α)] to significantly improve the therapeutic efficacy of cancer vaccines. Co-delivery of these modulating agents using PLGA nanoparticles significantly potentiated the cancer vaccine antitumor effects. The long-term survival of mice with established large carcinoma tumors was improved to 75%–100%, and the progression free survival of the mice nearly doubled. The potent adjuvant effect was associated with lymphoid and myeloid cell population alterations in the tumor and tumor-draining lymph node. Lu et al. (2021) developed a co-delivery immunotherapeutic strategy of the phagocytosis checkpoint (signal regulatory protein α, SIRPα) silencer and stimulator of interferon genes (STING) of APCs. A small interfering RNA targeting SIRPα (siSIRPα) and a STING agonist (cGAMP) were encapsulation into PLGA-based polymeric nanoparticles using the double emulsification method. In the ovalbumin-expressing B16-F10 (OVA-B16-F10) melanoma model, NPsiSIRPa/cGAMP stimulated the activation of OVA-specific CD8$^+$ T cells and induced holistic anti-tumor immune responses by reversing the immunosuppressive phenotype of APCs.

As effectors of innate immunity, natural killer (NK) cells represent 'the first line' of defense against pathogens and mediate potent antitumor cytotoxicity *in vitro* through secretion of cytotoxic lymphokines and disruption of the tumor vascular. Adoptive immunotherapy (AIT) with natural killer (NK) cells has emerged as a potential treatment strategy. However, their paucity in tumor infiltrates causes the low therapeutic efficacy of NK cell ATI. Park et al. (2017). demonstrate MRI-monitored

TABLE 1 PLGA-based nanoparticles used as delivery systems in cancer immunotherapy.

Nanocarrier	Payload	Tumor model	Outcomes	References
PLGA NPs	ICG, R837	4T1 breast cancer, CT26 cancer	Promoted generation of TAAs	Chen et al. (2016)
PLGA NPs	OVA, Riboxxim	EG7-OVA thymoma	Induced strong anti-tumor immune response	Koerner et al. (2021)
PLGA NPs	OVA, Hydroxychloroquine (HCQ)	EG7-OVA thymoma	Provided strong $CD8^+$ T cell immune responses	Liu et al. (2018)
PLGA NPs	HPV16 $E7_{44-62}$, ATP	TC-1 tumor	Induced strong anti-tumor immune response	Zhang et al. (2020)
DDAB/PLGA NPs	OVA	--	Enhanced the efficiency of nanovaccine	Han et al. (2021)
Cancer cell membrane-coated PLGA NPs	R837	4T1 breast cancer	Enhanced uptake of vaccine by DCs, which significantly promoted DCs maturation and antigen presentation	Yang et al. (2018)
Engineered peptide-expressed biomimetic cancer cell membrane-coated PLGA NPs	2′3′-cGAMP	B16-OVA melanoma, 4T1 breast cancer		Gou et al. (2021)
Lipid-coated PLGA NPs	mRNA, gardiquimod	B16-OVA melanoma		Yang et al. (2019)
Lipid-coated PLGA NPs	R837, OVA, ApoE3	B16-OVA melanoma		Zhou et al. (2020)
PLGA NPs	TLR7/8 agonists	Melanoma, Bladder, Renal Cell Carcinoma	Enhanced antigen specific immune response	Kim et al. (2018a)
PLGA NPs	TLR7/8 agonists, $NaHCO_3$	Melanoma	Resulted in higher loading of payload	Kim et al. (2018b)
PLGA NPs	Poly (I:C), R848, MIP3α	Carcinoma, Lymphoma	Enhanced the efficiency of nanovaccine	Da Silva et al. (2019)
PLGA NPs	siSIRPα, cGAMP	Melanoma	Induced strong anti-tumor immune response	Lu et al. (2021)
PLGA NPs	IFN-γ, Iron oxide nanocubes	Liver tumor	Enabled MRI-guided transcatheter IA delivery to liver tumor	Park et al. (2017)
PLGA NPs	Paclitaxel (PTX)	Melanoma	Enhanced the tumor inhibition capability	Hao et al. (2020)
M2pep modified cancer cell membrane-coated PLGA NPs	R848	B16-OVA melanoma	Inhibited tumor growth by reporamming TAMs	Zhang et al. (2021)

transcatheter intra-arterial (IA) local delivery of IFN-γ and iron oxide nanocubes (IONC) co-encapsulated PLGA nanoparticles to induce efficient NK cells infiltration to tumor sites for the targeted treatment of liver cancer. In an orthotopic liver tumor VX2 rabbit model, the prepared nanoparticles showed a sustained IFN-γ release and highly sensitive MR T2 contrast effects, significantly increased NK-cell infiltration into the liver tumor site.

Neutrophils can exert antitumoral functions especially in early stage of tumor development. However, they have been also shown to facilitate tumorigenesis and mediate immunosuppression. Due to their innate phagocytic functions and oriented migration capabilities in response to chemoattractants, nanoparticle-loaded neutrophils were used as "Trojan horses" (Hao et al., 2020). The pre-implantation of chemokine *CXCL1*-laden hydrogels could trigger and induce a targeted signal to attract an influx of neutrophils carrying the therapeutic goods to the desired position, thus the effectiveness of neutrophil-mediated nanoparticles drug delivery system is improved. *In vivo* studies showed that the combinatorial regimen of using the paclitaxel (PTX) loaded PLGA nanoparticles with the *CXCL1* chemokine laden PLGA-PEG-PLGA thermosensitive hydrogels exhibited superior tumor inhibition capability in mouse models of melanoma.

Tumor-associated macrophages (TAMs) are abundant in most human and experimental murine cancers, they are the major contributors to tumor angiogenesis, and also influence lymphocyte infiltration, leading to immunosuppression (Balkwill et al., 2012). Zhang et al. (2021) developed an M2-like macrophage-targeting nanoparticles to switch the tumor-promoting immune suppressive microenvironment by reprogramming TAMs. In these nanoparticles resiquimod (R848, a potent driver of macrophage reprogramming) loaded PLGA nanoparticles were coated with the B16-OVA cancer cell membrane. The membrane can increase the expression of CD47, which could avoid the nanoparticles being cleared by the reticuloendothelial system. The membrane was further modified with poly (ethylene glycol) (PEG) to achieve better long blood circulation and finally modified with M2pep to improve the selectivity and specificity for M2-like macrophages. The nanoparticles provide an effective and selective reprogramming strategy for macrophage-mediated

cancer immunotherapy. More *in vivo* studies showed that the loading nanoparticles reduced tumor size, and prolonged survival compared to the control groups.

Conclusion

This article briefly reviewed recent studies directed to improve the efficiency of PLGA-based delivery systems in cancer immunotherapy. In Table 1, we summarized these studies. Biocompatibility, biodegradability, and feasibility of modification are the most interesting features of PLGA-based nanoparticles, which can support these materials to have a good position in the development of nanomedicines. However, the high cost of production, the difficulty of the scale-up, fast *in vivo* degradation of non-coated nanoparticles and the relatively low drug loading efficiency are the main limitations of PLGA-based delivery systems. Although further research and clinical studies are still needed to improve the efficacy of drug delivery, the recent studies presented in this mini review clearly illustrate the promise of PLGA-based nanoparticles for novel treatments of cancer in the future.

Author contributions

LG and JL designed and wrote the manuscript, designed the pictures, and critically reviewed the manuscript. LG and TS searched articles relating to the subject. All authors contributed to the article and approved the submitted version.

Conflict of interest

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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Design, synthesis and biological evaluation of a novel colchicine-magnolol hybrid for inhibiting the growth of Lewis lung carcinoma *in Vitro* and *in Vivo*

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Colchicine is a bioactive alkaloid originally from Colchicum autumnale and possesses excellent antiproliferative activity. However, colchicine-associated severe toxicity, gastrointestinal side effects in particular, limits its further therapeutic use. In the current study, we thus designed and synthesized a novel hybrid (CMH) by splicing colchicine and magnolol, a multifunctional polyphenol showing favorable gastrointestinal protection. The antitumor activity of CMH in Lewis lung carcinoma (LLC) was then evaluated *in vitro* and *in vivo*. Biologically, CMH inhibited the growth of LLC cells with an IC_{50} of 0.26 μM, 100 times more potently than cisplatin (26.05 μM) did. Meanwhile, the cytotoxicity of CMH was 10-fold lower than that of colchicine in normal human lung cells (BEAS-2B). In C57BL/6 mice xenograft model, CMH (0.5 mg/kg) worked as efficacious as colchicine (0.5 mg/kg) to inhibit tumor growth and 2 times more potently than cisplatin (1 mg/kg). In terms of mortality, 7 out of 10 mice died in colchicine group (0.75 mg/kg), while no death was observed in groups receiving CMH or cisplatin at 0.75 mg/kg. Mechanistic studies using Western blot revealed that CMH dose-dependently suppressed the protein expression of phosphorylated ERK. Molecular docking analysis further indicated that CMH was well fitted in the colchicine binding site of tubulin and formed several hydrogen bonds with tubulin protein. These results enable our novel hybrid CMH as a potential antineoplastic agent with lower toxicity, and provide perquisites for further investigation to confirm the therapeutic potentiality of this novel hybrid.

KEYWORDS

colchicine-magnolol hybrid, lewis lung carcinoma cells, extracellular signal-regulated kinase, colchicine binding site, tumor growth inhibition

1 Introduction

Lung cancer represents a kind of very common malignant tumor that seriously threatens human life with a persistently high morbidity and mortality rate (Sun and Yan, 2020). Among them, non-small cell lung cancer accounts for the vast majority proportion. Although there are multiple avenues of therapeutic interventions in recent decades, including surgery, chemotherapy and radiation, alone or in combination, the compromised or even destroyed immune system of patients could be often observed in clinical practice (Zhou et al., 2022). Therefore, there is an urgent need to develop an alternative anti-cancer drug or therapy with increased efficacy and reduced toxicity.

Colchicine 1) is a bioactive alkaloid originally isolated from Colchicum autumnale and has long been used as a treatment for gout (Deng et al., 2021). Besides, there is extensive evidence that colchicine has displayed excellent antiproliferative potential in a variety of cancer cell lines against colon, breast, skin melanoma, liver and pancreas (Malik et al., 2022; Song et al., 2022; Wang et al., 2022), and thereby entered into different stages of clinical trials as an anti-cancer agent. Mechanistic studies have revealed that colchicine arrests cell division and kills tumor cells by favorably binding to the colchicine binding site of tubulin and interfering with microtubule formation (Dubey et al., 2017). However, colchicine treatment is always accompanied by serious gastrointestinal side effects (Papageorgiou et al., 2017), including vomiting, diarrhea and abdominal pain nausea, which limits its further clinical application or even causes treatment discontinuation in patients.

Magnolol 2) is a polyphenolic compound from Magnolia officinalis and possesses various pharmacological activities including antioxidation, anti-inflammation and antiangiogenesis (Peng et al., 2022; Xie et al., 2022). Most notably, magnolol has shown favorable gastrointestinal protection in a wide range of experimental paradigms associated with acute gastrointestinal injury and diarrhea (Xia et al., 2019; Lin et al., 2021; Mao et al., 2021).

Since that colchicine exhibits strong antiproliferative ability and magnolol is able to protect against gastrointestinal injury, a common side effect with colchicine treatment, we assume that the drug combination of colchicine and magnolol may provide additive antitumor potential with lower toxicity. Recently, we completed a concise asymmetric synthesis of (–)-colchicine (Pu et al., 2022a) and developed a series of new C-10-modified colchicinoid and evaluated their inhibitory activity on key proteases of 2019-nCoV replication and acute lung injury (Pu et al., 2022b).

While, in order to find new antitumor colchicine analogues with improved activity and lower toxicity, we expect to create a novel C-7-modified colchicinoid with single structure by splicing colchicine and magnolol (Figure 1). In this current study, we thus developed a novel colchicine-magnolol hybrid (CMH) and further evaluated its anti-proliferative potential *in vitro* and *in vivo* as well as the molecular mechanisms involved.

2 Results and discussions

2.1 Efficient synthesis of novel synthesized a novel hybrid

Small molecules that hit colchicine binding site could exert their efficient biological effects by inhibiting tubulin assembly and suppressing microtubule formation (Lu et al., 2012), numerous modifications of the colchicine chemical structure have been thus made to develop new anti-cancer candidate drug molecules (Gracheva et al., 2020). However, there were few reports about the hybridization of colchicine with other bioactive natural molecules. In this current study, we designed a new type of C-7-modified colchicinoid which was a hybrid of colchicine and magnolol with simple amino acid as the linker.

As shown in Scheme 1, the synthesis of CMH was commenced with the S_N2 reaction of magnolol with ethyl bromoacetate, and compound 3 was isolated in 78% yield. The ester was hydrolyzed with NaOH to generate the acid 4 which was used directly without further purification. In

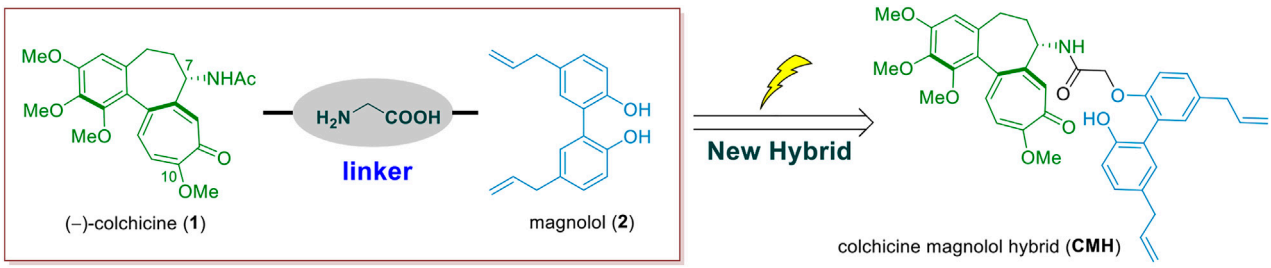

FIGURE 1
The design of the colchicine-magnolol hybrid (CMH).

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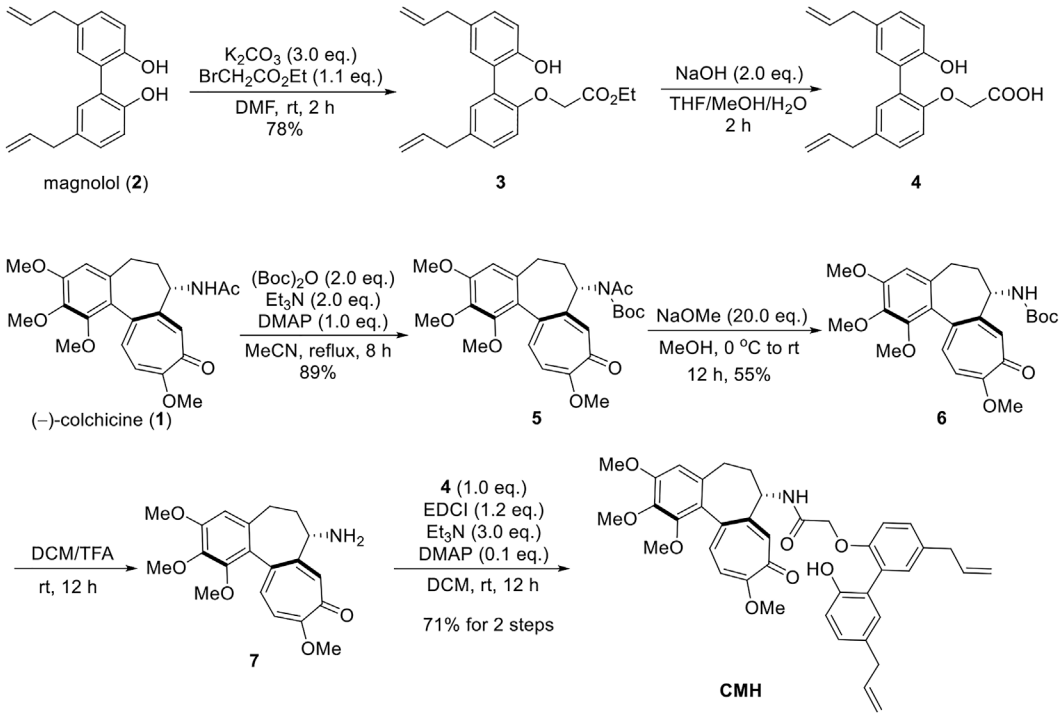

SCHEME 1
The synthetic route of novel hybrid CMH.

addition, a three-step sequence was employed to remove the -Ac group at C(7) of colchicine (Lagnoux et al., 2005; Yasobu et al., 2011). Furthermore, the amino group was then acylated with compound 4 to give the novel hybrid CMH.

2.2 CMH inhibited the proliferation of Lewis Lung Carcinoma cells 100 times more potently than cisplatin did

We then evaluated the antitumor activity of colchicine, CMH and cisplatin (positive control) in Lewis lung carcinoma (LLC) cells. As shown in Figure 2, the cell viability of LLC cells was greatly reduced after 24 h treatment with three compounds. Specifically, the half-maximal inhibitory concentration (IC_{50}) of colchicine, CMH and cisplatin against LLC cells was 0.06, 0.26 and 26.05 μM, respectively. It was evident that our novel hybrid CMH inhibited proliferation of LLC cells 100 times more potently than cisplatin did. In consistent with the cell viability results, images from confocal microscopy showed that compared to the control group, a lower density and rounder shape were observed in LLC cells incubated with different concentrations of CMH (Figure 3). Moreover,

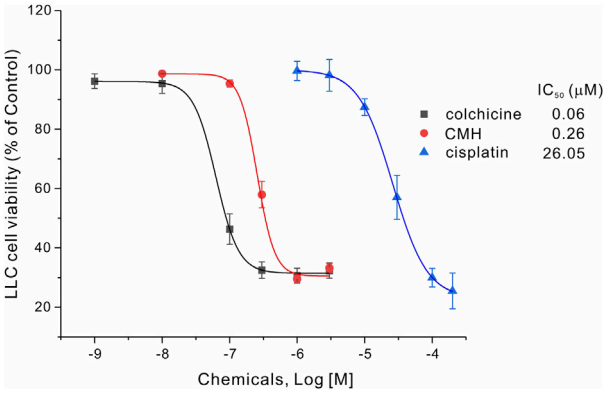

FIGURE 2
CMH inhibited the proliferation of LLC cells 100 times more potently than cisplatin did. LLC cells were incubated with colchicine, CMH, or cispatin (positive control) for 24 h, and then measured for cell viability.

CMH remarkably decreased the number of FDA-stained viable cells (Figure 3). These results indicated that the proliferation of LLC cells was inhibited to a larger extent by CMH treatment.

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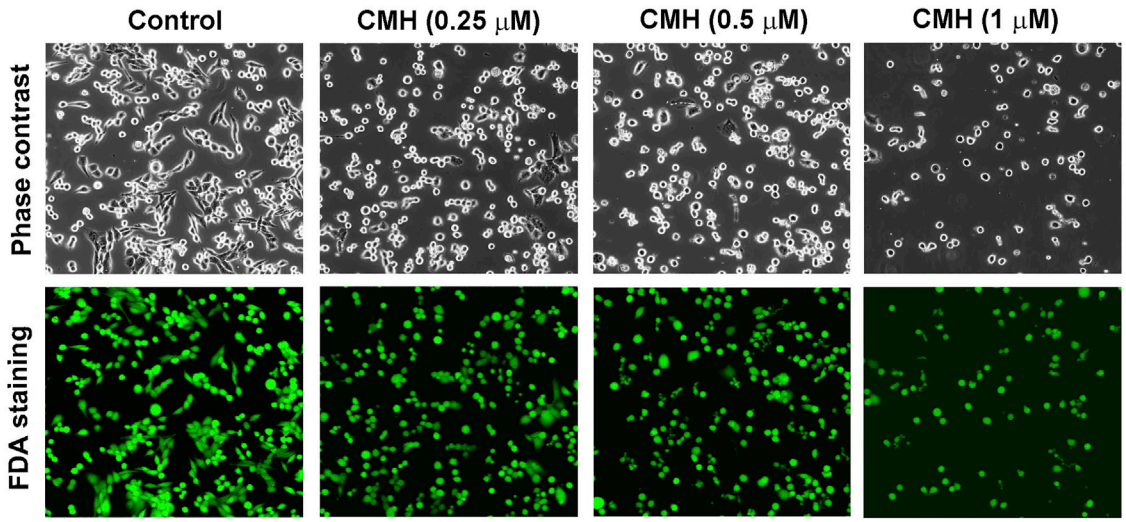

FIGURE 3
CMH markedly decreased the number of FDA-stained viable cells in a dose-dependent manner. LLC cells were incubated with CMH (0.25 μM, 0.5μM, 1 μM) for 24 h, and then stained with FDA for 5 min, and observed under a confocal laser scanning microscopy.

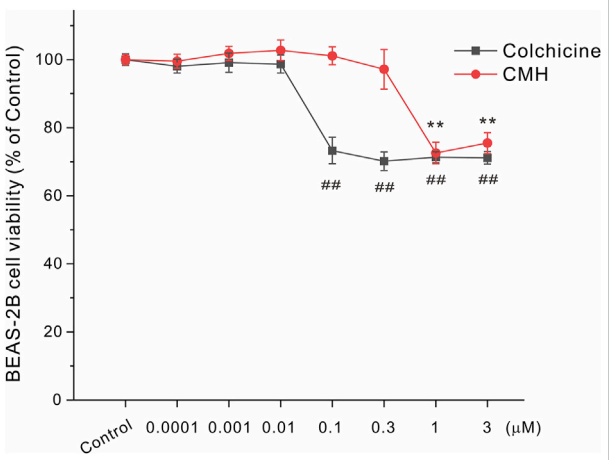

FIGURE 4
CMH induced lower toxicity in normal BEAS-2B cells compared to its parent molecule colchicine. BEAS-2B cells were incubated with dimethyl sulfoxide (Control), equal concentrations of CMH or colchicine for 24 h, and then examined for cell viability. ##, $p < 0.01$, colchicine group *versus* Control; **, CMH group $p < 0.01$ *versus* Control.

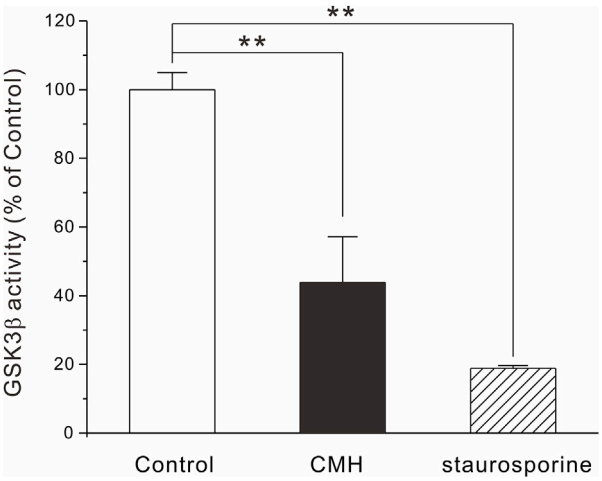

FIGURE 5
CMH at high concentration inhibited GSK3β activity. 1 ng GSK3β, 0.2 μg/μl substrate, 25 μM ATP and CMH (final concentration, 100 μM) or staurosporine (0.1 μM, positive control) were incubated in each well of 384-well plate for 60 min. ADP-Glo™ and kinase detection reagent was introduced successively into the well and luminescence was read. **, $p < 0.01$ *versus* Control.

2.3 CMH caused lower toxicity to BEAS-2B cells compared to colchicine

Next, we further used normal lung epithelial BEAS-2B cells to evaluate the toxicity of CMH and its parent molecule colchicine. As shown in Figure 4, CMH did not show any toxicity until its concentration reached 1 μM. Specifically, 24 h exposure of BEAS-2B cells to CMH at 1 μM decreased cell viability from (100.00 ± 1.48) % to (72.59 ± 3.17) %. In comparison, colchicine began to induce toxicity at the concentration of 0.1 μM, colchicine at 0.1 μM decreased cell viability to (73.27 ± 3.89) %. These results indicated the cytotoxicity of CMH might be 10-fold lower than that of colchicine in normal human lung cells.

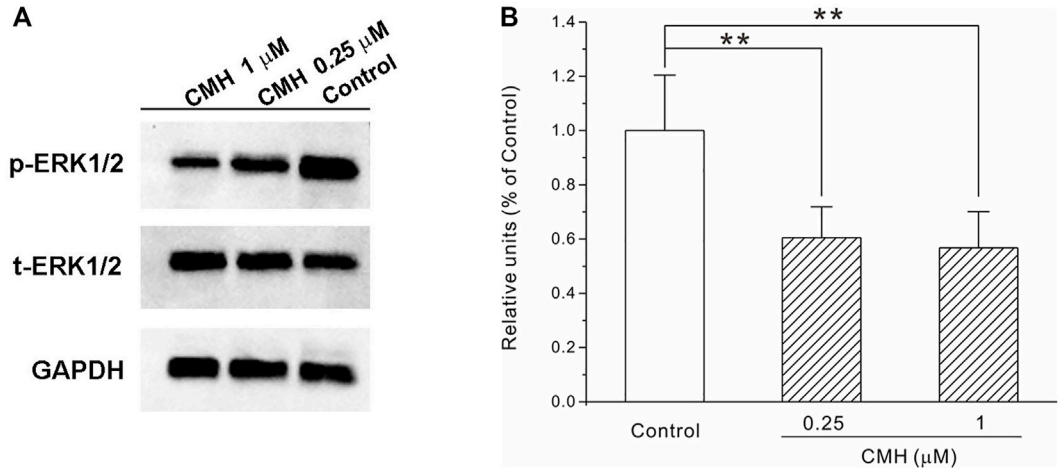

FIGURE 6
CMH strongly down-regulated the protein expression of phospho-ERK in LLC cells. LLC cells were incubated with CMH (0.25 μM, 1 μM) for 24 h, and then measured for protein expression. **, $p < 0.01$ *versus* Control. **(A)** Representative bands of p-ERK, t-ERK and GAPDH. **(B)** The statistical analysis.

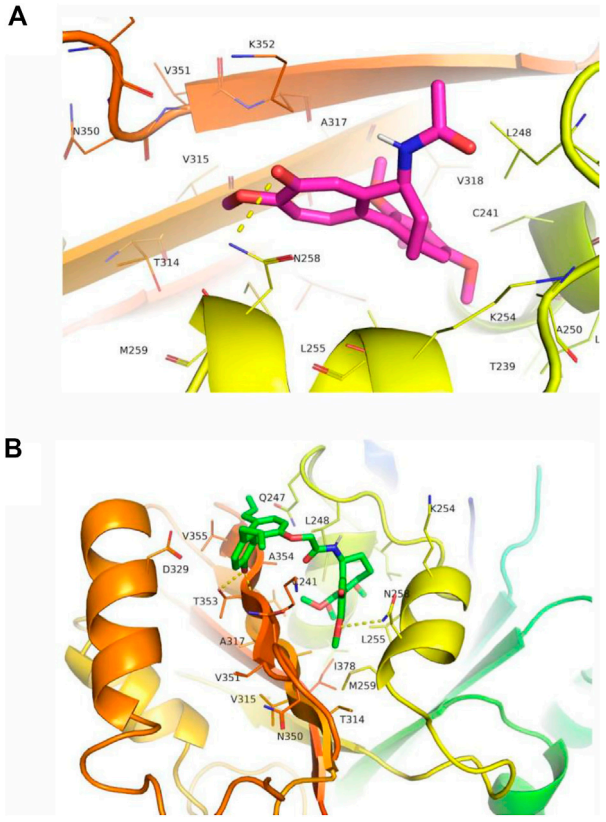

FIGURE 7
CMH interacted with tubulin at the colchicine binding site. The interaction between colchicine **(A)** or CMH **(B)** with tubulin. CMH was well fitted in the colchicine binding site of tubulin with binding energies of -8.04 kcal/mol, in comparison to the colchicine ligand (-5.93 kcal/mol).

2.4 CMH at high concentration inhibited the activity of GSK3β

Glycogen synthase kinase-3β (GSK3β) is a serine-threonine kinase that is responsible for promoting cancer cell survival, growth and proliferation (Domoto et al., 2020). It has been well documented that aberrant GSK3β activity is firmly associated with multiple tumor-related diseases and that GSK3β is generally accepted as a potential anti-tumor target (Bai et al., 2022; Fan et al., 2022; You et al., 2022). In light of this, we extended our effort to test the possibility that CMH may provide antiproliferative capacity through the direct inhibition of GSK3β enzyme activity. As shown in Figure 5, CMH at 100 μM decreased enzyme activity to approximately 40% of control, while failed to inhibit GSK3β at concentrations below 100 μM (data not shown). Since CMH showed toxicity in normal lung epithelial BEAS-2B cells when its concentration exceeded 1 μM, we speculate that GSK3β may not be the critical mechanism underlying CMH-mediated antitumor efficacy and some other potential targets are expected to be explored.

2.5 CMH downregulated the protein expression of phospho-ERK in LLC cells

There is extensive evidence that the phosphorylation of mitogen-activated protein kinases (MAPKs), extracellular signal-regulated kinase (ERK) subtype in particular, is closely associated with the growth and proliferation of tumor cells in cellular and animal experimental paradigms (Yang et al., 2022; Yu et al., 2022). Specifically, the increase of

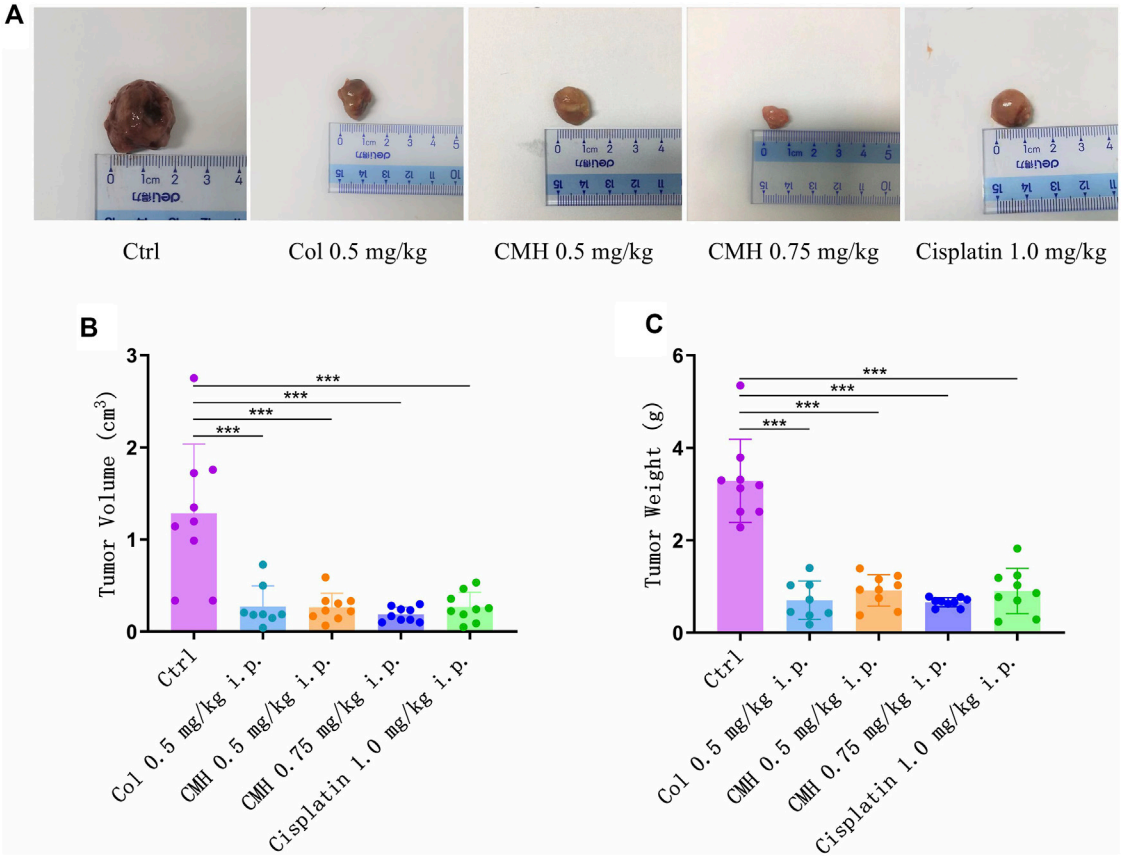

FIGURE 8
Effects of colchicine (Col, 0.5 mg/kg), CMH- (0.5 and 0.75 mg/kg) and cisplatin (1.0 mg/kg) on the tumor volume and weight in the xenografted mice during the entire experimental period. **(A)** Representative macroscopic view of LLC tumors in different groups. **(B)** Quantitative analysis of tumor volume. **(C)** Quantitative analysis of tumor weight. ***$p < 0.001$ *versus* control group.

TABLE 1 Inhibitory effects of compounds on tumor weights and tumor volume in the C57BL/6 mice xenografted LLC cells.

Groups	Avg. tumor weights (g)	Avg. tumor volume (cm^3)	%TGI	Mortality
Control	3.29 ± 0.90	1.29 ± 0.75	0	0/9
Col (0.5 mg/kg)	0.70 ± 0.42[a]	0.27 ± 0.23[a]	78.81	1/9
Col (0.75 mg/kg)[b]	—	—	—	7/9
CMH (0.5 mg/kg)	0.92 ± 0.34[a]	0.27 ± 0.15[a]	79.37	0/9
CMH (0.75 mg/kg)	0.66 ± 0.10[a]	0.19 ± 0.079[a]	85.44	0/9
Cisplatin (1 mg/kg)	0.90 ± 0.49[a]	0.27 ± 0.16[a]	79.22	0/9

[a]$p < 0.001$ versus the Control group.
[b]Colchicine was extremely toxic at 0.75 mg/kg, %TGI was thus not determined.

phospho-ERK level was usually observed in cancer cell lines such as LLC cells, particularly those treated with pro-tumorogenic compounds (Stoyanov et al., 2012), bio-molecules that could down-regulate ERK phosphorylation may thereby offer effective anti-tumor efficacy (Shi et al., 2022; Yuan et al., 2022).

In our cell system, the protein level of phospho-ERK (p-ERK) was evaluated using Western blot and the results in Figure 6 showed that CMH at 0.25 μM and 1 μM declined this phosphorylated protein to (0.61 ± 0.11) and (0.57 ± 0.13), respectively, compared to the control group (1.00 ± 0.20). This phenomenon was consistent with earlier findings where

this phosphorylated protein level in LLC cells greatly declined in the presence of anti-tumorogenic chemicals (Kim et al., 2007; Xie et al., 2018) and indicated that the inhibition of p-ERK may be a critical mechanism that underlied the anti-tumor effects of CMH.

2.6 CMH was well fitted in the colchicine binding site of tubulin and formed several hydrogen bonds with tubulin

Microtubules, which maintain the shape of the cell through the dynamic assembly of tubulin heterodimers, are generally accepted as an attractive target for the development of anti-cancer drugs (Andres et al., 2022). Specifically, colchicine binding site agents bind to colchicine binding domain and prevent the polymerization of tubulin proteins, thereby destabilize microtubules and provide antitumor potential (Wang et al., 2016; Sueth-Santiago et al., 2017). We then tested the possibility that our novel hybrid CMH could occupy colchicine binding site. Results from docking studies of compounds with tubulin protein (PDB entry: 1SA0) showed that both colchicine and CMH were well fitted in the colchicine binding site of tubulin. Specifically, colchicine molecule formed a hydrogen bond to Asn258 of tubulin protein with an estimated binding free energy of -5.93 kcal/mol, an observation consistent with an earlier study (colchicine docking score: −5.5 kcal/mol) (Dwivedi et al., 2022). In contrast, the best-docked conformation of CMH in the tubulin showed that the methoxy and the phenolic hydroxyl group of this ligand from the colchicine fragment and B fragment interacted with Asn258 and Thr353 through several hydrogen bonds with an estimated binding free energy of -8.04 kcal/mol (Figure 7). These results suggested that our novel CMH could be served as an effective colchicine binding site inhibitor.

In this docking system, CMH showed better docking score than the standard inhibitor colchicine, on the other hand, CMH (IC_{50} = 0.26 μM) displayed anti-proliferative potential 4 times less potently than colchicine (IC_{50} = 0.06 μM, Figure 2). This discrepancy could be explained by the existence of some other possible targets, such as taxane, vinca, laulimalide binding domains of tubulin, that CMH or colchicine may hit. Such interesting topics will be further investigated in our future projects.

2.7 CMH exhibited robust suppression of tumor growth in C57BL/6 mice xenografted with LLC cells

Finally, the antitumor ability of CMH was verified in C57BL/6 mice xenograft model. As shown in Figure 8, CMH exhibited promising antitumor efficacy, with a tumor growth inhibition (TGI) of 79.37% and 85.44% at the dosages of 0.5 mg/kg and 0.75 mg/kg, respectively. No mortality was observed in the group treated with CMH. For a reference, cisplatin (positive control) at 1.0 mg/kg inhibited tumor growth by 79.22% and colchicine at 0.5 mg/kg inhibited tumor growth by 78.81%. However, colchicine treatment was accompanied with a high risk of mortality. It was obvious that 7 out of 10 mice (colchicine group, 0.75 mg/kg) and one out of 9 mice (colchicine group, 0.5 mg/kg) were dead during 10 days of drug treatment (Table 1). These findings taken together suggested that CMH inhibited tumor growth 2 times more potently than cisplatin, and that CMH displayed antitumor capacity with a lower mortality and an efficacy comparable or even superior to colchicine.

3 Conclusion

In conclusion, we herein designed and synthesized a novel hybrid CMH by splicing colchicine and magnolol. CMH exhibited excellent antiproliferative effects in LLC cells (IC_{50} = 0.26 μM) and robustly suppressed tumor growth in C57BL/6 mice xenograft model. Meanwhile, CMH showed lower toxicity in normal human lung BEAS-2B cells and in mice when compared to its parent molecule colchicine. Mechanistic studies revealed that CMH provided its antitumor potential mainly through suppressing ERK signaling pathway and occupying colchicine binding site of tubulin concurrently. These results identify our novel hybrid CMH as a potential antineoplastic agent with lower toxicity, and provide perquisites for further investigation to confirm the therapeutic potentiality of this novel hybrid.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was reviewed and approved by Ethics Committee of Shenzhen Second People's Hospital.

Author contributions

ZL: Animal study and acquisition of data. SH: conception, design and drafting of the manuscript LP: Synthetic work. YC: Molecular docking analysis work. ZL, GZ, YM, ZL, XL, GL: Cell biology studies. LL, Enzyme activity assay. KC and ZW: supervision, revision and editing.

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Conflict of interest

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.

The handling editor FH declared a shared affiliation with the author KC at the time of review.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fchem.2022.1094019/full#supplementary-material

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Development of novel polymeric nanoagents and their potential in cancer diagnosis and therapy runing title: Polymeric nanoagents for cancer theranostics

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Cancer has been one of the leading factors of death around the world. Cancer patients usually have low 5-year survival rates and poor life quality requiring substantial improvement. In clinic, the presenting diagnostic strategies lack sensitivity with only a small proportion of patients can be accurately identified. For diagnosed patients, most of them are at the advanced stages thus being delayed to receive treatment. Therefore, it is eager to investigate and develop highly effective and accurate techniques for cancer early diagnosis and individualized therapy. Various nanoplatforms are emerging as imaging agents and drug carriers for cancer theranostics recently. Novel polymeric nanoagents, as a potent exemplar, have extraordinary merits, such as good stability, high biosafety and high drug loading efficacy, showing the great prospect for cancer early diagnosis and precise treatment. Herein, we review the recent advances in novel polymeric nanoagents and elucidate their synthesis procedures. We further introduce the applications of novel polymeric nanoagents in cancer diagnosis, treatment, and theranostics, as well as associated challenges and prospects in this field.

KEYWORDS

polymeric nanoagents, nanotechnologies, nanoparticles, cancer diagnosis, cancer therapy

1 Introduction

Cancer has been threatening human life and healthy, as a predominant driver to cause high morbidity and mortality worldwide (de Oliveira et al., 2020; Temkin et al., 2022). In the past few decades, substantial efforts have been devoted in cancer research, but the diagnosis and prognosis have not been improved much (de Oliveira et al., 2020). The main challenge behind is to identify the cancer patients in their early stage, so as to start personalized treatment in time.

Most cancer patients are diagnosed at the advanced stages because of lacking typical clinical symptoms. Conventional methods for cancer diagnosis mainly contain biopsy, magnetic resonance imaging (MRI), computed tomography (CT), positron emission tomography (PET), single photon emission computed tomography (SPECT) and ultrasound (US) (Solnik et al., 2022). Biopsy is still the gold standard, PET has a low resolution, and MRI causes a high false positive signal (Jin et al., 2021; Kowalchuk et al., 2021). Traditional cancer therapies, chemotherapy, radiotherapy, immunotherapy, and surgery face great challenges, such as low permeability, defective specificity, severe systemic side effects, and drug resistance (Liu et al., 2022a; Yazbeck et al., 2022). Therefore, it is imperative to investigate more effective therapeutic strategies for fighting against cancer.

Nanoagents have attracted great attention due to the easy-modified hydrophobic segments and rich functional groups, which are designed with the assistance of nanotechnologies and nanoparticles (NPs) (Huang et al., 2017). NPs dominantly comprise metal and metal oxide-based NPs, liposomes, dendrimers, magnetic NPs, quantum dots, and polymeric NPs (Selmani et al., 2022). Especially, Polymeric nanoagents, as polymeric NPs-assembled nanoagents, have made great progress for cancer diagnosis and treatment (Wang et al., 2020b; Yuan et al., 2021). Because of the high surface to volume ratio and the nanoscale size of NPs, polymeric nanoagents are capable of navigating through microvasculatures and across various biological barriers (Yuan et al., 2021). Hence, polymeric nanoagents can rapidly accumulate in cancer cells with the enhanced permeability and retention (EPR) effect as well as regulating the systemic biodistribution of therapeutic medicines (Wang et al., 2020b; Yuan et al., 2021).

Since the easy-modified hydrophobic segments and functional groups of nanoagents, lots of innovative polymeric nanoagents are developed by simultaneously conjugating with imaging agents and therapeutic molecules, such as fluorescent dyes, photosensitisers, aptamers, peptides, antibodies, chemotherapeutic drugs, or other biological molecules (Wang et al., 2020b; Haider et al., 2020; Yuan et al., 2021). Most importantly, growing preclinical trials have proved the increased biosafety, high selectivity, reduced systemic side effects, better solubility and stability of novel polymeric nanoagents (Li et al., 2022). These features have allowed polymeric nanoagents to perform specific imaging and precise therapy of cancer tissues, further developing into personalized polymeric theranostic nanoplatforms. Compared to conventional imaging methods, novel polymeric nanoagents-based imaging has a high temporospatial resolution, and numerous polymeric nanoagents have been developed for the early detection of cancer, such as indocyanine green (ICG) (Lv et al., 2022), lanthanide ion neodymium (Nd) (Deng et al., 2022), and chlorin e6 (Ce6)-based nanoagents (Odda et al., 2020). Recently, emerging methods for cancer treatment include novel polymeric nanoagents-based chemotherapy, gene therapy, photothermal therapy (PTT), and photodynamic therapy (PDT). PTT triggers irreversible pyroptosis, apoptosis, and necrosis of cancer cells *via* converting near-infrared (NIR) light into heat and further triggering hyperthermia (Yu et al., 2022). PDT stimulates cancer cells death through absorbing light and producing reactive oxygen species (ROS), especially singlet oxygen (Wang et al., 2022c). These polymeric nanoagents-based theranostic approaches are characterized with non-invasiveness, increased specificity, and low off-target toxicity (Wang et al., 2020a). Hence, this review would introduce the design of novel polymeric nanoagents, and their promising applications for the early diagnosis, treatment, and combined theranostics of cancer. Also, the unsolved problems of novel polymeric nanoagents in the field of oncology would be discussed.

2 The design and structure of novel polymeric nanoagents

Polymeric NPs lay a solid foundation for the development of novel polymeric nanoagents. Polymeric NPs originate from natural polymers and synthetic polymers in a core-shell structure, with hydrophilic blocks forming the shell and hydrophobic blocks forming the core of the NPs (Crucho and Barros, 2017). Generally, the synthesis of polymeric NPs is prepared by the two groups of methods, including preformed polymers and monomers polymerization-based encapsulating polymers (Crucho and Barros, 2017). Since the limitations of polymerization techniques, preformed polymers are more extensively utilized, where organic solvents are applied for dissolving the polymer in the first step (Zielinska et al., 2020). The preparation methods are composed of two-step and one-step procedures. Two-step procedures involving emulsification preparation and nanoparticles formation, mainly include emulsification-solvent evaporation, emulsification-solvent diffusion, and emulsification–reverse salting-out. One-step procedures consist of nanoprecipitation, dialysis, and supercritical fluid technology without emulsification to form nanoparticles (Crucho and Barros, 2017; Zielinska et al., 2020). In solvent evaporation method, the oil-in-water (o/w) emulsion is required for producing nanospheres. Emulsification diffusion requires the formation of an o/w emulsion that consists of a partially hydro-miscible organic solvent in the internal phase. The salting-out method separates a hydro-miscible solvent from an aqueous solution, and salting-out effect may facilitate the formations of nanospheres. Nanoprecipitation is considered as solvent displacement, depending on the interfacial deposition of a polymer after the organic solvent displacing from a lipophilic solution to the aqueous phase. During the synthesis of polymeric NPs, multiple impurities can exist in NPs suspension or adsorb into the surface of NPs, including

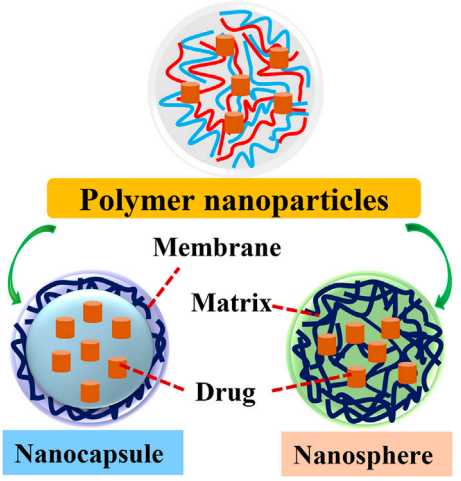

FIGURE 1
The structure of two types of polymer nanoparticles. Polymer NPs are classified into nanocapsules and nanospheres. Polymeric nanosphere is a matrix type, insoluble solid-colloidal particle in which drugs are distributed throughout the chemical compound matrix. Polymeric nanocapsule is a colloidal-vesicular system where the drug is confined to a cavity surrounded by a distinctive compound membrane.

organic solvents, salts, and particle residues. These toxic impurities must be removed with filtration, centrifugation, and dialysis techniques (Drozdz et al., 2020). Taken together, it is essential for choosing the preferable preparation method based on the drug's characteristics and the desired properties of polymeric NPs.

According to the morphology of polymeric NPs, they are classified into nanocapsules and nanospheres. Polymeric nanosphere is an insoluble solid-colloidal particle in which drugs are dissolved, entrapped, encapsulated, chemically bound or adsorbed to the constituent polymer matrix. Polymeric nanocapsule is a colloidal vesicle where drugs are confined to an oily reservoir or within an aqueous cavity surrounded by the polymer membrane (Crucho and Barros, 2017) (Naseef, P.P et al., 2021) (Figure 1). The special core-shell structure allows the selective delivery of drugs or fluorescent molecules to cancer tissues. Then, the release of drugs is achieved by modulating the rates of polymer biodegradation and drugs diffusion in the polymer matrix as well as induced by exogenous and endogenous stimuli in the specific disease microenvironments (Kamaly et al., 2016; Singh et al., 2021). Ultimately, the polymer matrix is degraded into non-hazardous molecules and excreted from the body.

Polymeric NPs-based nanoagents are characterized by effective drug-polymer interactions and can inhibit the premature release of drugs (Villamizar-Sarmiento et al., 2021). Moreover, they exhibit non-toxicity, good water-solubility, extensive biocompatibility, and easy modification (Crucho and Barros, 2017; Villamizar-Sarmiento et al., 2021). In virtue of these unique features, novel polymeric nanoagents have served as prospective candidates for cancer precise diagnosis and therapy *via* guiding MRI, SPECT/CT, NIR, X-ray imaging, photodynamic diagnosis (PDD), PTT, and PDT after conjugating with imaging agents and therapeutic drugs (Figure 2).

3 The applications of novel polymeric nanoagents in cancer diagnosis

In clinic, a number of cancer patients suffer from metastasis and advanced cancer stages due to the delayed diagnosis. Increasing evidence has shown that the early detection of cancer can significantly decrease mortality rate and improve therapeutic efficacy (Ribeiro et al., 2022; Zhu et al., 2022). However, conventional imaging techniques and biomarkers detection are not sufficient for the early diagnosis. Fortunately, polymeric nanoagents-based accurate bioimaging can detect small tumors at the early stage as well as reveal the biological processes involved in early carcinogenesis after conjugating with non-invasive optical imaging agents (Casas, 2020). Herein, novel polymeric nanoagents would be extensively utilized for cancer diagnosis due to their high sensitivity, non-invasiveness and good biocompatibility, and they will greatly advance the field of oncology (Shanavas et al., 2017; Tiwari et al., 2017) (Table 1).

Chitosan is a biocompatible, biodegradable, and minimized invasive polymer, and it has the widespread application for cells optical imaging. It is demonstrated that most epithelial cancer cells highly express folate receptor, and folate-chitosan (fol-cht)-based polymeric nanoagents can be applied for SPECT/CT imaging to detect folate receptor-overexpressed cancer tissues after radiolabeling with technetium-99m (Polyak et al., 2014). In addition, a study synthesizes hybrid nanoagents with a magnetic poly (lactide-co-glycolide) (PLGA) core and a fol-cht-based shell, and the shell surface's hydroxyl (-OH) and amine ($-NH_2$) functional groups can be easily modified through complicated chemical reactions. The biocompatible hybrid nanoagents provide super paramagnetic iron oxide NPs (SPIONs) delivery for targeting to folate receptor-overexpressed oral cancer cells, further bettering MRI contrast with the shortened T2 relaxation time and enhanced nanoparticle relaxivity (Shanavas et al., 2017). Taken together, chitosan shell-based polymeric nanoagents have showed a prospective potential for the early diagnosis of various cancers with better imaging contrast.

5-aminolaevulinic acid (5-ALA) is a hydrophilic and zwitterionic imaging drug as well as a precursor of heme and chlorophyll (Harada et al., 2022). Exogenous administration of 5-ALA ultimately metabolizes into protoporphyrin IX (PpIX), and accumulated PpIX in cancer cells generates PpIX fluorescence, further performing 5-ALA-based PDD (Beika et al., 2021; Harada

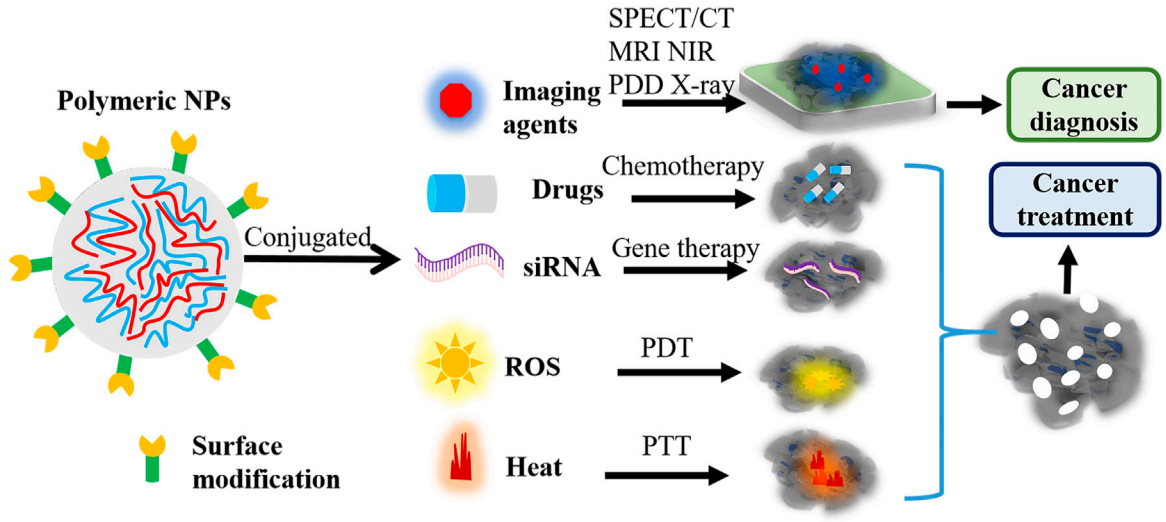

FIGURE 2
The function of polymeric nanoagents in cancer diagnosis and treatment. Polymeric NPs-based nanoagents would be widely used to guide SPECT/CT, MRI, NIR, PDD, and X-ray imaging through integrating with imaging agents. Also, these novel polymeric nanoagents can guide chemotherapy, siRNA therapy, PTT and PDT through integrating with chemotherapeutic drugs, siRNA, and imaging agents. NPs, nanoparticles; PDD, photodynamic diagnosis; NIR, near-infrared; PTT, photothermal therapy; PDT, photodynamic therapy; NPs, nanoparticles; ROS, reactive oxygen species; MRI, magnetic resonance imaging; CT, computed tomography; SPECT, single photon emission computed tomography.

et al., 2022). Nevertheless, 5-ALA shows a poor affinity toward the cell membrane, and cancer cells are difficult to take up 5-ALA (Yang et al., 2013). Considering the folic acid receptor-mediated endocytosis promoting the uptake of 5-ALA, 5-ALA is conjugated with succinate-modified chitosan (SCHI) and fol-cht-based polymer NPs (Casas, 2020). Once taken up by cancer cells, 5-ALA releases from lysosome due to the wakened attraction intensity between chitosan and 5-ALA, which strengthens the accumulation of PpIX fluorescence and facilitates the PDD of cancer cells (Yang et al., 2013). Numerous research have indicated the great prospect of the folic acid-based polymeric nanoagents for diagnosing cancer by delivering 5-ALA, such as oral cancer (Yang et al., 2013), cervical intraepithelial neoplasia (CIN) (Xu et al., 2021), prostate cancer (Casas, 2020) and glioblastoma (Babic et al., 2018).

Semiconducting polymer nanoparticles (SPNs) are also identified as excellent optical agents and applied for fluorescence, chemiluminescent, and photoacoustic (PA) imaging for the early detection of cancer due to the excellent biocompatibility and structural versatility (Cui et al., 2020; Tang et al., 2022). Poly (p-phenylenevinylene) (PPV)-based SPNs could emit afterglow luminescence after removing light excitation. Hence, the tetraphenylporphyrin (TPP) photosensitizer-copolymerized PPVs SPNs are synthesized to function as near-infrared (NIR) afterglow nanoagents. The novel copolymer nanoagents produce red-shifted NIR luminescence and amplified afterglow signals, allowing the *in vivo* tiny cancer imaging *via* sensing low oxygen in the cancer microenvironment (Cui et al., 2018). In general, cancer-associated fibroblasts are recognized as the key barriers for cancer therapy. Thereby, activated fibroblasts (AF)-camouflaged SPNs (AF-SPNs) are emerged as effective biomimetic nanoagents for optimizing cancer phototheranostics, where a SPN and AF membrane serve as the core and shell, respectively (Li et al., 2018). The AF-SPNs would generate stronger PA signal and dramatically strengthen photodiagnostic efficacy (Li et al., 2018). As well, RBC membrane-coated SPNs have the enhanced NIR light absorption and photostability for PA imaging (Zheng et al., 2020), which can deeply penetrate into cancer tissues and be rapidly cleared due to the small size. Both RBC and AF membrane-modified polymeric nanoagents provide the remarkable PA imaging contrasts for diagnosing cancer tissues (Li et al., 2018; Zheng et al., 2020).

In clinic, ICG is a usually used NIR fluorescence contrast agent. But the poor optical stability and clearance efficacy of ICG limit its applications. To overcome these demerits, ICG is encapsulated into hyaluronic acid (HA) for NIR imaging, HA is a natural and biodegradable polysaccharide polymer (Souchek et al., 2018). The ICG-lorded HA nanoagents have showed the prominent value in detecting prostate cancer and CD44-positive cervical cancer (Souchek et al., 2018). Bovine serum salbumin (BSA) is another common natural polymer, and BSA-coated ICG nanoagents exhibit non-toxicity, good water-solubility and strong NIR-I

TABLE 1 The applications of novel polymeric nanoagents in cancer diagnosis.

Types	Conjugation	Imaging techniques	Indications	Advantages	Ref
Fol-cht-based polymer	Technetium-99m	SPECT/CT	Folate receptor-overexpressed cancers	Targeting to folate receptor-overexpressed cancer cells	Polyak et al. (2014)
Fol-cht-based polymer	Magnetic PLGA	MRI	Oral cancer cells	Providing SPIONs delivery, further shortening T2 relaxation time and enhancing nanoparticle relaxivity	Shanavas et al. (2017)
Fol-cht-based polymer	5-ALA	Performing 5-ALA-based PDD	Oral cancer, CIN, prostate cancer, glioblastoma	Increasing the uptake of 5-ALA, the accumulation of PpIX fluorescence and facilitating the photodynamic detection	Yang et al. (2013); Babic et al. (2018); Casas, (2020); Xu et al. (2021)
SPNs	TPP	NIR	Various cancers	Producing shifted NIR luminescence, allowing the *in vivo* tiny cancer imaging *via* sensing low oxygen	Cui et al. (2018)
SPNs	AF	PA	Various cancers	Generating stronger PA signal and improving photodiagnostic efficacy	Li et al. (2018)
SPNs	RBC	PA	Various cancers	Enhancing NIR light absorption and photostability	Zheng et al. (2020)
HA	ICG	NIR	Prostate cancer	Augmenting optical stability and reducing systemic toxicity	Souchek et al. (2018)
BSA	ICG	NIR-I	Cervical cancer, neuroblastoma	Producing strong NIR-I fluorescence emission	Ma et al. (2021); Clutter et al. (2022)
PSMA	ICG	NIR	Cervical cancer	Improving the chemical stability and biocompatibility	Chen et al. (2021)
PLGA	ICG	NIR	Cervical cancer	Enhancing the NIR stability and internalization, reducing cytotoxicity	Choi et al. (2021)
PEG	ICG	NIR	Various cancers	Showing more sensitive imaging and strong targeting ability	Wang et al. (2022b)
PNMs	ICG	NIR	Various cancers	Inhibiting ICG leakage and indicating favorable biocompatibility	Hsu et al. (2020)
PAH	ICG	NIR	Ovarian cancer	Enhancing the fluorescence stability and targeting ability	Bahmani et al. (2014)
PCL	ICG	NIR, X-ray	Various cancers	Boosting the fluorescence stability, and having minimized adverse effects on the surrounding tissues	Gorecka et al. (2022)

fluorescence emission for cancer imaging, such as cervical cancer (Ma et al., 2021) and neuroblastoma (Clutter et al., 2022). Besides natural polymers, a study encapsulates ICG with synthetic poly (styrene-co-maleic anhydride) (PSMA) to construct ICG@PSMA nanoagents. Poly PSMA is an amphiphilic polymer that can encapsulate ICG to improve its chemical stability and biocompatibility. Further, ICG@PSMA nanoagents exhibit strong NIR fluorescence as well as reduced systemic toxicity for cervical cancer cells (Chen et al., 2021). Also, ICG can be encapsulated into other synthetic polymers to perform NIR imaging, including PLGA (Choi et al., 2021), polyethylene glycol (PEG) (Wang et al., 2022b), polymeric nanomicelles (PNMs) (Hsu et al., 2020), poly (allylamine hydrochloride) (PAH) (Bahmani et al., 2014), poly caprolactone (PCL) (Chien et al., 2018; Gorecka et al., 2022).

4 The role of novel polymeric nanoagents in cancer treatment

The progression of cancer is a relatively complex process, involving in cellular and genetic alterations. Although, advancements in cancer therapy have improved the survival rates and reduced the deaths, there are undesirable side effects. In recent, polymeric nanoagents-guided chemotherapy, nucleic acid therapy, PDT and PTT, emerge as remarkable approaches for achieving cancer treatment (Wang et al., 2022c; Yu et al., 2022) (Table 2). These advanced strategies selectively target into cancer tissues with minimal invasion into healthy tissues as well as prominently enhance the therapeutic efficacy (Liu et al., 2022b; Macchi et al., 2022).

It is worth noting that premature drug leakage, low drug delivery efficiency and defective cellular uptake are considerable

TABLE 2 The role of novel polymeric nanoagents in cancer treatment.

Types	Conjugation	Treatments	Indications	Advantages	Ref
ACC-SF	DOX	Chemotherapy	Various cancers	Blocking the premature efflux of DOX and preventing the protonation of DOX within the lysosome, displaying the excellent therapeutic performance	Tan et al. (2019)
LPNPs	Sal	Chemotherapy	Pancreatic cancer	Increasing oral absorption and cancer cells uptake	Fang et al. (2014)
Polymeric micelles	PTX	Chemotherapy	Breast cancer	Enhancing water-solubility, bioavailability, and reducing toxicity	Wang et al. (2017)
Triacontanol polymer	DTX	Chemotherapy	Pancreatic cancer, breast cancer, prostate cancer	Improving water-solubility and oral bioavailability, further enhancing the cancer cells-killing effects	Almawash et al. (2018); Lu et al. (2020); Xiao et al. (2022)
GC-based polymer	PTX, cisplatin, CPT, DTX	Chemotherapy	Various cancers	Resulting in maximized therapeutic efficacies	Hao et al. (2019); Dutta et al. (2021); Ren et al. (2021); Wang et al. (2021)
GC-based polymer	C60	PDT	Various cancers	Generating ROS and guiding PDT	(Kim et al. (2014); Gunduz et al. (2022)
PSMA NPs	ICG	PTT	Cervical cancer	Augmenting PTT efficiency under 808 nm laser irradiation	Chen et al. (2021)
SPNs	AF	PTT, PDT	Various cancers	Releasing single oxygen and heat for strengthening PDT and PTT	Li et al. (2018)
SPNs	RBC	PTT	Various cancers	Exert dramatic photothermal conversion and photothermal killing efficacy against cancer cells	Zheng et al. (2020)
PEG-PCL	C3, ICG	PDT, PTT	OSCC	Combining PTT and PDT treatment with better photothermal conversion stability	Ren et al. (2017)
HSA	Cisplatin, ICG	Chemotherapy, PTT, PDT	Various cancers	Displaying the synergistic anti-cancer effects of PDT, PTT and chemotherapy	Wang et al. (2019b)
GC-PEI	RFP-siRNA	Gene therapy	Various cancers	Delivering siRNA to the cell cytoplasm and exerting remarkable silencing effects	Huh et al. (2010)
tGC polymer	VEGF-siRNA	Gene therapy	Various cancers	Achieving VEGF knockdown and performing anti-cancer effects	Kim et al. (2017)
PEG-PEI-Ce6	Wnt1-siRNA	PDT, Gene therapy	Oral cancer	Hindering EMT process and enhancing the killing effects	Ma et al. (2017)
Polymeric micelles	PTX	Gene therapy, chemotherapy	Various cancers	Co-Delivering PTX and siRNA, and boosting the synergistic anti-cancer effects	Shi et al. (2021)

barriers for cancer chemotherapy, and assembling chemotherapeutic drugs with polymer NPs might solve these problems. Herein, the tumor microenvironment (TME)-responsive biocompatible nanoagents are constructed using amorphous calcium carbonate (ACC) cores and silk fibroin (SF) shells. Upon entering into TME, SF shells concurrently inhibit premature drug release and target to the acidic lysosomes. And the sensitive ACC NPs are gradually degraded, further producing a majority of Ca and CO and resulting in lysosomal collapse. Doxycycline (DOX) is a widely used anti-cancer drug. ACC-SF NPs-conjugated DOX nanoagents block the premature efflux of DOX from cancer cells and prevent the protonation of DOX within the lysosome, displaying the excellent therapeutic performance (Tan et al., 2019).

Salidroside (Sal) is a potent anti-cancer drug with high water-solubility. The clinic application of Sal in cancer therapy has been restricted by poor oral absorption and low cancer cells uptake. To overcome this impediment, Sal is lorded with lipid-shell and PLGA-PEG-PLGA triblock polymer-core NPs (Sal-LPNPs) by a double emulsification method (Fang et al., 2014). Sal-LPNPs nanoagents have a distinct inhibitive effect on the growth of human pancreatic cancer cells, but its clinical use needs more profound exploration. Paclitaxel (PTX) is an effective anti-cancer drug for various solid cancers, but it has low solubility, poor bioavailability, and inevitable toxicity. Encapsulation of PTX in polymeric micelles can boost its water-solubility and bioavailability, which has the promising applications in breast cancer therapy (Wang et al., 2017). Docetaxel (DTX) is a taxane-

based anti-cancer drug with low water-solubility and oral bioavailability. Encapsulating DTX with PLGA, liposomes and PEGylated triacontanol polymer systems can dramatically enhance the DTX delivery efficacy and cancer cells-killing effects, like pancreatic cancer (Almawash et al., 2018), breast cancer (Lu et al., 2020), and prostate cancer (Xiao et al., 2022). In addtion, glycol chitosan (GC)-based polymeric nanoagents have been widely used for delivering chemotherapeutic drugs *via* hydrophobic interactions, such as paclitaxel (Hao et al., 2019), cisplatin (Wang et al., 2021), camptothecin (CPT) (Dutta et al., 2021), or DTX (Ren et al., 2021), resulting in maximized therapeutic efficacies.

Besides chemotherapy, PDT-produced ROS and PTT-generated heating specifically trigger phototoxic death of cancer cells with the assistance of optical nanoagents (Liu et al., 2022b; Macchi et al., 2022). Fullerene (C60) possess remarkable photophysical properties, thereby it can be used as a potentially strong photoactivatable agent for PDT *via* triggering ROS production (Gunduz et al., 2022). In detail, C60 is conjugated with GC to form GC-C60 or GC-2,3-dimethylmaleic acid (DMA)-C60, the solubilized C60 nanoagents produce tremendous fluorescence signals, further accumulating in various cancer tissues to guide PDT (Kim et al., 2014). Also, ICG@PSMA shows good PTT efficiency in cervical cancer cells under 808 nm laser irradiation. Therefore, ICG@PSMA nanoagents might serve as photothermal nanoagents (PTN) for other different types of cancer treatment, providing a solid basis for following *in vivo* experiments (Chen et al., 2021). Additionally, GC-based polymeric nanoagents are preferable carriers for PTT and PDT (Rhee et al., 2014).

SPNs is stand out as superb optical agents for PDT and PTT, and cell membrane-coated SPNs have indicated superior therapeutic effects (Tang et al., 2022). For instance, the AF-SPNs nanoagents generate not only NIR fluorescence and PA signals for imaging but also single oxygen and heat for strengthening photodynamic and photothermal therapeutic efficacies *via* promoting NPs accumulation in cancer cells (Li et al., 2018). As well, RBC membrane-coated SPNs have excellent photothermal conversion efficiency for PTT. These data demonstrate that RBC membrane-coated SPNs exert dramatic photothermal killing efficacy against cancer cells, which would become a promising phototheranostic agent for clinical translation (Zheng et al., 2020).

In particular, recent studies have proved that the combined treatment of chemotherapy, PTT and PDT has the stronger therapeutic effects. For instance, ICG is conjugated with organic compound (C3) and polyethylene glycol-polycaprolactone (PEG-PCL) to construct hybrid nanoagents (PEG-PCL-C3-ICG) for combined PTT and PDT treatment. *In vitro* and *in vivo* experiments of oral squamous cell carcinoma (OSCC) cells show that PEG-PCL-C3-ICG nanoagents have better performance than PTT or PDT separately, together with better photothermal conversion stability, lower cytotoxicity, and faster metabolic rate (Ren et al., 2017). In line with these, human serum albumin indocyanine green-cisplatin nanoagents (HSA-ICG-DDP) are designed to combine PDT, PTT with chemotherapy. *In vitro* and *in vivo* experiments have demonstrated that the synergistic anti-cancer effects of PDT, PTT and chemotherapy are remarkably heightened compared to ICG, HSA-ICG and DDP treatments, showing the favorable value of polymeric nanoagents in combination therapy (Wang et al., 2019b).

Small interfering RNA (siRNA), as one of nucleic acid therapy, is an effective therapeutic agent due to its specific gene silencing ability. However, the application of siRNA is hindered due to its susceptibility to nuclease degradation and low internalization by cancer cells. To develop novel delivery systems of siRNA, GC-PEI NPs are assembled by combining GC-5β-cholanic acid and polyethylenimine (PEI) polymers-5β-cholanic acid at a 1:1 weight ratio, then mixing RFP-siRNA and GC-PEI NPs at a 1:5 weight ratio (Huh et al., 2010). The generated GC-PEI NPs can effectively deliver siRNA to the cell cytoplasm and exert remarkable silencing effects, which are verified in RFP-expressing B16F10 cells (Huh et al., 2010). Besides, the thiolated GC (tGC)-polymerized siRNA is developed to enhance the stability of the siRNA (Kim et al., 2017). *In vivo* fluorescence imaging results reveal that VEGF-siRNA-tGC nanoagents have the increased serum stability, and they can quickly internalize and localize into the cytosol, further achieving VEGF knockdown and performing anti-cancer effects (Kim et al., 2017).

Most importantly, siRNA represents the enhanced synergistic anti-cancer effects when combining with chemotherapeutic drugs or PDT. For instance, siRNA Wnt1 is introduced into polyethylene glycol-polyethyleneimine-chlorin e6 (PEG-PEI-Ce6) nanoagents. Further, siRNA Wnt1-PEG-PEI-Ce6 nanoagents target into the cytoplasm of PDT-treated oral cancer cells to hinder EMT process and boost the killing effects against cancer cells (Ma et al., 2017). For another thing, siRNA integrated with PTX is co-delivered by pH-sensitive polymeric micelles, which can not only achieve gene silencing but also block premature drug release and perform chemotherapeutic effects (Shi et al., 2021). These combined treatments bring great advancements in nanotechnology, nanomedicine, drug delivery, and cancer therapy.

5 The role of novel polymeric nanoagents in cancer theranostics

In order to simultaneously deliver therapeutic drugs and diagnostic imaging agents as well as real-timely monitor of therapeutic responses, imaging-guided theranostic nanoagents are developed (Hu et al., 2021a) (Table 3). The strategy of treatment with simultaneous visual diagnosis benefits the

TABLE 3 The role of novel polymeric nanoagents in cancer theranostics.

Types	Conjugation	Theranostics	Indications	Advantages	Ref
Cy@Silk	Tc	SPECT and real-timely monitoring	Various cancers	Visualizing the distribution of nanoagents, real-timely monitoring cancer progression and maximizing the therapeutic efficacy	Wang et al. (2019a)
SPNs	Peroxidase	NIR-II-based PA and PTT	Various cancers	Elevating the sensitivity of diagnosis and the efficacy of PTT with the stronger PA signals	Lyu et al. (2018)
SPNs	—	NIR-II-based PA and PTT	Gliomas	Augmenting the absorption and inducing cancer cells death in both shallow and deep tissues	Wen et al. (2020)
AIE	ApoE	NIR-II-based PTT	GBM	Showing a higher PTT efficiency	Wang et al. (2022a)

formulation of individualized therapy planning and the development of precise medicine. Nevertheless, it is required for figuring out the whole-body 3D information and the dynamic biological processes of theranostic nanoagents for *in vivo* applications, including absorption, distribution, metabolism, and excretion.

Presently, the NIR cypate-induced SF self-assembly (Cy@Silk) nanoagents are designed and labeled with the radionuclides (Tc) for SPECT imaging. The multimodal SPECT imaging can offer the whole-body 3D information about nanoagents' distribution *in vivo*, substantially facilitating the real-timely monitoring of cancer progression and maximizing the therapeutic efficacy (Wang et al., 2019a). Besides, biodegradable SPNs are developed with the ability to augment PA imaging and PTT efficacy. The biodegradable SPNs are designed based on the enzymatically oxidizable nature of vinylene bonds, and they can be transformed into water-soluble nanoparticles (SPNV). The vinylene bonds within the polymer backbone endow SPNV with an excellent mass absorption coefficient (1.3-fold) and a photothermal conversion efficacy (2.4-fold). Hence, SPNV shows the stronger PA signals and higher photothermal maximum temperature, dramatically elevating the sensitivity of diagnosis and the efficacy of PTT for various cancers (Lyu et al., 2018).

Considering the restricted absorption of the first NIR window (NIR-I) nanoagents, SPNs under the second NIR window (NIR-II) are assembled to augment the absorption. In light of the excellent PA and photothermal performance, high photostability, proper size, and low toxicity of SPNs, NIR-II-based SPNs nanoagents are assimilated by U87 glioma cells. Then, they lead to efficient cells death under NIR-II light irradiation, allowing PA imaging and PTT toward gliomas in both shallow and deep tissues (Wen et al., 2020). In another study, brain-targeted NIR-IIb aggregation-induced-emission (AIE) nanoagents are synthesized to graft apolipoprotein E peptide (ApoE), which is termed as ApoE-Ph nanoagents (Wang et al., 2022a). ApoE-Ph nanoagents have a higher PTT efficiency for glioblastoma (GBM) by keeping the balance of radiation-modulated NIR-fluorescence imaging at 1,550 nm and non-radiation NIR-PTT, opening a novel window for boosting theranostics in other cancers (Wang et al., 2022a). Also, nanoagents under NIR-II have the precise multimodel imaging capability and concurrent PTT, such as dual-model (NIR-II/MRI)-guided cancer theranostics (Hu et al., 2021b), and tri-modal (PA/NIR-II/MRI) imaging-guided PTT (Hu et al., 2019).

6 The challenges of novel polymeric nanoagents

Novel polymeric nanoagents have been investigated for a long time and provide new insights for the diagnosis and treatment of cancer, and some polymeric nanoagents are entering clinical trials. However, novel polymeric NPs also have several drawbacks requiring further attention. Neutral or negatively charged and larger nanoagents are prone to escape the immune system with the reduced EPR effects, and positively charged and smaller NPs are poorly excreted and have the potential toxicity (Liang et al., 2021). These properties of nanoagents might affect the diagnostic and therapeutic efficacy for cancer (Crucho and Barros, 2017). Thereby, the size, charge, shape, and hydrophilicity of polymeric nanoagents should be further optimized.

On the other hand, the change of TME (like temperature and pH) is an important issue that attenuates the effectiveness of polymeric nanoagents-based theranostic systems, and the corresponding mechanisms remain unclear. In order to timely adjust and optimize the polymeric nanoagents-based theranostic strategies, the advanced equipment can be used to monitor the change of TME. On the other side, an innovative wearable electronic strain sensor might be applied for timely assessing therapeutic response of cancer patients by discerning differences in tumor volume dynamics (Abramson et al., 2022). Another pressing drawback of nanoagents-based systems is the rapid initial or burst release, which is often attributed to the weakly bound to the surface (Yang et al., 2014). Hence, supramolecular chemistry, especially host-guest chemistry might markedly strengthen the interaction between drugs and polymer chains (Yang et al., 2014).

Thirdly, the relatively large dose of polymeric nanoagents is administrated *in vitro* mouse models, but showing a relatively short circulating lifetime. Further clinical trials should try to reduce the dose and extend lifetime *via* adjusting medication plan, modifying with human serum albumin (HSA), polysaccharides or PEG (Hu et al., 2018). Meanwhile, the large discrepancies between *in vivo* and *in vitro* experiments should also be concerned, thereby, the more homologous animals should be chosen for experiments.

Lastly, the manufacturing processes of theranostic nanosystems for simultaneous diagnosis and therapy are generally complicated, and the productivity of novel nanoagents needs to be improved. So, the technical challenges, such as cost, colloidal stability, and reproducibility, must be taken into account. Future experiments should be focused on investigate cost-effective nanomaterials and simply the productive procedures.

7 Conclusion

The advanced polymeric nanoagents have obtained increasing attention in oncology research and biomedicine, wherein nanoagents not only function as imaging agents but also as the delivery carriers of drugs. Most importantly, novel polymeric nanoagents-based theranostic systems have the great potential to achieve the simultaneous treatment and diagnosis of cancer; accordingly, there are future prospects to prolong the survival of cancer patients. In the coming years, more profound research would pay attention to optimizing the physicochemical properties of nanoagents, improving productivity, lowering the production costs, and rapidly translating polymeric nanoagents into clinical applications. Although there is a long way to go until clinical translation, novel innovative polymeric nanoagents offer a great opportunity for improving current strategies for early cancer detection, diagnosis and treatment procedures, and they will be welcome in the future.

Author contributions

GH, QL and LL conceived and wrote the article. EW revised and reviewed the article. All authors contributed to the article and approved the submitted version.

Conflict of interest

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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Glossary

Fol-cht, folate-chitosan;
PLGA, poly (lactide-co-glycolide);
PDD, photodynamic diagnosis;
SPIONs, super paramagnetic iron oxide NPs;
CIN, cervical intraepithelial neoplasia;
5-ALA, 5-aminolaevulinic acid;
PpIX, protoporphyrin IX;
SPNs, semiconducting polymer nanoparticles;
PA, photoacoustic;
TPP, tetraphenylporphyrin;
HA, hyaluronic acid;
ICG, indocyanine green;
BSA, bovine serum salbumin;
PSMA, poly (styrene-co-maleic anhydride);
PEG, polyethylene glycol;
PNMs, polymeric nanomicelles;
PAH, poly (allylamine hydrochloride);
PCL, poly caprolactone;
NIR, near-infrared;
MRI, magnetic resonance imaging;
CT, computed tomography;
SPECT, single photon emission computed tomography;
ACC, amorphous calcium carbonate;
SF, silk fibroin;
DOX, doxycycline;
Sal, salidroside;
LPNPs; lipid-shell and PLGA-PEG-PLGA triblock polymer-core nanoparticles;
PTX; paclitaxel;
DTX, docetaxel;
GC, glycol chitosan;
CPT, camptothecin;
C60, fullerene;
ICG, indocyanine green;
PSMA, poly (styrene-co-maleic anhydride);
SPNs, semiconducting polymer nanoparticles;
AF, activated fibroblasts;
C3, organic compound;
OSCC, oral squamous cell carcinoma;
HAS, human serum albumin;
PEI, polyethylenimine;
tGC, thiolated GC;
Ce6, chlorin e6;
siRNA, small interfering RNA;
PEG, polyethylene glycol;
PTT, photothermal therapy;
PDT, photodynamic therapy

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"Smart" drug delivery: A window to future of translational medicine

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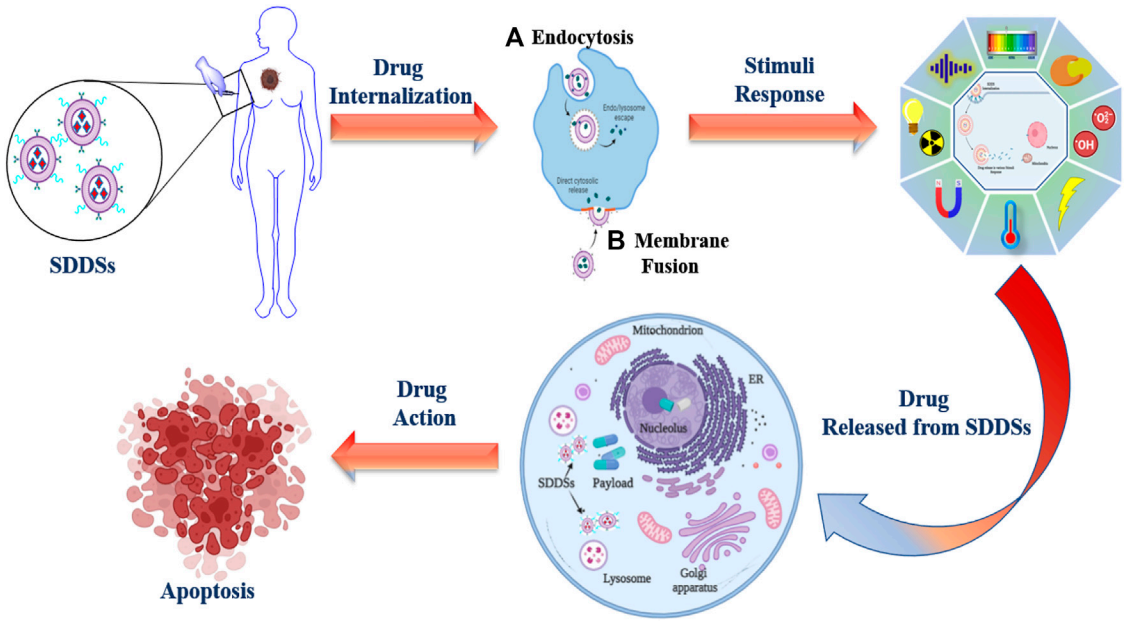
Chemotherapy is the mainstay of cancer treatment today. Chemotherapeutic drugs are non-selective and can harm both cancer and healthy cells, causing a variety of adverse effects such as lack of specificity, cytotoxicity, short half-life, poor solubility, multidrug resistance, and acquiring cancer stem-like characteristics. There is a paradigm shift in drug delivery systems (DDS) with the advent of smarter ways of targeted cancer treatment. Smart Drug Delivery Systems (SDDSs) are stimuli responsive and can be modified in chemical structure in response to light, pH, redox, magnetic fields, and enzyme degradation can be future of translational medicine. Therefore, SDDSs have the potential to be used as a viable cancer treatment alternative to traditional chemotherapy. This review focuses mostly on stimuli responsive drug delivery, inorganic nanocarriers (Carbon nanotubes, gold nanoparticles, Meso-porous silica nanoparticles, quantum dots *etc.*), organic nanocarriers (Dendrimers, liposomes, micelles), antibody-drug conjugates (ADC) and small molecule drug conjugates (SMDC) based SDDSs for targeted cancer therapy and strategies of targeted drug delivery systems in cancer cells.

KEYWORDS

smart drug delivery systems (SDDSs), cancer, translational medicine, nano-therapy, active targeting, passive targeting, targeted drug delivery, targeted cancer therapy

1 Introduction

Cancer has emerged as a leading health concern of the 21st century, with over 10 million new patients diagnosed each year (Sung et al., 2021). In 2020, more than 19 million people worldwide were diagnosed with cancer, with nearly 10 million dying as a result (Mao et al., 2022). By 2040, the number of new cases and deaths is expected to be around 28 million and 16 million, respectively (Khazaei et al., 2021; Sung et al., 2021). Currently, surgical resection, radiation therapy (RT), and chemotherapy are the three major treatment modalities of cancer treatment. The comparative usefulness of various procedures are determined on the basis and type of cancer and stage of development. Despite recent advances in treatment strategies and targeted treatment, the survival rate has not improved significantly. As a result, innovative cancer treatment approaches are required. Chemotherapy has been one of the most effective treatments for both localised



GRAPHICAL ABSTRACT

and metastatic tumours for more than 50 years. The issue of systemic side effects from chemotherapy has yet to be addressed. Conventional drug delivery systems frequently have systemic adverse effects due to non-specific biological distribution and uncontrolled drug release features. Exploration of innovative drug delivery technology can have commercial as well as therapeutic value for health products, is needed to have significant expansion (Liu et al., 2016). Moreover, many drugs are difficult to administer using traditional drug delivery techniques due to a lack of therapeutic effectiveness and a variety of challenges such as limited bioavailability, sensitive toxicity, insufficient specificity, and so on (Majumder and Minko, 2021). Additionally, challenges to be consider and overcome, include the attack of enzymes, the poor permeability of some tissues, and the difficulty of access to the target once arriving at the destination cells, among others (Alvarez-Lorenzo and Concheiro, 2014). There is a need to investigate new innovative ways of drug delivery that can minimize side effects. A drug delivery system (DDS) is a method or process that releases the drug at a pre-selected site in a controlled manner to achieve therapeutic effect. Drug delivery systems can in principle provide enhanced efficacy and/or reduced toxicity for a therapeutic agents. An ideal DDS in cancer achieves two goals: tumor-specific delivery and tumor-specific drug release from delivery systems (Nkepang et al., 2014). Smart Drug Delivery Systems (SDDSs) were developed to circumvent these limitations, allowing payloads to be delivered to target areas in a spatially controlled way. SDDS have many other applications and can be developed into smart systems, encasing therapeutic and imaging agents as well as bearing stealth property. SDDSs can also be used to develop diagnostics tools, PET scanning, MRI-CAs for efficient and early diagnosis of cancer (Wu and Wang, 2016).

Targeted treatments aim to block specific biologic transduction pathways or cancer proteins that are involved in tumour growth and progression, i.e., molecular targets (receptors, kinase cascades, growth factors, or molecules related to angiogenesis and apoptosis) that are found overexpressed or mutated in cancer (Chabner et al., 2005; Hanahan and Weinberg, 2011). The primary objective of these revolutionary therapies is to either block the signals that lead cancer cells to grow and divide uncontrollably, induce apoptosis in cancer cells, stimulate the immune system, or target the delivery of chemotherapy agents specifically to cancer cells, minimising the death of normal cells and avoiding the negative side effects (Perez and Fernandez-Medarde, 2015).

SDDSs can preferentially accumulate and bind to the disease target, allowing for controlled release. It is common knowledge that drugs should be released at target areas in a regulated way to maximise therapeutic effectiveness while minimising negative effects. The loaded medicines can act “smart” by inheriting from the controlled release (Liu et al., 2016).

SDDSs are designed to take advantage of the different conditions (e.g., temperature, pH, and enzyme concentration) that occur in pathological tissues rather than in normal tissues in a “smart” way, enabling them to trigger drug release in the targeted tissue, overcome intermediate barriers, and increase bioavailability, blood circulation time, and overall therapeutic

efficacy (AlSawaftah et al., 2021). A better understanding of tumour biology, combined with the increased availability of versatile materials such as polymers, lipids, inorganic carriers, polymeric hydrogels, and bio-macromolecular scaffolds, has led to the development of systems that can deliver chemotherapeutics to tumour sites with improved therapeutic efficacy in recent years (Senapati et al., 2018).

Drug delivery efficiency refers to the safe delivery of a drug to target locations without significant off-target effects (Sanadgol and Wackerlig, 2020). SDDSs are efficient tools to ensure the release of the therapeutic agent at the target and in the right dosage for the needed duration in order to optimise its efficacy by accumulating at the site of action and achieve the therapeutic effective concentration level within the therapeutic window while minimising adverse effects on healthy tissues. This delivery method must be biocompatible and biodegradable in order to penetrate the tissue and cells without causing specific toxicity, immunogenicity, or accumulation in organs other than the tumour. SDDSs have the potential to deliver medicines to precise and targeted locations. The most reported carriers mainly are Liposomes, micelles, dendrimers, mesoporous silica nanoparticles (MSNs), and gold nanoparticles, carbon nanotubes (CNTs), quantum dots (QDs), vitamins (Folic acid (B9) (Rana and Bhatnagar, 2021) and Biotin (B7) (Saha et al., 2013) and monoclonal antibodies (Kimiz-Gebologlu et al., 2018).

In this review article, we highlight the recent development of various SDDSs used in cancer therapeutics to increase the therapeutic index of chemotherapeutic drugs. We highlighted the components and classification of SDDSs, example of target nanocarriers, antibody based smart drug delivery systems, small molecule based smart drug delivery systems. In the context of the current oncological developments, the contribution of fundamental research to clinical practices with respect to SDDSs is explored.

2 smart drug delivery systems (SDDSs)

SDDSs have the exciting potential to vastly improve the efficiency and precision of treatment across a wide range of disorders. Smart drug delivery is a means of administering treatment to a patient in a targeted and controlled release manner. SDDSs can efficiently lower dosage frequency while maintaining drug concentrations in certain organs or tissues for a longer period of time when compared to conventional DDSs. In this way, SDDSs offer a wealth of possibilities for lowering drug concentration fluctuations, reducing drug toxicity, and enhancing therapeutic efficacy.

Most anticancer drugs are given at the maximum tolerated dose, cancer patients frequently suffer from severe cytotoxic side effects, limiting their treatment options. SDDSs allow for lower drug doses while maintaining effective intracellular concentrations, therefore expanding the therapeutic window of anticancer drugs. SDDSs have several advantages, including improved specific localization, patient compliance, reduced toxic side effects, and controlled biodistribution (Naziris et al., 2016).

2.1 Components of SDDS

Successful drug delivery requires that drugs should be released at desired target sites in a controlled manner to maximise therapeutic efficacy while minimising side effects. SDDSs, are made up of the following components: carriers/targeting ligand, linker and cytotoxic drug payload. A smart drug delivery system (SDDS) using liposomes as smart carrier (Figure 1), consists of (Liu et al.) Smart Carriers/Targeting Ligands that transport anti-cancer drugs to the targeted cancer site, (ii) targeting mechanisms that locate the cancerous site, and (iii) stimulus techniques that release the payload drugs at the pre-located cancer cell site. The following sections go over the various SDDSs, as well as their targeting mechanisms and stimulus techniques.

2.1.1 Targets utilized by SDDS

The drug target is a crucial part of SDDSs. Commonly explored drug targets in the body: **i)** Receptors on cell membranes which enable drug carriers to engage specifically with cells, boosting drug absorption *via* receptor-mediated endocytosis. For example, folate receptors (FRs), which are differentially overexpressed in epithelial cancer cells, are used to deliver tumor-specific drugs in cancers such as breast, ovarian, brain, and lung cancers (Rana and Bhatnagar, 2021). G protein coupled receptors (GPCRs), Integrins, sigma receptors, Epidermal growth factor receptor, Sigma receptors (SRs) these over expressed receptors are frequently used in preclinical cancer models for selective drug delivery *via* receptor–ligand pairs (Rana and Bhatnagar, 2021). Follicle-stimulating hormone receptors, C-type lectin receptors, biotin receptors, and neuropilin receptors are some of the less common receptors that have lately been utilised (Kim et al., 2018). Other targets for tumor-selective accumulation of drug carriers include those expressed on tumour vasculature endothelial cells. The dependence of tumour growth on angiogenesis is a potential target for the development of therapies to prevent the production of new tumor-feeding blood vessels to reduce tumour progression (Xu J. et al., 2017). Anti-angiogenesis strategies are successful in reducing tumour development, with endothelial cells in tumour blood arteries being the primary targets. Cancer cells are denied of nutrition and oxygen, resulting in the tumor death (Teleanu et al., 2019). Targeting ligands are coupled to drug-loaded nanocarriers. These ligands find their corresponding target on the cancer cell surface, which is overexpressed. A wide variety of synthetic and natural

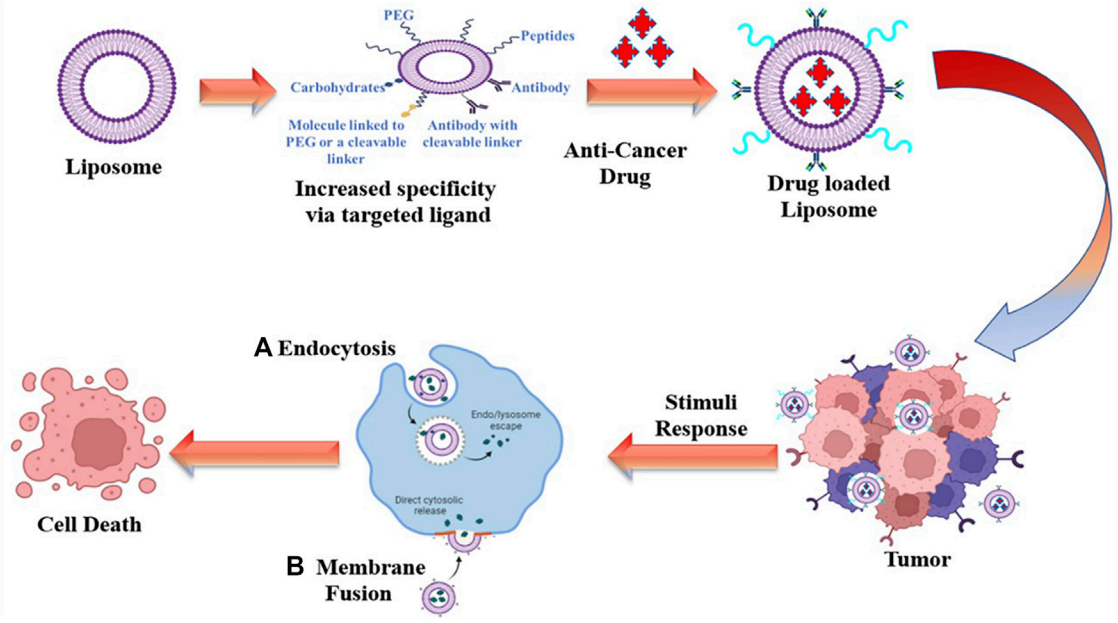


FIGURE 1
Overview of a SDDSs using liposomes as smart carrier.

chemicals of various chemical classes have been utilised to target nano systems against cancer cells. Antibodies (Ab) and other proteins (such as transferrin), Aptamers, tiny molecules like folic acid, and peptides are among the most utilised. It is important to identify optimum targets to maximise the efficacy of active targeting. Identifying receptors expressed at greater levels on target cancer cells than on normal cells is the justification for picking optimal targets (Gui et al., 2017).

ii) The Cell Membrane Lipid Components: When synthetic phospholipid analogues interact with biological membranes, they change the lipid content, membrane permeability, and fluidity. As a result, signal transduction pathways are disrupted, resulting in apoptosis (Torres et al., 2021). iii) Cell Surface Antigens or Proteins: The diseased cells either produce novel proteins or show differential (under/over) expression of proteins seen in healthy cells. Against such proteins, monoclonal antibodies are employed. The tumor-specific antigen that may be used to target drugs is one that is expressed exclusively and uniformly by all tumour cells (Khanna, 2012).

2.1.2 Targeting ligands

Sugars, folic acid, peptides, monoclonal-antibodies and specifically designed antibodies are examples of ligands that bind with specific receptors found on certain cell types with some degree of exclusivity. Nucleic acids like aptamers, tiny compounds like vitamins, and sugars like galactose, mannose, and other sugars have also been described as cellular targeting components (Figure 2) (Khanna, 2012).

2.1.3 Carrier and Targeting ligand (TL)

Special carrier systems are required for cancer-targeted drug delivery applications. A SDDSs carrier is a special molecule, particle, composite, or system that can hold the drug, either through encapsulation or using a spacer. The TL is one of the most significant components for the successful delivery of drug payload in a SDDS. They segregate, transport, and hold drug payloads while delivering them to a specific targeted site. SDDSs require different carrier systems depending on the type of targeting mechanism. SDDSs carriers are specially designed vectors capable of encapsulating and/or bonding with a spacer moiety to keep the drug inside or on them (Figure 3). The medication delivery vehicle utilised must be non-toxic and non-immunogenic, stable, biocompatible, biodegradable, readily eliminated from the body, and unrecognizable by the host's defence mechanisms. Other characteristics of drug carriers include high loading/encapsulation quantity with zero premature release of drug molecules, cell type or tissue specificity and site directing ability, and appropriate regulated release rate of drug molecules with an effective local concentration (Vallet-Regi et al., 2001). SDDSs based on carriers/TL provide benefits such as a larger surface-to-volume ratio, more reactive activity centres, more adsorption capacity, and other characteristics such as morphological preferences. The mechanism of control and drug secretion by these carriers at the target locations is very distinct and special. The reason is that the SDDS cleaves at first upon exposure to a particular stimulus, leading to continuous release for a long time afterwards (Rai et al., 2022).

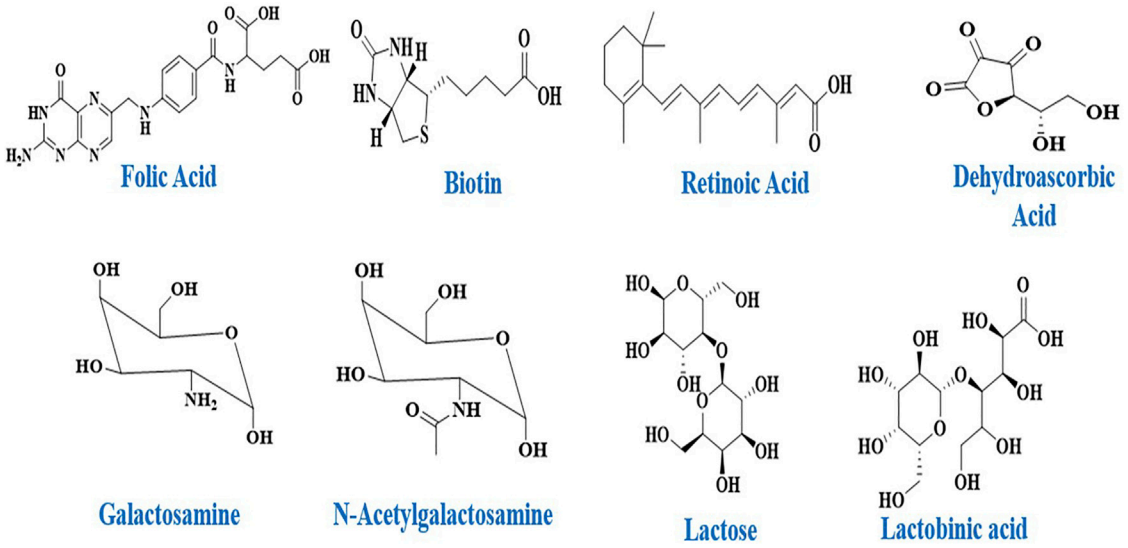


FIGURE 2
Some example of Targeting Ligands utilized in SDDSs.

2.1.4 Therapeutic drug payload

SDDSs for targeted chemotherapy usually consist of the carrier, a cleavable linker, and the chemotherapeutic agent (Rana and Bhatnagar, 2021). The chemotherapeutic agent is chosen to be inactive in its conjugated form, which makes the SDDS a prodrug that is activated only in the tumor tissues.

This allows the chemotherapeutic agent to exert its desired toxic activity on the cancer cells in a fast and effective manner (Figure 4). Drug delivery systems having the ability to attach targeted moieties can be given locally or systemically. The drug payload might be delivered either outside or within the target cells. Larger drug-delivery systems can provide high local drug concentrations, whereas smaller drug-delivery systems can be directly endocytosed. The precise architecture of the SDDSs allows drug payloads to be delivered to particular tissues in systemic administration. The main focus of SDDSs platform that the drug does not easily extravasate during blood circulation, but rather only releases at the sites where the drug carriers concentrate *via* an active or passive targeting approach (Liu et al., 2016).

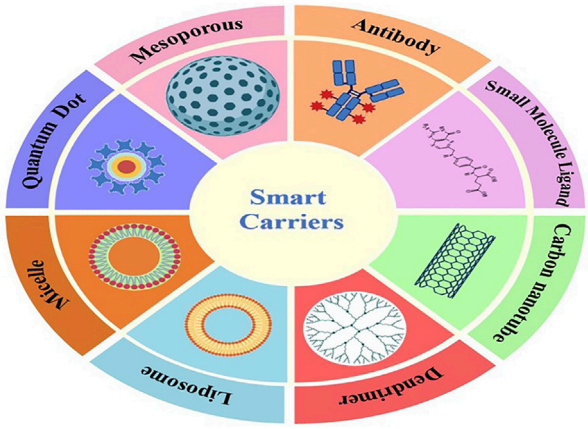


FIGURE 3
Some examples of Smart Drug Delivery Carriers.

3 mechanism of action of smart drug delivery system

Early detection, location of the original tumour and metastases, killing cancer cells as effectively as possible while limiting harm to the patient (i.e., maximising therapeutic index), and high accumulation in tumour lesion are all important factors in cancer treatment (Kue et al., 2016). Drug targeting can be an effective strategy to address these challenges and overcome some of the drawbacks of non-targeted treatments. To deliver therapeutic payloads to tumour locations, there are two main mechanisms of drug targeting (Figure 5) (Torchilin, 2010).

Passive targeting relies on the use of large, generally polymeric molecules as carriers to increase permeability and retention. Targeting moieties such as ligands and antibodies are used in active targeting. These approaches differ from mechanistic or direct targeting options, which use monoclonal antibodies (mAbs) or small-molecule drugs to bind to surface proteins or interfere with elevated metabolic processes in cancer (Kue et al., 2016).

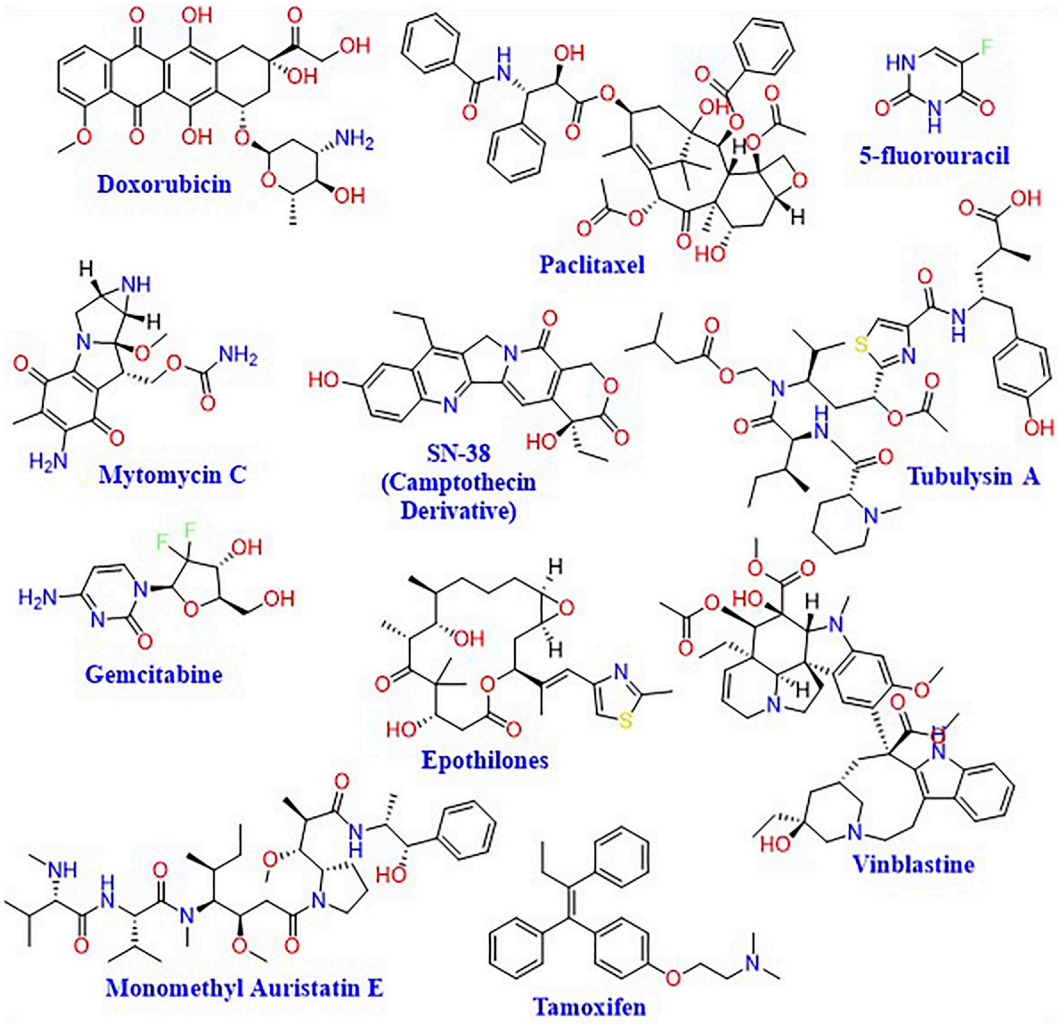


FIGURE 4
Chemical structures of representative cytotoxic agents used in SDDSs.

3.1 Active targeting delivery systems

Active targeting entails the identification of cancer cells, which leads to increased drug accumulation and cellular internalisation (Figure 6) (Kim et al., 2018). In active drug targeting, antibodies, antibody fragments, and peptides are linked to drugs and delivery systems to function as homing devices, allowing them to bind to receptor structures expressed at the target region. In terms of receptors on the cell surface and antigen expression, cancer cells vs healthy cells can be distinguished (e.g., Folate Receptors, transferrin's and Prostate-Specific Membrane Antigen (PSMA)). Trans-membrane communication is facilitated by cell surface receptors, which are proteins anchored in the cell membrane. Active targeting refers to the employment of externally coupled targeting moieties to improve carrier distribution. Because a quickly developing tumour needs a wide range of minerals and vitamins, tumour cells overexpress several tumor-specific receptors. Nanoparticles are tethered with ligands such antibodies, peptides and folic acid that serve as targets that bind to those receptors, which may aid internalisation following engagement, to provide efficient tumor-specific drug delivery. G-protein-coupled receptors (GPCRs), integrins, folate receptors, transferrin receptors, epidermal growth factor receptor (EGFR), fibroblast growth factors (FGFRs), and sigma receptors are all used to target medicines to tumour tissues and microenvironments (Rana and Bhatnagar, 2021).

Antibodies have long been recognised to detect malignancies, particularly receptors or surface antigens with a high level of expression on cancer cells. In 1975, the first tumour antigen-targeting monoclonal antibody (mAb) was produced and since then, several mAbs have been FDA-approved for cancer therapy

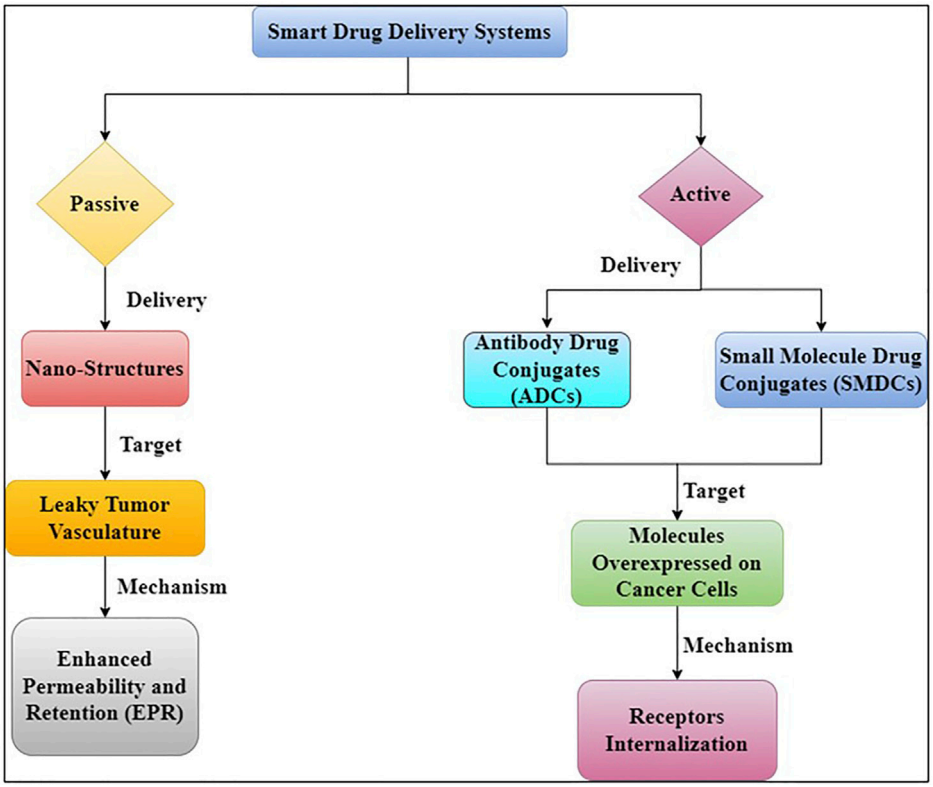


FIGURE 5
Different targeting strategies for anticancer therapeutics.

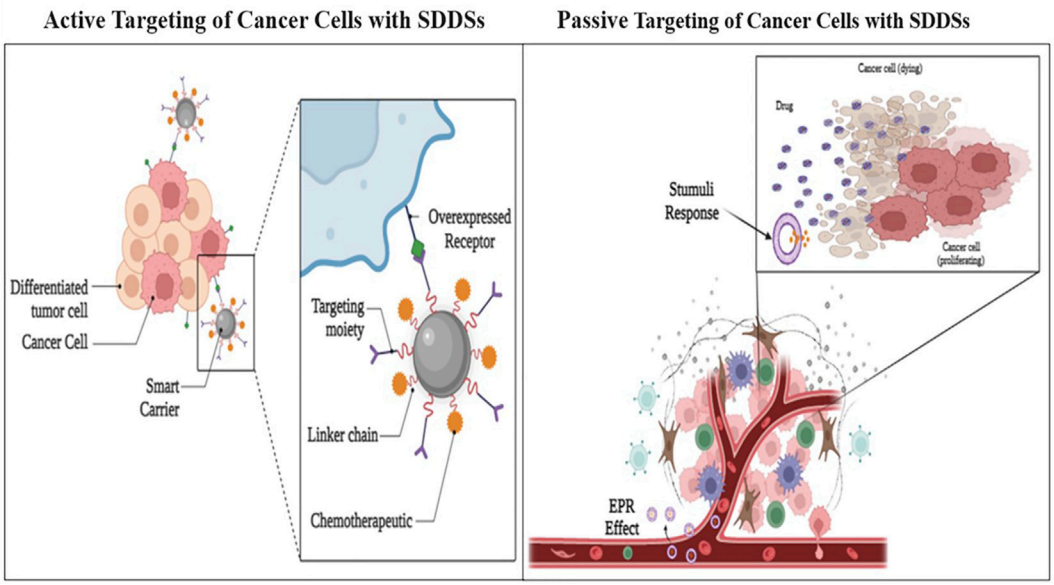


FIGURE 6
Active and Passive targeting delivery of drug payload by SDDSs.

(Baah et al., 2021). Long-term administration of mAb-conjugated drug carriers is thought to create immunological memory against antibodies, although targeted treatment using mAb-conjugated drug carriers is regarded a key possibly curative approach (Kim et al., 2018). Antibody fragments or chimeric antibodies have the potential to significantly lower immunogenicity when compared to full antibodies (Singh et al., 2018). Cetuximab, a recombinant chimeric mAb with a murine variable region and a human constant region that has been successfully used to treat cancer by targeting the epidermal growth factor receptor (Singh et al., 2018). Dual targeting antibodies, which have two epitope binding sites and may respond with single or dual targets, are an emerging approach for improving tumour targeting capabilities (Kim et al., 2018). Trastuzumab, a humanised mAb for the treatment of HER2-positive breast cancer, was developed in 1998 (Piccart-Gebhart et al., 2005). Bevacizumab, a tumour angiogenesis inhibitor that binds to vascular endothelial growth factor, was authorised in 2004 for the treatment of colorectal cancer (VEGF) (Ferrara et al., 2004). Recent research has attempted to encapsulate chemotherapeutic medicines in nanoparticles and then functionalize the particle surface with mAbs to preserve targeted effectiveness. The nanoparticles' absorption and cytotoxic efficacy are improved by conjugated antibodies (Sanchez-Moreno et al., 2018).

When metabolic activities are enhanced, transferrin receptors are overexpressed on cell surfaces (Shen et al., 2018). The primary route of cellular iron absorption *via* clathrin-coated pits, with subsequent traffic to endosomal compartments, has been shown to involve membrane transferrin receptor-mediated endocytosis of the complex of transferrin-bound iron and transferrin receptor (Tortorella and Karagiannis, 2014). Anti-tumor medicines, proteins, and therapeutic genes have all been effectively delivered through this absorption route (Sanchez-Moreno et al., 2018). Due to high iron needs, transferrin receptors have been found to be increased in malignant cancer cells, including those of bladder, brain, breast, and lung cancers, as well as lymphoma (Kim et al., 2018).

Aptamers are three-dimensional DNA or RNA sequences that are short and single-stranded. Aptamers are nucleic acid molecules that fold into complex 3D shapes that bind to specific targets, much like antibodies (Dunn et al., 2017). They're gaining a lot of attention in clinical trials for a variety of reasons, including their prolonged storage life, narrow batch-to-batch differences, low immunogenicity, and the ability to make chemical modifications for improved stability, serum half-life extension, and targeted delivery (Ni et al., 2021). Aptamers are more stable ligands *in-vivo* than antibodies, as they are produced chemically *via in-vitro* selection, a simple and inexpensive process and the time required to generate aptamers is comparatively short. Unlike antibodies, aptamers do not require any specific biological systems for their production (Thiviyanathan and Gorenstein, 2012). According to Zhang et al., cell-based SELEX has a lot of potential since cancer cells may be targeted specifically without knowing the proteins expressed on their surfaces; hence, different aptamers can be produced to target different kinds of cancer (Zhang et al., 2020). Aptamers, have limitations, on the other hand as their affinity is lower than the one of antibodies. The most significant success of aptamers thus far has been the development of FDA-approved aptamers that can bind to VEGF, a protein involved in angiogenesis (Kaiser, 2006). The coupling of aptamers to drug-delivery nanoparticles resulted in better targeting, more effective treatments, and more selective diagnostics (Sanchez-Moreno et al., 2018).

Folate is a B9 vitamin that is water soluble and interacts with folate receptor to aid cellular uptake. The folate receptor has the advantage of having low expression in normal tissues, but it is strongly expressed by numerous malignancies, particularly cancers that afflict women, such as cervical, breast, and ovarian cancer (Rana and Bhatnagar, 2021). Folic acid binds 20 times more to tumour cells than it does to normal epithelial cells or fibroblasts. Folate conjugation has been a popular approach for targeting drug delivery systems due to these appealing features (Rana and Bhatnagar, 2021).

CTPs (cell targeting peptides) are peptides that are short and have been chemically synthesised from peptide libraries and utilised as targeting ligands (Vives et al., 2008). CTPs are less than 10 amino acids in length and are more stable than traditional antibodies (Dissanayake et al., 2017). The amino acid sequences of CTPs identify targets, and the best sequences for interacting with specific cancer cell surface receptors are important for target identification. The most well-studied CTP is the Arginylglycylaspartic acid (RGD) peptide, which has a high affinity for integrin receptors overexpressed on the surfaces of 21 different types of cancer cells (Sanchez-Moreno et al., 2018).

3.2 Passive targeting delivery systems

Passive targeting refers to the accumulation of a drug or drug-carrier system at a specific location, which can be caused by physicochemical and pharmacological variables (Eckmann et al., 2014). There are few universally applicable methods for targeting tumours and tumour cells due to the phenotypic diversity of malignant cells and tumours (or their organelles). The most important approach is since many cancerous cells and vascularized solid tumours, as well as some vascularized metastatic tumour nodules, have an enhanced permeability and retention (EPR) effect that can be used for antitumor drug "passive targeting" (Maeda et al., 2000). Because many solid tumours have a leaky vasculature and absent or limited lymphatic drainage, high molecular weight molecules (such as polymers) and small particles with a diameter of ~20–500 nm accumulate within the tumour tissue (Ulbrich et al., 2016). This form of targeting is based on the pathophysiology of the disease

and the characteristics of tumour tissues, which may encourage drug accumulation in target tissues, reducing non-specificity (Rabanel et al., 2012). The vasculature of tumours is thought to differ from that of surrounding tissue. In comparison to typical well-organized arteries, tumour angiogenesis has featured that aid drug retention, such as high vascular density and permeability, defective vascular architecture, and poor lymph drainage from tumour tissue interstitial spaces. The Enhanced permeability and retention effect (EPR) effect is used in passive targeting to detect cancer spots (Sanchez-Moreno et al., 2018). The accumulation rate of drug-loaded nanocarriers in a tumour is much higher than in normal tissue because to the leaky endothelium of the tumour vasculature (Hossen et al., 2019). The concentration of anti-cancer drugs in the tumour might be raised several times when compared to healthy bodily tissue using this EPR effect. The passive targeting of gelatin (typeB) –based NPs was extremely effective in the delivery of genes at tumour locations, according to Kommareddy et al. (Kommareddy and Samiji, 2007). Another study utilised gelatin (type B) for the creation of NP-based DDSs that included plasmid DNA (pDNA) (Kaul and Amiji, 2002). Encapsulating DNA with PEGylated gelatine NPs improved the efficiency of targeting pDNA-expressed green fluorescent proteins and -galactosidase *in vitro* as well as *in vivo*. PEGylated-gelatin NPs have also been utilised to focus on DNA moieties in lung carcinomas, suppressing tumour development and angiogenesis in breast cancer cells (Das et al., 2020; Mi, 2020).

4 Stimuli responsive smart drug delivery systems

The active drug can be released at the location in released under strict restraint systems in response to specific physical, chemical, or biological processes, some of which are triggered internally and some of them are induced externally (Sershen and West, 2002). Stimulus-based drug delivery techniques have showed a lot of promise in terms of successfully targeting active drug moieties. The first time thermo-sensitive liposomes were utilised for medication delivery was in 1978 (Mazzotta et al., 2018). Over the years, scientists have created and widely used stimuli-responsive biomaterials for regulated drug administration, culminating in the development of the area of stimuli-responsive polymers (Mi, 2020). As a result, they may be divided into two types of responsive DDS (Figure 7).

1. Exogeneous stimuli-responsive SDDSs (Open-loop system): Externally controlled systems, or pulsatile systems, are also

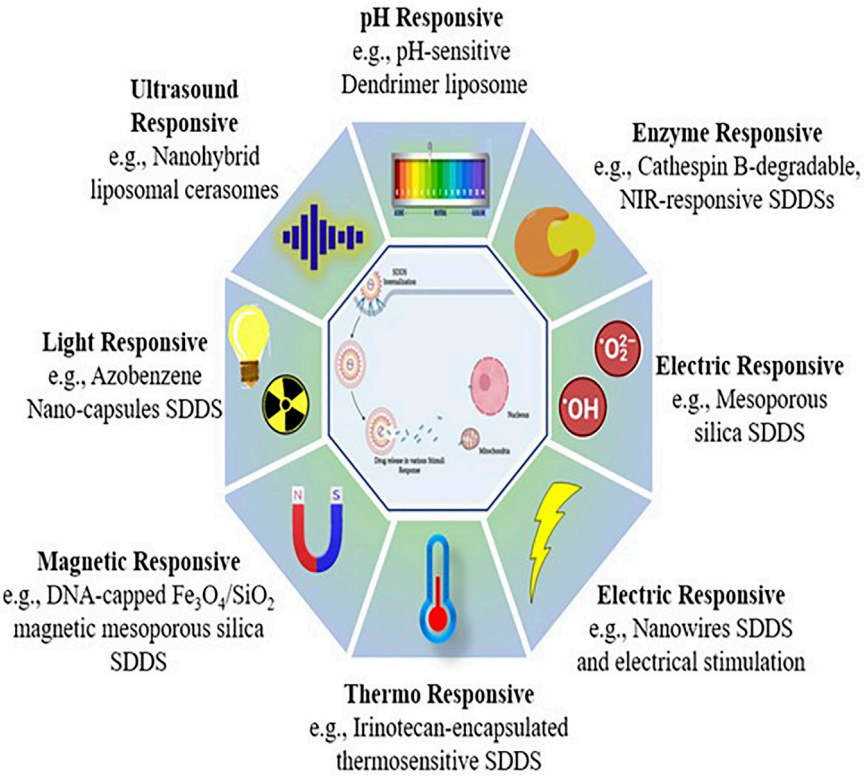


FIGURE 7
Stimuli responsive smart drug delivery.

known as open-loop systems. Magnets, temperature, ultrasound, electric effects, and in these systems, external triggers were employed to deliver the drug (Wen et al., 2018).

2. Endogenous stimuli-responsive SDDSs (Close-loop system): These are also known as self-regulating or responsive medication delivery systems. pH, enzyme-responsive drug delivery systems, and other internal triggers like redox-responsive drug delivery systems, *etc.*, controls the drug release from a closed loop control system (Wen et al., 2018). Drug release needs structural changes across the carrier or in specific layers or channels due to the fact that stimulation, according to SDDS (Mi, 2020). There are two types of stimuli: exogenous and endogenous. The utilisation of endogenous cues such as pH, glutathione (GSH), and certain enzymes allows for non-invasive, spatiotemporally regulated medication delivery (Mousavi et al., 2020). Different stimuli-based energy sources (light, ultrasonic, magnetic) that efficiently trigger drug release from nano cargos for effective delivery to specific locations (Table 1).

4.1 pH responsive stimulus

pH is one of the most commonly used triggers for drug release because of the significant pH difference seen at the cellular level between the cytosol (7.4), the Golgi apparatus (6.40), the endosome (5.5–6.0), and the lysosome (4.5–5.5) of cancer cells and in the tumour microenvironment (Mi, 2020). In general, the pH of cytoplasm, blood, and normal tissues is around 7.0 to 7.4, while endosomal/lysosomal organelles have a pH of 6 to 4, and the tumour microenvironment has a pH of 6.5–6.8 (Mi, 2020). The use of polymers with weak acids (e.g., carboxylic acid) or bases (e.g., primary and tertiary amines) groups is used to create pH-responsive systems that produce rapid changes in ionisation at the appropriate pH. The pH responsiveness of the polymer may be readily tweaked by changing the type of the co-monomers employed to make it (Darvin et al., 2019). A pH-responsive medication delivery system may be created by hydrazone bonding an anticancer agent to carriers or targeting ligands. A medication delivery system like this reacts to acidity inside tumour cells and releases drugs in a regulated manner. Following this technique, Du et al. developed PCC-Hyd-DOXDA, a custom-made dual pH-triggered polymer drug attached system. PCC-Hyd-DOX-DA has been found to be easily absorbed by MDA-MB-231 tumour cells at pH 6.8, whereas absorption at pH 7.4 is negligible (Du et al., 2011). The polyacidic pH-responsive system includes polyacrylic acid (PAAc) and polymethacrylic acid (PMAAc) (Chen et al., 2016a). The invention of a pH-triggered auto-fluorescent polymeric nanoplatform for the delivery of non-fluorescent aromatic nitrogen mustard chlorambucil (CBL) to cancer tumours was reported by Saha et al. (Saha et al., 2019). In another study, Zhang and others incorporated doxorubicin and dextran with a hydrazone linker, targeting hepatocytes with folate (Zhang et al., 2015). While pH is widely utilised in smart medicine administration, it should be combined with other stimuli such as temperature or redox to achieve extremely exact and precise release at the target locations. The use of acidic pH as a tumour microenvironment trigger has certain drawbacks. To begin with, the acid pH in perivascular areas is often remote from the blood flow, resulting in a lack of reaction of nanoparticles. In addition, pH changes in healthy tissues and malignant tissues are frequently similar (Pan et al., 2012; Cheng R. et al., 2013). For regulated release of doxorubicin, Nikravan et al. created a pH sensitive cross-linked nanoparticle system generated from various molar ratios of poly (acrylic acid) (PAA) and ethylene glycol dimethacrylate. With increasing cross-linking degrees, the pH-responsive behaviour of this nanocarrier was less effective. At pH levels of 1.2, 5.3, and 7.4, the release of the model drug doxorubicin was investigated (Dhanasekaran, 2015).

4.2 Redox responsive stimulus

The redox-sensitive drug delivery system has sparked a lot of attention in the field of therapeutic strategies, because of its close ties to a variety of diseases, and it is being investigated a lot (Mura et al., 2013). Additionally, the redox-sensitive delivery system has the benefit of drug release within the cancer cell. Redox hemostasis is a crucial process for cell survival that involves glycolysis, glutathione synthesis, fatty acid oxidation, and glutaminolysis (Panieri and Santoro, 2016). However, in tumour cells, dysregulated redox hemostasis resulted in a shift in redox balance and an increase in ROS levels. An increase in ROS levels was caused by mitochondrial dysfunction, overexpression of NADPH oxidases, and changes in antioxidant enzymes (Arcucci et al., 2016). The redox potential in microenvironments tends to vary depending on the tissue, which may be exploited to develop redox-responsive delivery systems. The reducing environment of tumour cells is largely determined by NADPH/NADP+ and glutathione (GSH, GSH/GSSG), both of which have different reduction potentials and capacities (Wu et al., 2004). GSH levels differ between normal and cancerous cells. It ranges from 2 to 20 μM in blood and normal extracellular matrices, whereas it ranges from 2 to 10 mM in cancer cells which is 100- to 500- fold higher than the normal ranges (Liu et al., 2016). To produce redox-responsive carriers, the disulfide bond has been proven to be the major redox-sensitive linker (Liu et al., 2017). GSH levels in intracellular compartments (cytosol, mitochondria, and nucleus) are two to three orders of magnitude greater (2–10 mM) than in external fluids (2–20 mM). As a result, GSH is a well-known intracellular molecule that may be used to induce drug release within cells (Indermun et al., 2018). Many studies on the redox responsiveness of disulfide bonds are currently in progress, and diselenide bonds are also getting a lot of attention as well. Diselenide redox-sensitive bonds delivery systems are similarly

TABLE 1 Examples of Stimuli-Sensitive various smart-carriers developed for SDDS.

Stimulus	SDDSs	Smart Carrier/ Ligand	Target	Drug Payload	Cancer	*In vitro* Cell lines	*In vivo* tumor Model	Ref
pH	Dox-loaded RGD-modified GQDs (Dox-RGD-GQDs)	RGD	$\alpha_v\beta_3$ integrins receptors	Doxorubicin	Prostate cancer	DU-145, PC-3, and MC3T3-E1 cell lines	****	Qiu et al. (2015)
	HA/α-TOS@ZIF-8 nanoplatform	Hyaluronic acid	CD44 receptors	D-αTocopherol succinate (α-TOS)	Cervical cancer	HeLa cell line	Kunming mice	Sun et al. (2019)
	ATRAM-BSA-PLGA NPs	ATRAM	Membrane surface	Doxorubicin	Breast cancer, Cervical cancer, and human pancreatic carcinoma	MCF-7, HeLa, MIA PaCa-2 cells and mouse neuroblastoma Neuro-2a cell lines	Female C3H/HeJ mice	Palanikumar et al. (2020)
	TfR ligand (7pep; amino-acid sequence: HAIYPRH) conjugated micelle	TfR ligand (7pep; amino-acid sequence: HAIYPRH)	Transferrin receptors	Doxorubicin	Breast cancer	MCF-7/Adr cell line	Nude mice bearing drug-resistant MCF-7 xenografts (MCF-7/Adr)	Gao et al. (2017)
	DHA-GO-Tf	Transferrin	Transferrin receptors	Dihydroartemisinin	Breast cancer	Murine mammary tumor EMT6 Cell line	Balb/c female mice	Liu et al. (2015)
	Tri-Dox-FA-A-NPs	Folic acid and the AS1411 aptamer	Folate receptor and nucleolin receptor	Doxorubicin	Breast and pancreatic cancer	MCF-7, PANC-1 and L929 cell lines	****	Lale et al. (2014)
	D-Biotin/DOX-loaded mPEG-OAL/ N-CQDs	D-Biotin	Biotin receptor	Doxorubicin	Cervical cancer	Hela cell line	****	Bao et al. (2019)
	FA-BSA-CAD	Folic acid	Folate receptor	Doxorubicin	Breast cancer, Hepatic cancer, and Lung cancer	MDA-MB-231, MCF-7, Bel-7402, HELF cancer cell lines	Kunming mice	Du et al. (2013)
	IgG1 (XE114)-vc-MMAE—ADC (+)	IgG1 (XE114) Monoclonal antibody	Carbonic Anhydrase IX (CAIX)antigen	Monomethyl Auristatin E	Human renal cell carcinoma	SKRC-52 cell lines	Female BALB/c nu/ nu mice	Cazzamalli et al. (2018)
	AAZ- CA-IX-vc-MMAE SMDC	CAIX ligand	Carbonic Anhydrase IX (CAIX)	Monomethyl Auristatin E	Human renal cell carcinoma	SKRC-52 cell lines	Female BALB/c nu/ nu mice	Cazzamalli et al. (2018)
	EC2220	Folic acid	Folate receptor	Vinca alkaloid	Squamous cell carcinoma, Lung cancer and Breast cancer	KB M109, and 4T1 cell lines	Female BALB/c nu/ nu mice	Leamon et al. (2006)
Redox	DOX-loaded HPAEG-AS1411 nanoparticles	Aptamer AS1411	Nucleolin receptor	Doxorubicin	Breast cancer	L929, MCF-7 cell lines	****	Zhuang et al. (2016)
	DOX-loaded star-PECLss-FA	Folic acid	Folate receptor	Doxorubicin	Breast cancer and Cervical cancer	HeLa, 4T1 cell lines	Female BALB/c mice	Shi et al. (2014)
	DOX@MSNs-S-S-Tf	Transferrin	Transferrin receptors	Doxorubicin	Hepatic cancer	Huh7 cell line	****	Chen et al. (2017)

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10.3389/fchem.2022.1095598

TABLE 1 (*Continued*) Examples of Stimuli-Sensitive various smart-carriers developed for SDDS.

Stimulus	SDDSs	Smart Carrier/ Ligand	Target	Drug Payload	Cancer	*In vitro* Cell lines	*In vivo* tumor Model	Ref
	DOX@MSN-ss-GHA	Hyaluronic acid	CD44 receptor	Doxorubicin	Breast cancer and Cervical cancer	4T1 and HUVEC cell lines	female Balb/c mice	Chen et al. (2016b)
	Folate-Vinca Alkaloid Conjugate (EC145)	Folic acid	Folate receptor	Vinca alkaloid	Human nasopharyngeal carcinoma	KB, 4T1 cell lines	female nu/nu mice and female BALB/c mice	(Vlahov et al., 2006; Reddy et al., 2007)
	DOX@MSN-S-S-RGD	RGD	$\alpha_v\beta_3$ integrins receptors	Doxorubicin	glioblastoma	U87 MG cell lines	****	Li et al. (2015)
	HA9.5-ss-PTX	Hyaluronic acid	CD44 receptor	Paclitaxel	Breast cancer and Skin cancer	MCF-7, B16F10 and VERO cell lines	Male BALB/c nude mice	Yin et al. (2015)
	Inotuzumab ozogamicin	Anti-CD22 mAb (G544, IgG4 isotype)	CD22 antigen	Calicheamicin	B-cell malignancy	Ramos (CRL-1923), Raji (CCL-86), Daudi (CCL-213), RL (CRL-2261), and HL-60 (CCL-240) cell lines	Female, athymic BALB/c nu/nu (nude) mice	DiJoseph et al. (2004)
	DOX@MSNs-CAIX	Anti-carbonic anhydrase IX antibody (A-CAIX Ab)	Carbonic Anhydrase IX (CAIX)antigen	Doxorubicin	Breast cancer	4T1-Luc (Luciferase), Mef cells (mouse embryo fibroblast) cell lines	BALB/C mice	Chen et al. (2020a)
Enzyme	PTX-loaded PEG-GPLGVRGDG-PDLLA nanoparticle	RGD	$\alpha_v\beta_3$ integrins receptors	Paclitaxel	Breast cancer	4T1 cell line	Female CD-1 (ICR) mice	Ke et al. (2017)
	MSNs-Peptide-BSA-LA@DOX	Lactobionic acid	asialoglycoprotein receptor (ASGP-R)	Doxorubicin	Hepatocellular carcinoma	BEL7402 cell lines	Balb/c mice	Bansal et al. (2016)
	Ac-La-G (4)-PAMAM-FITC dendrimer loaded with sorafenib	Lactobionic acid	asialoglycoprotein receptor (ASGP-R)	Sorafenib	Hepatocellular carcinoma	HepG-2 and HLE cell lines	****	Iacobazzi et al. (2017)
	FA-GFLG-SN38	Folic acid	Folate receptor	SN38	Cervical cancer, Lung cancer and liver cancer	HeLa, Siha	****	Jin et al. (2020)
						A549, and SK-Hep-1 cell lines		
	FA-GFLG-MMC	Folic acid	Folate receptor	Mitomycin C (MMC)	Cervical cancer and Lung cancer	HeLa, SiHa, PC9, A549, and 16HBE cell lines	****	Xu et al. (2020)
	FA-conjugated CDDP-loaded Mal-PEG-b-PLG-FITC vesicles	Folic acid	Folate receptor	cisplatin (CDDP)	Cervical cancer	HeLa and NIH-3T3 cell lines	****	Shirbin et al. (2015)
	Hyaluronic acid coating caspase 3 loaded drug nanoparticles	Hyaluronic acid	CD44 receptor	Paclitaxel	Breast cancer	4T1 and MCF-7 cell lines	MCF-7 tumor-bearing Balb/C nude mice	Xin et al. (2018)
	PEGylated lysine peptide dendrimer-gemcitabine conjugate	****	****	Gemcitabine	Breast cancer	4T1 and COS-7 cell lines	Female BALB/C mice	Zhang et al. (2017)
		CycloRGD	$\alpha_v\beta_3$ integrins receptors	Gemcitabine	Pancreatic cancer	BxPC-3 cell lines		Han et al. (2017)

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10.3389/fchem.2022.1095598

TABLE 1 (*Continued*) Examples of Stimuli-Sensitive various smart-carriers developed for SDDS.

Stimulus	SDDSs	Smart Carrier/ Ligand	Target	Drug Payload	Cancer	*In vitro* Cell lines	*In vivo* tumor Model	Ref
	Gemcitabine (GEM) nanovectors (RGD-GEM-GELG- CdSe/ZnS)						BxPC-3 xenograft models in nude mice	
	Folate bound poly (ethylene glycol)-distearoylphosphatidylethanolamine (FA-PEG-DSPE)	Folic acid	Folate receptor	Paclitaxel	Breast cancer	MDA-MB-231, MDA-MB-468, BT-20, and T47-D cell lines	Female athymic nude mice	Satsangi et al. (2015)
Light	Photocaged folate nanoconjugates	Folic acid	Folate receptor	Paclitaxel	Cervical cancer	KB cell lines	****	Fan et al. (2012)
	FA adsorbed $PC_{12}NB$ polymersomes ($PC_{12}NB$ + DOX + FA + hn)	Folic acid	Folate receptor	Doxorubicin	Cervical cancer	HeLa cell lines	****	Zhou et al. (2020)
	Folate-targeted gold nanorods (AuNRs@ PHEA-EDA-FA)	Folic acid	Folate receptor	Nutlin	Human osteosarcoma and Lung Cancer	U2OS, 16HBE and HDFa cell lines	****	Li Volsi et al. (2017)
	HMS/C18/PRMS-FA	Folic acid	Folate receptor	Doxorubicin	Cervical cancer and Lung Cancer	KB and A549 cell lines	****	Xing et al. (2014)
	AuNPs with the folate PEG-SH and PSS (Au@folate-PEG-PSS)	Folic acid	Folate receptor	Doxorubicin	Breast cancer	MCF-7 and MDA-MB-231 cell lines	****	Banu et al. (2015)
	GNR-embedded Diblock copolymer [PEG-bpoly (2-hydroxyethyl	Folic acid	Folate receptor	GW627368X	Cervical cancer	SiHa, ME180, HaCat, and 3T3 cell lines	S180 bearing Swiss albino mice	Parida et al. (2017)
	acrylate)–lipoic acid–folic							
	acid] micelles							
	Dopamine-adipic acid dihydrazide-hyaluronic	Folic acid/Hyaluronic acid	Folate/CD44 receptor	Doxorubicin	Breast cancer	MCF-7 cell lines	female BALB/c nude mice	Xu et al. (2017b)
	acid trifuncitionalized gold							
	nanorod (GNRs-HA-FA-DOX)							
	DOX-EGCG/DPA-FA NPs	Folic acid	Folate receptor	Doxorubicin	Breast cancer	4T1 cell line	4T1 tumor-bearing BALB/c mouse model	Fan et al. (2021)
	DOX-MUCNP@C18@PSMN-FA	Folic acid	Folate receptor	Doxorubicin	Cervical cancer and Lung cancer	KB, A549 and Beas2B cell lines	KB tumor bearing nude mice	Xing et al. (2015)
	PDA-RGDC/DOX	Arginine glycine-aspartic-cysteine acid (RGDC) peptide	$\alpha_v\beta_3$ integrins receptors	Doxorubicin	Cervical cancer	HeLa cell line	HeLa tumor-bearing BALB/c mouse model	Li et al. (2017a)
	Biotin-PEG-GNR-DNA/DOX (BPGDD)	Biotin	Biotin receptors	Doxorubicin	Breast cancer	MCF-7 and MCF-7/ADR cell lines	****	Zhang et al. (2016)
	PB@PDA@PEG-FA-DOX	Folic acid	Folate receptor	Doxorubicin	Cervical cancer	HeLa and HL-7702 cell lines	Hela tumor-bearing nude mice	Lin et al. (2019)

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10.3389/fchem.2022.1095598

TABLE 1 (*Continued*) Examples of Stimuli-Sensitive various smart-carriers developed for SDDS.

Stimulus	SDDSs	Smart Carrier/ Ligand	Target	Drug Payload	Cancer	*In vitro* Cell lines	*In vivo* tumor Model	Ref
Ultrasound	Paclitaxel-liposome–microbubble complexes (PLMC)	Biotin	Biotin receptors	Paclitaxel	Breast cancer	4T1 cell lines	4T1 tumor-bearing female BALB/c mice model	Yan et al. (2013)
	Paclitaxel loaded hyaluronic acid targeted liposome (HA-Lipo/PTX)	Hyaluronic acid	CD44 receptor	Paclitaxel	Breast cancer	4T1 and T47D cell lines	4T1 tumor-bearing female BALB/c mice model	Ravar et al. (2016)
	Microbubble-liposome complex (IRMB-OxLipo)	Biotin	Biotin receptors	FOLFIRINOX (Irinotecan and Oxaliplatin)	Pancreatic cancer	Panc-01 3D spheroid	BxPC-3 human xenograft murine models	Gao et al. (2020)
	PTX@FA--CD/H-MSN (DESN)	Folic acid	Folate receptor	Paclitaxel	Breast cancer	4T1 cell lines	4T1 tumor-bearing female BALB/c nude mice model	Wang et al. (2018)
	A10-3.2/siCAT-1/3WJ-NDs	A10-3.2 aptamer	Prostate specific membrane antigen (PSMA)	siCAT-1 (siRNA)	Prostate cancer	22RV1, PC-3 and 16HBE	****	Guo et al. (2022)
	Span–PEG with FA–CNT–PTX	Folic acid	Folate receptor	Paclitaxel	Breast cancer	MCF-7 cell lines	MCF-7 tumor-bearing mice model	Zhang et al. (2019)
	TRAIL-Dox-Nanoshards	Tumor necrosis factor-related apoptosis inducing ligand (TRAIL)	TRAIL–receptor	Doxorubicin	Breast cancer	MDA-MB-231, TRAIL-resistant MCF7 and MCF-12A	****	Jablonowski et al. (2018)
	ALN/FA-decorated PTX-loaded nanoparticles	Folic acid	Folate receptor	Paclitaxel	Breast cancer	4T1 cell lines	4T1 tumor-bearing female BALB/c nude mice model	Chen et al. (2020b)
	ANP-D/P	Angiopep-2	Lipoprotein receptor-related protein (LRP)	Doxorubicin	Glioblastoma	U87 MG and BCEC cell lines	U87 MG tumor-bearing female BALB/c nude mice model	Luo et al. (2017)
	LHRH-ELP-DOX	LHRH	Luteinizing hormone releasing hormone (LHRH) receptor	Doxorubicin	Breast cancer	MCF-7 and MCF-7/ADR cell lines	MCF-7/ADR tumor-bearing female BALB/c nude mice model	Wang et al. (2017)
Magnetic	DOX-FA-MN-MWCNTs	Folic acid	Folate receptor	Doxorubicin	Glioblastoma	U87 cell lines	****	Lu et al. (2012)
	Fe3O4@OCMC@IRMOF-3/FA	Folic acid	Folate receptor	Doxorubicin	Cervical cancer	HeLa cell line	****	Chowdhuri et al. (2016)
	DOX–SPION– (P(NIPAAm-coAAm)-b-PCL) micelles	Integrin β4 antibody	A9 antigen	Doxorubicin	Head and Neck cancer	SQ20B cell line	****	Kim et al. (2013)

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10.3389/fchem.2022.1095598

TABLE 1 (*Continued*) Examples of Stimuli-Sensitive various smart-carriers developed for SDDS.

Stimulus	SDDSs	Smart Carrier/ Ligand	Target	Drug Payload	Cancer	*In vitro* Cell lines	*In vivo* tumor Model	Ref
	MSCN-PEG-HB5/DOX	HB5 aptamer	HER2 receptor	Doxorubicin	Breast cancer	SK-BR-3 cell lines	SK-BR-3 tumor-bearing female BALB/c nude mice model	Wang et al. (2015)
	MagO2MB-RB-Gem conjugate	Biotin	Biotin receptors	Gemcitabine	Pancreatic cancer	BxPC-3 and Mia-PaCa-2 cell lines	Xenograft ectopic BxPC-3 tumours in SCID mice	Beguin et al. (2020)
	HER2-paclitaxel-GMO-MNPs	HER2 antibody	HER2 receptor	Paclitaxel	Breast cancer	MCF-7	****	Dilnawaz et al. (2010)
	Dox loaded-CD105-conjugated SWCNTs	Mouse Endoglin/ CD105 mab	Endoglin/CD105	Doxorubicin	Breast cancer	4T1-Luc2 cell line	4T1-Luc2 tumor-bearing female BALB/c mice	Al Faraj et al. (2015)
	Casein-CFNP-CNA-BT	Biotin	Biotin receptors	Cinnamaldehyde	Lung cancer	L929 and A549 cell lines	****	Purushothaman et al. (2021)
	DGNP Loaded and Folate	Folic acid	Folate receptor	Doxorubicin	ovarian	A2780, OVCAR3 and SKOV3 cell lines	CD-1 female nude mice	Ak et al. (2018)
	Attached Erythrocyte							
	Vesicles (FVzDGNP)							
	PFH/DOX@PLGA/Fe3O4-FA	Folic acid	Folate receptor	Doxorubicin	liver cancer	Bel-7402 cells, SKOV-3 cells, and MB-231 cell lines	Bel-7402 tumor-bearing female nude mice	Tang et al. (2018)

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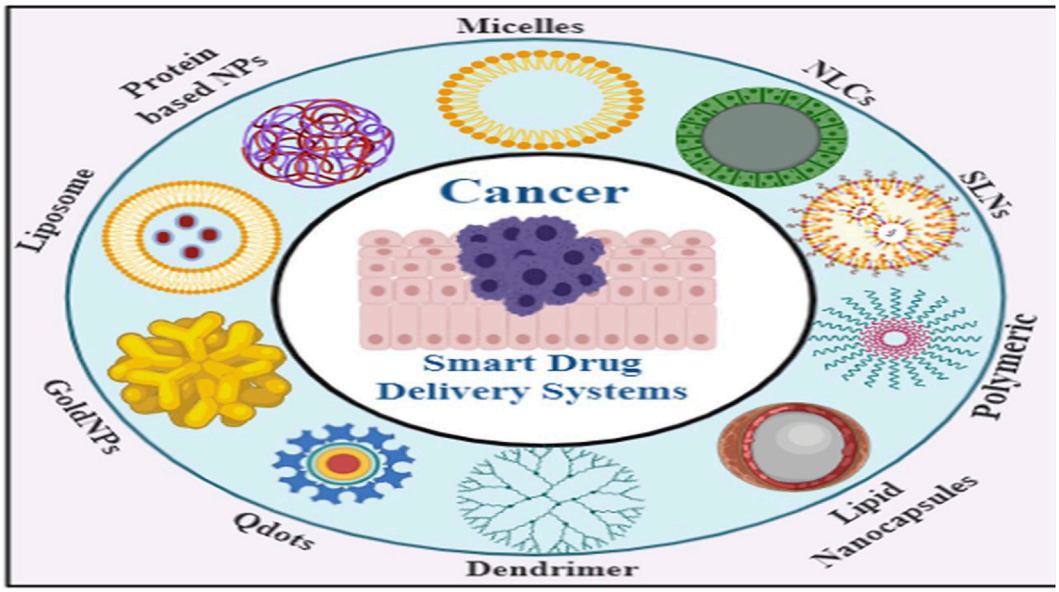

FIGURE 8
Smart nanocarriers used in SDDSs.

sensitive to reduction and have characteristics similar to disulfide connections. Diselenide bonds can be used to create a more sensitive redox-responsive delivery system in tumour therapy because their bond energies are lower than S–S bonds (Se–Se 172 kJ/mol; C–Se 244 kJ/mol; S–S 268 kJ/mol) (Guo et al., 2018). Gang Cheng et al. synthesised the polycationic carrier OEI_{800}-$SeSe_x$ by adding the active ester containing diselenide bonds to the branched oligoethyleneimine 800 Da (OEI_{800}) (Cheng et al., 2012). The ability of SDDSs to respond to reactive oxygen or nitrogen species (ROS or RNS) is still barely explored (Alvarez-Lorenzo and Concheiro, 2014). The main contributors to the intra- and extracellular redox potential associated with stress conditions, signalling cascades, diabetes, hypertension, atherosclerosis, or cancer are ROS such as hydrogen peroxide, superoxide, or OH radicals (Lallana and Tirelli, 2013). Oxidation-responsive SDDSs are a subset of redox-sensitive drug delivery systems that rely on reactive oxygen species (Torres et al.), primarily H_2O_2 and OH radicals (Darvin et al., 2019). A redox-sensitive polymeric nanoparticle for tumor-targeted medication delivery was described by Cho et al. The paclitaxel-incorporated nanoparticle was prepared using a redox-responsive biodegradable polymer that was capable of delivering paclitaxel in response to a reduction process (Das et al., 2020).

4.3 Enzyme responsive stimulus

Due of its distinct benefits, such as substrate, specificity and excellent selectivity under moderate circumstances, enzymes employed as triggers in the construction of SDDSs have been a growing topic in recent years (Liu et al., 2016). Many enzymes have been put to work to enhance medication transport to cancer cells, including lipase, protease, trypsin, glycosidase, phospholipase, oxidoreductase, and others (De La Rica et al., 2012). The drugs will be released at the target locations by site-specific enzymatic cleavage by smart carriers/ligands bearing drug payload linked/conjugated to them *via* encapsulation or covalent bonding. The drug-release mechanism is triggered by several enzymes (Darvin et al., 2019). Proteases which degrade protein and peptides, a fantastic alternative for releasing medicines from liposomes (Hossen et al., 2019). Radhakrishnan et al. developed hollow nanocarriers triggered by the trypsin/hyaluronidase enzyme to deliver anticancer agents intracellularly (Radhakrishnan et al., 2014).

The phospholipase A2 (PLA2) enzyme is used to release medicines or expose target ligands from SDDSs that use liposomes or small unilamellar vesicles (SUVs) (Sanchez-Moreno et al., 2018). With the presence of Cathepsin B, an intracellular cysteine protease that was particularly overexpressed in tumour locations, the H-Phe-Lys-OH peptide could be broken. Hollow nanocarriers activated by the trypsin/hyaluronidase enzyme to deliver anticancer drugs intracellularly. MMPs (matrix metalloproteases) are a zinc-dependent family of endopeptidases that are well-known for their role in cancer prognosis (Liu et al., 2017) and have been extensively studied for drug delivery and imaging applications (Kessenbrock et al., 2010). Zhu et al. Developed MMP2-sensitive; PEG lipid

conjugated liposomes with anti-nucleosome monoclonal antibodies modified on their surface to improve cancer targeting (Zhu et al., 2012). In another study, Chen et al. manufactured multifunctional poly (ethylene glycol)- blocked-poly (L-lysine) Biotin 6-maleimido-caproic acid (Biotin-PEG-b-PLL (Mal)-peptide) polymeric micelles enclosing doxorubicin to improve cancer cell uptake by endocytosis (Chen WH. et al., 2015). Despite its utility, enzyme responsive SDDSs lacks precise control over the system's initial response time.

4.4 Light responsive stimulus

Light-responsive SDDSs have received much interest as a way to take advantage of either daily and seasonal exposure to natural solar irradiation or artificial sources of electromagnetic radiation with very specific wavelengths between 2500nm and 380 nm (Alvarez-Lorenzo and Concheiro, 2014). These photo-responsive drug delivery systems offer many advantages over other stimuli-responsive formulations for drug delivery because photochemical processes do not require additional reagents or catalysts, and the majority of by-products, if any, are harmless (Pan et al., 2021). Photosensitive carriers light-responsive smart drug delivery devices have an on/off drug release mechanism in response to irradiation stimulation. A photosensitive biomaterial is generally conjugated or encapsulated to a therapeutic agent in such SDDSs. The photosensitive material absorbs light (photons), which causes a conformational change in these smart-carriers, dramatically altering their structure and allowing the encapsulated/conjugated agent to be released at the desired site in a spatio-temporal controlled manner (Sanchis et al., 2019; Pan et al., 2021). UV and visible light can cause medication release from formulations that are applied to the skin or circulate through blood vessels near the body's surface (e.g., eye structures). Drug release is usually initiated by reversible or irreversible photo-induced structural changes in smart-carriers. Photo-cleavable bonds can be used to conjugate medicines for light sensitive release (Alvarez-Lorenzo and Concheiro, 2014). For example, doxorubicin-encapsulated poly (lactic-co-glycolic acid) (PLGA)matrix particles with a gold over-layer, NIR-triggered release was observed. When cancer cells were exposed to NIR light, doxorubicin was released abruptly, resulting in high cancer cell toxicity and tissue ablation (Zhu et al., 2012). Specifically, carbon nanotubes and graphene nanoparticles (GNPs) are excellent candidates for light-triggered stimuli, in particular, the near-infrared (Prasanna et al.) range (Hossen et al., 2019). In order to destroy cancer cells, metallic nanocarriers are capable of absorbing light and convert it to heat (Alvarez-Lorenzo and Concheiro, 2014).

Azobenzene and its derivative-based nanocarriers are frequently utilised to regulate drug delivery at its target by using ultraviolet–visible light and/or visible light to facilitate structural change and drug release (Yan et al., 2012). By encapsulating doxorubicin and ammonium bicarbonate inside nanocarriers, Chen et al. created a bubble-generating thermo-responsive liposomal system. Ammonium bicarbonate decomposes at high temperatures, releasing carbon dioxide bubbles that generate permeable holes in the lipid bilayer of liposomal nanocarriers, allowing the loaded medication doxorubicin to be released quickly (Chen et al., 2013).

4.5 Ultrasound responsive stimulus

Ultrasound is a type of high-frequency sound wave that may have an impact on carriers used for controlled drug release at diseased sites (i.e., tumors). The ultrasound intensity could be adjusted for various applications. At low ultrasound frequencies (less than 20 kHz), it could be used for imaging, as well as disrupting smart-carriers to release cargos or increasing the permeability of cancer cell membranes at high ultrasound frequencies (greater than 20 kHz) (Mi, 2020). Ultrasound has become quite popular as a stimulus in clinical investigations because to its various benefits, including intrinsic tissue penetration, improved spatiotemporal control, and increased safety. Ultrasound has recently been popular in clinics as a diagnostic and therapeutic technique (Mi, 2020). The invention of nanocarriers with ultrasonic sensitivities for ultrasonography has expanded ultrasound procedures to become a unique and successful tool for capturing drug carriers and triggering drug release at the target locations by adjusting the ultrasound frequency, duty cycles, and exposure duration (Liu et al., 2016). Kruskal et al. used a nanocarrier-DOX-encapsulated delivery technique, followed by ultrasonic tumour irradiation, to accomplish tumour targeting, resulting in the drug's systemic distribution. Wang et al. created amphiphile segments with ultrasound-sensitive oxyl-alkylhydroxylamine(-oa) linkages. To improve medication transport to hepatocellular carcinoma cells, hydrophobic DOX was encased between the hydrophobic amphiphile portion (Mi, 2020). Jung et al. created dual-functional Gd(III)-DOTA-modified sono-sensitive liposomes for doxorubicin administration and magnetic resonance imaging acquisition (Jung et al., 2012). In the realm of cancer treatment, ultrasonic therapy has been utilised in combination with micelles. Husseini et al. (Bulbake et al., 2017) examined the release of doxorubicin from Pluronic P105 micelles at various ultrasonic frequencies.

4.6 Magnetic responsive stimulus

Magnetic-responsive drug delivery systems offer a non-invasive method of controlling the carriers' spatiotemporal proximity to their targets. The use of magnetic particles for the delivery of anti-cancer drugs or antibodies to organs or tissues altered by disease has become an active and appealing

field of research since the pioneering idea proposed by Freeman et al. (Freeman et al., 1960) that fine iron particles could be transported through the vascular system and concentrated at a particular point in the body with the aid of a magnetic field (Estelrich et al., 2015). This aids the device in releasing payloads under programmed external magnetic field exposure. MNPs (magnetic nanoparticles) have an abundance of active sites for bio molecule conjugation, allowing for accurate design and engineering to achieve their intended smart functions by applying a localised external magnetic field, such as long-term circulation in the bloodstream, target specificity to lesion tissues, and therapeutic delivery (Darvin et al., 2019). The most widely used core/shell magnetic nanoparticle has a wide range of magnetic properties. The drug is combined with a pharmaceutically stable ferromagnetic carrier in this complex. There are a number of ways to create magnetic-responsive systems, such using nanoparticles, or magneto-liposomes (Madaan et al., 2014). Jiang et al. created magnetically tunable BSA (Fe_3O_4/BSA) particles coated with negatively charged iron oxide nanoparticles. The release of these particles from bone marrow mesenchymal stem cells, where they were internalised with the help of an external magnetic field, was delayed (Freeman et al., 1960). Li et al. created a magneto-thermally responsive nanocarrier/doxorubicin (MTRN/Dox) using Mn-Zn containing ferrite magnetic nanoparticles (MZF-MNPs) to form a thermosensitive copolymer coating with absorbed chemotherapeutic combined with the magnetothermal effect of MZF-MNPs to allow controlled drug release at the tumour site under an alternating magnetic field (AMF) (Li et al., 2018). When compared to free Dox and MTRN/Dox treatment without the use of an AMF, the authors found that magnetic targeting of MTRN/Dox increased accumulation in tumour tissues and that AMF treatment was required for MTRN/Dox increased cytotoxicity. The MTRN/Dox with combined magnetic targeting and AMF treatment showed the greatest tumour volume reduction compared to the MTRN/Dox with only magnetic targeting or AMF treatments after injection into nude mice bearing tumours, indicating that it has potential as a liver cancer therapy. Fang, Xiuqi et al. developed a highly controllable process of Carbon Encapsulated Magnetic Nanoparticles (CEMNs) synthesis in arc discharge plasma. With an external magnetic field, CEMNs have been made more controllable with respect to both their size distribution and purity and with an external magnetic field, CEMNs have been made more controllable with respect to both their size distribution and purity. For the purpose of assessing the potential for CEMNs to be used in biomedicine, the human breast cancer cell line MDA-MB-231 was used to determine the cytotoxicity of CEMNs. Based on this finding, it is concluded that specific CEMN dosages can be utilized in biomedical settings such as MRI, cell migration control, hyperthermia, and medication administration (Fang et al., 2018).

5 Smart drug delivery systems using smart nano-carriers in cancer therapy

Nanotechnology is a cutting-edge, innovative, and promising method of delivering a drug payload to tumour tissue. Nanoparticles (NPs), which range in size from 1 to 100 nm, can reveal both physical and chemical properties; are more likely to be accumulated in solid tumors by passively extravasation from the hyperpermeable tumor blood vasculature (Cabral et al., 2011). Nanoparticle delivery systems are broadly evaluated preclinically with other nanoparticle-constructed formulations and technologies that have been used so far in the clinic setup (Peer et al., 2007; Shi et al., 2010; Wicki et al., 2015; Navya et al., 2018; Murugan et al., 2021). There are two types of therapeutic and diagnostic nanoparticles: [a] inorganic nanoparticles (such as gold, silica, and iron oxide) and [b] organic nanoparticles (e.g., polymeric, liposomes, and micelles) (Figure 8) (Murugan et al., 2021). Conventional nanocarriers are unable to transport and release drugs in the desired concentration at the targeted site when stimulated externally or internally. They must be improvised or functionalized to make them smart (Lee et al., 2015). The following qualities should be present in smart nanocarriers. To begin, smart nanocarriers should avoid the immune system's cleaning process. Second, they should only be gathered at the targeted site. Third, upon external or internal stimulation, the intelligent nanocarrier should release the cargo at the correct focusing on the targeting site (Hossen et al., 2019). Finally, they must supply chemotherapeutics as well as other things for example, genetic materials and imaging agents, and other similar compounds (Peer et al., 2007; Lee et al., 2015; Liu et al., 2016). Depending on the type and application of conventional nanocarriers, there are a few methods for transforming them to smart nanocarriers (Bhatia, 2016; Sur et al., 2019; Sirisha, 2020). First, nanocarriers must overcome a number of biological obstacles, including cleaning, making their way to the desired targeted site through the reticuloendothelial system (RES). The RES quickly removes the nanocarrier from circulation and it is then stored in the liver, spleen, or bone marrow as anti-cancer drug payload carrying nanocarriers (Nie, 2010). Second, nanocarriers may be functionalized to distinguish cancer cells from normal cells with pinpoint accuracy. Some proteins are overexpressed on surface-level of cancer cells (Kubler and Albrecht, 2018; Antignani et al., 2020). The smart primary targets are overexpressed proteins (Perez and Fernandez-Medarde, 2015). Nanocarriers are equipped with ligands that match the overexpressed proteins. Smart nanocarriers use ligands to detect cancer cells that have overly expressed receptor proteins of their surface (Sabir et al., 2021). Third, delivering the drug to the target cancerous cells does not imply that the operation is finished. The next major issue will be releasing the drug from the smart carrier while it is being stimulated. The surface of nanocarriers can be grafted with a variety of chemical

groups to make them sensitive to the stimuli system (Alvarez-Lorenzo and Concheiro, 2014). Fourth, changes are made to allow anti-cancer drugs to be delivered when combined with another material such as genetic materials (Xu et al., 2014), imaging agents (Das et al., 2020), or even more anti-cancer therapies (Bose et al., 2018).

Smart NPs materials utilized in SDDSs can be classified into Organic and inorganic NPs based on number of organic and inorganic materials have been used to fabricate them with their own distinctive architecture and attached functionalities, and they have been evaluated for effective drug delivery to tumors (Srinivasan et al., 2015). Liposomes, dendrimers, micelles, are example of organic nanoparticles and carbon nanotubes, mesoporous silica NPs (MSNs), gold/silver NPs and Quantum Dots are example of inorganic nanoparticles.

5.1 Organic nanocarriers based SDDS

5.1.1 Liposomes

Liposomes are tiny, artificially created vesicles that are completely enclosed by phospholipid bilayer membranes of varying sizes (20–10,000 nm) (Prasanna et al., 2018). Gregoriadis et al. were the first to employ liposomes as an example drug delivery device in 1971 (Gregoriadis et al., 1971). The large unilamellar liposomes (LUV) may then be produced by extrusion of multilamellar vesicles *via* polycarbonate filters, thanks to the invention of novel preparation technique (Prasanna et al., 2018). Liposomes have been widely used as advanced DDSs in numerous clinical trials, especially when the diameter of the liposome was reduced to less than 100 nm (Torchilin, 2012; Akbarzadeh et al., 2013).

The physico-chemical nature of lipids allows drug molecules to be encapsulated or intercalated into phospholipid bilayers, extending the medication's location. Liposomes have been extensively studied for the delivery of imaging and therapeutic agents in a sustained and controlled manner for cancer diagnosis and treatment, with high diagnostic and therapeutic efficiency and minimal side effects (Koren et al., 2012).

Traditional liposomes have a number of flaws, including instability, insufficient drug loading, faster drug release, and shorter blood circulation times; therefore, they are not smart (Bozzuto and Molinari, 2015). The conventional liposomes need to be Traditional liposomes have been functionalized, making them ideal for use as SDDSs in order to makes them smart for utilized as SDDSs. Liposomes, like other nanocarriers, must overcome the challenge posed by the RES. Liposomes are helped to escape the RES by PEGylation. PEGylated liposomes have a longer blood circulation time as a result (Allen and Cullis, 2013). Smart nanocarriers can distinguish between cancerous and healthy cells. To actively target the cancer site, monoclonal antibodies, antibody fragments, proteins, peptides, vitamins, carbohydrates, and glycoproteins are usually attached/conjugated on the liposome (Sapra and Allen, 2003; Ruoslahti, 2012; Sawant and Torchilin, 2012; Noble et al., 2014). Smart liposomes drug delivery systems are responsive to various external and internal stimulation, including pH change, enzyme transformation, redox reaction, light, ultrasound and microwaves (Jin et al., 2016; Lee and Thompson, 2017). As a smart drug carrier system, ThermoDox, temperature-sensitive DOX liposomes developed by the company Celsion may be the closest formulation to the clinic so far. The doxorubicin may be liberated from ThermoDox at 41.5°C by taking advantage of the dipalmitoylphosphatidylcholine (DPPC) lipid crystallisation melting point (Chen et al., 2014). A novel range of cationic liposome-based systems has also been developed by integrating different cationic lipids for targeted delivery of anionic therapies such as small interfering RNA (siRNA), antisense oligonucleotides, and aptamers etc (Yingchoncharoen et al., 2016). For example, Peddada et al. created a complex nanocarrier by combining a cationic DOTAP (1,2-dioleoyl-3-trimethylammonium-propane) liposome, an anionic copolymer, and an antisense oligonucleotide with a poly (propyl-acrylic acid) (PPAA) polymer backbone. In human ovarian cancer A2780 cells, this complex nanocarrier with grafted poly (alkylene oxides) (g-PAO) increased antisense gene silencing activity. The authors also observed increased antisense oligonucleotide delivery in ovarian tumour xenografts, demonstrating that the DOTAP/PPAA-g-PAO nanocarrier system can be used for antisense oligonucleotide delivery for gene silencing (Peddada et al., 2014). Kang et al. created a dual-targeted liposomal system that used the Pep-1 peptide as a cell penetrating peptide and folic acid as an affinity ligand for the folate receptor (FR). The authors created this dual ligand (Pep-1 and folate)-modified liposome by using a short (PEG-2000) and long (PEG-3400) polymer linker to attach both ligands to the liposomal surface. In FR-positive HeLa and FR-negative HaCaT cells, cellular uptake of various fluorescent tagged liposomes was investigated. In FR positive cells, cellular uptake was higher than in FR negative cells, indicating that this multifunctional liposomal system is suitable for FR-selective drug targeting (Kang et al., 2015).

5.1.2 Micelles

Polymer micelles are thermodynamically stable colloidal solutions formed by self-assembly of amphiphilic block copolymers (O'Reilly et al., 2006). Polymeric micelles are created using block copolymers, which are composed of two or more polymer chains with distinct hydrophilic characteristics. In an aqueous environment, these copolymers spontaneously combine into a core-shell structure. The core is made up of hydrophobic blocks, which may carry any hydrophobic medication, while the shell of hydrophilic blocks (Hibino et al., 2021). To develop therapeutic carriers, a variety of polymeric molecules have been investigated. Polymer-protein conjugates, drug-polymer conjugates, and supramolecular drug delivery systems are just a few examples.

Only a few polymers have been accepted into clinical practise out of the many that have been proposed (Sanchez-Moreno et al., 2018). Biodegradable polymers, in particular, are highly preferred due to their high bioavailability, better encapsulation, controlled release, and low toxicity. Wang et al. demonstrated that paclitaxel-loaded micelles bound specifically to an MCF-7 cell-specific phage and found the cytotoxicity of the targeted paclitaxel-loaded phage micelles was significantly higher than that of the free drug or non-targeted micelle formulations against target MCF-7 cells, but not against non-target C166 cells (Wang et al., 2010). Ke et al. created micelles containing both thioridazine (which has been proven to kill cancer stem cells) and doxorubicin, presenting a promising method for breast cancer treatment that targets cancer as well as the cancer stem cells (Ke et al., 2014). Site-specific drug delivery smart nanocarriers are sought in the field of cancer therapy, with different molecules located in the external part of the nanoparticles that favour receptor-mediated cell-internalization (Wang et al., 2014). Different types of ligands, for example, folic acid and peptides, carbohydrates, antibodies, aptamers are utilised to adorn the micelle surface in order to aggressively target cancer cells (Sutton et al., 2007). The core of the micelle can be functionalized to release the anti-cancer medication at the correct concentration. pH gradients, temperature fluctuations, ultrasound, enzymes, and oxidation are among stimuli utilised in micelle based SDDSs (Sutton et al., 2007; Hossen et al., 2019). Co-delivery strategies in cancer treatment are very important for synergetic effects using multifunctional micelles. Seo et al. described a temperature-responsive micelle-based co-delivery system capable of carrying genes and anti-cancer drugs (Seo et al., 2015).

5.1.3 Dendrimers

Dendrimers are synthetic polymers with a high degree of branching made composed of an initiator core and several layers of active terminal groups. Each layer is referred to as a generation (the core is referred to as generation zero), and it is made up of repeating units (Fischer et al., 2010). Dendrimers are great candidates for developing smart nanocarriers for biological applications due to their distinct chemical structure and ability to incorporate a large number of functional groups at spatially precise locations (Nanjwade et al., 2009). Dendrimers are versatile due to their branched structure. Furthermore, all of the surface's active groups face outward, resulting in a higher drug encapsulation rate. Several kinds of dendrimers have been reported, including poly (propylene-imine) (PPI or POPAM), polylysine dendrimer, dendritic hydrocarbon, carbon oxygen-based dendrimer, porphyrin-based dendrimer, ionic dendrimer, silicon-based dendrimers, phosphorus-based dendrimer, and Newkome dendrimer (Hossen et al., 2019).

Traditional dendrimers are cleared rapidly by the immune system and have a low uptake by cancer cells. The alternative to these limitations is to modify the dendrimer. Chemical modification, copolymerization with a linear polymer, and hybridization with other smart nanocarriers have all been suggested as ways to get around these limitations (Bugno et al., 2015). Peptides, proteins, carbohydrates, aptamers, antibodies, and other substances can be used to modify the surface of dendritic structures to actively target the cancer site (Sirisha, 2020). The surface of the dendrimer may also be changed to respond to various stimuli, such as light, heat, and pH shift protein, and enzyme transformation (Rajasekhar Reddy et al., 2012; Wang et al., 2016). The cationic character of PAMAM, among other dendrimers, makes it ideal for the transport of genetic elements. The production of PAMAM has an impact on delivery efficiency. PAMAM-based nucleic acid delivery was initially reported by Haensler and Szoka in 1993 (Madaan et al., 2014). The use of a dendritic contrast agent for tumour imaging has shown to be highly effective (Hossen et al., 2019). Researchers Zhang and Shi found a multifunctional system that may be used to target cancer treatment using G5-PAMAM dendrimers coated with folic acid and doxorubicin (Zhang et al., 2018). Kaminskas et al. investigated the use of a PEGylated polylysine dendrimer conjugated to doxorubicin to promote controlled and prolonged doxorubicin exposure of lung-resident cancers. After 2 weeks of treatment, they found a 95% reduction in lung tumour burden in rats (Kaminskas et al., 2014).

5.1.4 Polymer based

Smart polymers are extremely efficient polymers that adapt to their surroundings. Natural, semi-synthetic, or synthetic polymers are used to make polymeric NPs (Brighenti et al., 2020). Polymeric nanosystems are formed by the polymerization of numerous monomer units, and under specific conditions, they may be structured and self-assemble with a nanometric size (10–100 nm) (Joglekar and Trewyn, 2013). Drugs can be entrapped, encapsulated, or bonded to polymeric NPs in the form of a nanosphere, a nano-capsule, or a drug conjugate, depending on the production technique (Prabhu et al., 2015). Polymeric capsules may be created by conjugating targeting ligands, which boost selectivity for cancer cells and improve intracellular drug delivery while decreasing various side effects and medication toxicity (Prabhu et al., 2015). Monoclonal antibodies (mAbs) or antibody fragments, aptamers, peptides, and small compounds, such as folic acid, are widely used as targeting ligands for polymeric capsule (Avramovic et al., 2020). These ligands specifically bind to antigens or receptors overexpressed on cancer cells (Rana and Bhatnagar, 2021). The efficacy of polymeric carriers modified with targeting ligands is determined by ligand properties such as density and receptor binding affinities, which can improve receptor internalisation and drug biodistribution. A drug is chemically bonded to the polymer *via* a linker/spacer in drug-conjugates. When the drug is released at the target site, the bond drug-linker/spacer is a common breakage point. FA-PEG-b-PCL-hyd-DOX, a multifunctional polymeric-drug conjugate containing a di-block PEG-PCL copolymer linked to DOX through a labile

hydrazone bond and adorned with folic acid (FA), was developed by Guo et al. (Guo et al., 2016)]. Hu et al. created a nanoplatform with paclitaxel (PTX) encapsulated in a triblock PCL-PEG-PCL copolymer that confirmed sustained drug release and a lower cytotoxic effect when compared to free PTX injection (Hu et al., 2017). Guo et al. demonstrated the ability of the hydrophobic polymer PLGA to encapsulate the low-solubility medicine PTX in a poly (lactic-co-glycolic acid)-poly (ethylene glycol) (PLGA-PEG) nanoplatform, with longer circulation time and improved cancer inhibition confirmed when this SDDS was decorated with DNA aptamers in C6 glioma cells (Guo et al., 2011). In another study, Wang et al. found that methoxy PEG-PLGA NP co-loaded with hydrophilic DOX and hydrophobic PCT inhibited cancer development more effectively than polymeric micelles loaded with only one medication (either DOX or PCT), with the best anticancer effectiveness at a 2:1 concentration ratio (Yingchoncharoen et al., 2016). Duong et al. also developed a PEG-PLGA copolymer system for the delivery of DOX and PCT, which includes the targeting ligand folate and the TAT peptide, and which improves the cellular interaction between PEG-PLGA micelles in the kB cell line of a human oral cavity carcinoma (Duong, 2013). In essence, folate improves the drug carriers' targeting ability, whereas TAT peptide is a cell-penetrating peptide (CPP) used to modify the carrier surface. In PEG-PLGA micelles, different concentration ratios of DOX and PCT were used, and a concentration ratio of 1:0.2 was found to be more effective than a concentration ratio of 1:1 (Duong, 2013). Jin et al. recently developed a promising smart delivery system based on the cationic deblock poly (ethyleneimine)-poly (lactic acid) (PEI-PLA) copolymer, which was designed to deliver the drug PTX and siRNA in a synergistic strategy in chemo or gene therapy for non-small cell lung cancer (Jin et al., 2018). This PTX NPs formulation enhances the drug's effect by inhibiting target proteins involved in cancer cell metabolism and proliferation *via* siRNA. With high drug loading, a longer half-life in the circulation, lower toxicity, and an antiproliferative effect of PTX on A549 cells, this co-delivery system is a promising SDDS (Jin et al., 2018).

5.2 Inorganic based SDDSs

5.2.1 Carbon nanotubes (CNTs)

CNTs have attracted incredible interest in the biomedical field due both to their promising properties (such as high surface area, needle-like structure, considerable strength, flexible interaction with drug cargo, high drug loading capacity, outstanding optical and electrical features, high stability, biocompatibility, and ability to release therapeutic agents at targeted sites) and negative properties (most notably, lack of biodegradability and toxicity) (Alshehri et al., 2016; Costa et al., 2016; Singh et al., 2016; Azqhandi et al., 2017). CNTs are one-dimensional carbon allotropes with a nanostructure with a length-to-diameter ratio greater than one million that are made by rolling a thick sheet of graphene into a smooth cylinder with a diameter on the order of a nanometre (nm) (Rahamathulla et al., 2021). CNT can be fabricated in a number of ways, including rolling up a single layer of graphene sheet (single-walled CNT; SWCNT) or rolling up many layers to form concentric cylinders (multiwalled CNT; MWNT) (Rahamathulla et al., 2021). Traditional CNTs have difficulties dissolving in both aqueous and organic solvents, which makes it difficult to disperse homogeneously as compared with other nanoparticles. To make conventional CNTs smart, they must be functionalized chemically or physically (Li Z. et al., 2017). Several biological applications, including as proteins, nucleic acids, and drug transporters, have been successfully explored using CNTs that have been functional (Anzar et al., 2020). PEGylation is a critical step in increasing solubility, avoiding RES, and reducing toxicity (Kenchegowda et al., 2021). The polymer poly (N-isopropyl acrylamide) (PNIPAM) is temperature sensitive. PNIPAM could be used to modify CNTs for temperature stimulus because of their low critical stimulus temperature (LCST) (Schmaljohann, 2006). For enzyme-responsive drug release, a disulfide cross-linker based on methacrylate cysteine is used. An ionizable polymer with a pKa value of 3–10 is an ideal candidate for pH responsiveness. Weak acids and bases show a change in the ionization state upon pH variation (Schmaljohann, 2006). Researchers developed a PEGylated CNT complex loaded with paclitaxel for the treatment of breast cancer in an early study. When compared to free paclitaxel alone, the CNT-paclitaxel complex showed better treatment efficacy in a 4T1 murine breast cancer model (Liu et al., 2008). Jain et al. reported that chemical modification of CNTs by carbohydrate D-galactose can generate a novel cascade of chemical functionalization of MWCNTs (Jain et al., 2009). Galactosylated MWCNTs are utilised to deliver active ligands (like galactose) to tumour sites as a targeted drug (Jain et al., 2009). SWCNTs are more efficient in drug distribution than MWCNTs because their walls are more defined and MWCNTs have more structural flaws. CNTs have been studied as nanocarriers for medication delivery as well as biomolecules including DNA, siRNA, and others. Functionalized carbon nanotubes can be utilised as early cancer detection techniques (Hossen et al., 2019). Cheng et al. recently developed a PLGA-functionalized CNT system for delivering the proapoptotic protein caspase-3 (CP3) to bone cancer cells with reduced toxicity (Cheng Q. et al., 2013). This nanocomplex showed efficient transfection of CP3 in cells and suppressed their proliferation. In a CNT-PLGA system, transcription factors were well delivered with a good transfection rate, and the payload release profile could be modified by adjusting the PLGA polymer molecular weight and ratio (Cheng Q. et al., 2013). For the treatment of cancer, Mehra et al. created a multiwall PEG-CTN complex loaded with doxorubicin (DOX) (Mehra and NKJain, 2015). On the surface of this DOX/ES-PEG-MWCNT system, both folic acid (FA) and estrone (ES) were attached as targeting molecules. They observed a long survival of Balb/c mice with

MCF-7 tumors treated with DOX/ES-PEGMWCNT nano formulation (Mehra and NKJain, 2015).

5.2.2 Meso-porous silica nanoparticles (MSNs)

Mesoporous materials, which have pore sizes ranging from 2 to 50 nm, high surface areas, adjustable pore sizes and internal architectures, and a plethora of modifiable sites, are frequently utilised in catalyzer, sensor, and molecular sieve research (Shi et al., 2020). In the recent decades, mesoporous materials have shown significant potential for SDDS. Due to their drug loading capacity, desirable biocompatibility, and practical feasibility, MSNs have attracted the attention of researchers (Farjadian et al., 2019). The versatility of MSNs is due to their tuneable particle size (50–300 nm), tuneable pore size (2–6 nm), high surface area, and biocompatibility (Hossen et al., 2019). Tuneable particle size is an essential criterion to be a smart nanocarrier, and tuneable pore size allows drugs of different molecular shapes to be loaded. The high surface areas of the pores and external surface are suitable for grafting different functional groups on MSNs (Yingchoncharoen et al., 2016; Hossen et al., 2019). Typical MSNs have low circulation half-lives due to hemolysis of red blood cells, non-specific binding to human serum proteins, and phagocytosis of human THP-1 mono-cytic leukemia macrophages. PEGylation can help to reduce the negative impact of these variables (He et al., 2010). MSNs are used as stimulus-sensitive drug delivery systems, and the surface pores are also blocked to build gatekeeper-based delivery systems, thanks to their adaptability. For targeted administration of doxorubicin, Cheng et al. developed and synthesised a pH responsive multifunctional MSN system made up of poly dopamine, poly (ethylene glycol), and folic acid (Cheng et al., 2017). The findings revealed significant anticancer activity and release of the encapsulated drug payload from MSNPDA-PEG-FA nanosystems in acidic pH (Cheng et al., 2017). Yang et al. created disulfide-bridged 'degradable dendritic mesoporous organo-silica nanoparticles (DDMONs) to deliver therapeutic proteins to cancer cells (Yang et al., 2016). In B16F0 cancer cells, this DDMONs system demonstrated a greater rate of glutathione (GSH)-responsive degradation and release of the therapeutic protein, but in normal HEK293t cells, the nanoparticle degradation was modest (Yang et al., 2016). Targeted MSNs therapies work by interfering directly with specific molecules involved in cancer growth and progression or indirectly by activating the immune system to detect and destroy cancer cells to prevent cancer from spreading (Colilla et al., 2010). For example, many anticancer medicines require "zero release" before reaching the target site. Efficient distribution of doxorubicin (DOX) utilising MSNs coated with a PEG copolymer employing 50 nm MSNs, which can reach a size of 110 nm when coated with the copolymer (Gary-Bobo et al., 2012; Bharti et al., 2015).

5.2.3 Gold nanoparticles

As GNPs (gold nanoparticles) have a high drug loading capacity, biocompatibility, and stability, they can be used as nano-carriers to transport drugs. In order to create GNPs with the desired morphology, the seeded growth technique is used (Dhanasekaran, 2015). The size of GNPs can be controlled by adjusting the seed to chloroauric salt ratio and the pace at which reducing agents are added, and the form of GNPs may be controlled by using surfactant intelligently to tailor the end facets (Wang et al., 2020). Medicines are connected to the surface of GO in spherical or rod-shaped GNPs; in hollow-structured GNPs, drugs are enclosed in the hollow cave (Shi et al., 2020). Non-covalent and covalent interactions are involved in the conjugation of GNPs and medicines (Wang et al., 2020). Among the non-covalent interactions are electrostatic and hydrophobic interactions, which are weak forces linking drug payload molecules to GNPs (Shi et al., 2020). The gold–thiolate bond (Au–S) is primarily responsible for covalent connections, and thiol-containing molecules are connected on the surface of GNPs in this fashion (Xue et al., 2021). For example, thiol-linked drugs or genes are linked on the surface of GNPs to release drug delivery; thiol-linked targeting groups are also decorated on the surface to improve targeting efficacy; and polymers with stimuli responsibility are functionalized on GNPs *via* Au–S link or electrostatic attraction, endowing the system with TME responsibility (Cobley et al., 2011). The surface plasmon resonance (SPR) phenomena in GNPs is particularly fascinating, which allows them to change light into heat and disperse that heat to kill cancer cells (Sztandera et al., 2019). Ideally, SDDS should be chemically stable in biological media, biocompatible, and targetable. Traditional GNPs are unstable in blood and are more likely to be absorbed by RES. In order to overcome these limitations, gold nanocarriers must be PEGylated. PEGylated GNPs exhibit enhanced solubility and stability under physiological conditions (Qian et al., 2011). GNPs can be modified by ligands or tumor-specific recognition molecules to deliver targeted drugs such as transferrin, folic acid, epidermal growth factor (EGF), or any number of monoclonal antibodies can be conjugated to the surface of GNPs (Vines et al., 2019). Drugs can be released from GNPs through either (1) external stimulation (laser, ultrasound, X-ray, light) or (2) internal stimulation (pH, redox condition, matrix metalloproteinase) (Tian et al., 2016; Yao et al., 2016). Trastuzumab (anti-EGF receptor monoclonal antibodies) was conjugated with citrate-coated GNPs to target EGF receptors in human SK-BR-3 breast cancer cells, resulting in downstream expression of EGF receptors and a 2-fold increase in trastuzumab cytotoxicity, even at low GNP concentrations (Jiang et al., 2008). Another study used GNPs to treat pancreatic cancer with gemcitabine and cetuximab. The cancer site could be identified using GNPs conjugated with fluorescently labelled heparin (Bansal et al., 2020).

5.2.4 Quantum dots (QDs)

QDs are semiconductor nanoparticles with excellent photoluminescence properties, optical properties, and electronic properties that make them suitable for image guided drug delivery. (Mi, 2020). This smart carrier could be used to visualise the tumour and can be functionalized with the targeting ligands for tissue specific therapeutic delivery application for the drug is delivered to the desired location. Various targeted QDs have been studied for diagnosis and therapeutic delivery applications over the years (Badıllı et al., 2020). Chen et al., for example, developed a quantum dot-based FRET system for image-guided drug delivery in the nucleus (Chen H. et al., 2015). In this study, graphene quantum dots (GQDs) were prepared and decorated with TAT peptide to facilitate nuclear localization. The quantum dot-based FRET system enabled real-time monitoring of therapeutic delivery as well as image-based tracking of release (Chen H. et al., 2015). Recent research has shown that conjugating metal based QDs with lipid nanocarriers reduces their cytotoxicity and improves their safety (Olerile et al., 2017). Iannazzo et al. recently demonstrated the potential of graphene QD-based targeted drug delivery. To exploit the biotin receptor overexpressed on tumour cells, they covalently conjugated QDs to the tumour targeting ligand biotin. This system utilizes the pH stimuli to release the drug payload at desired targeted site (Iannazzo et al., 2017). The inherent florescence of QDs makes them ideal for cancer imaging. Ovarian cancer has been diagnosed using a folic acid complex (Zhao, 2016). A DNA aptamer was added up to the top of the created QDs to target mutant MUC1 mucin, which is overexpressed in ovarian cancer. Doxorubicin was attached to the surface of the QD by a pH labile hydrazine linker, which hydrolyses at the acidic pH of the tumour microenvironment, allowing for regulated drug release (Dutta et al., 2021).

5.3 Antibody based SDDSs

In the past 3 decades, monoclonal antibodies have evolved from scientific tools into powerful therapeutics. A monoclonal antibody (mAb) is covalently attached to a cytotoxic drug payload *via* a chemical linker in an antibody–drug conjugate (ADC). It combines the advantages of highly specific targeting and a highly potent killing effect to achieve accurate and efficient cancer cell elimination, and it has become one of the hotspots for anticancer drug research and development (Fu et al., 2022). The first ADC drug, Mylotarg® (gemtuzumab ozogamicin), was approved by the US Food and Drug Administration (FDA) in 2000 for adults with acute myeloid leukaemia (AML), signalling the start of the ADC era of cancer targeted therapy (Norsworthy et al., 2018).

By December 2021, 14 ADC drugs had been approved worldwide for both haematological malignancies and solid tumours. Furthermore, there are currently over 100 ADC candidates in various stages of clinical trials (Fu et al., 2022). Figure 9 depicts the general mechanism of action for an ADC. Following administration, the ADC's mAb component recognises and binds to the target tumour cells' cell surface antigens. After antigen binding, the ADC–antigen complex is internalised by the cancer cell through endocytosis (Ritchie et al., 2013). In the case of non-cleavable linkers, the internalised complex is broken down *via* proteolysis within lysosomes, releasing the cytotoxic payload inside the cell, whereas the mechanism of payload release for ADCs with cleavable linkers varies depending on the specific linker used (Sanadgol and Wackerlig, 2020). The liberated payload binds to its target in all cases, causing cell death through apoptosis (Tong et al., 2021).

5.4 Small molecule drug conjugate based SDDSs

Just like ADCs, Small Molecule Drug Conjugates (SMDCs) are another class of SDDSs. An SMDC comprises of a targeting ligand, a releasable bond, a hydrophilic spacer, and a therapeutic drug payload (Rana and Bhatnagar, 2021). SMDCs which use biomarker-targeted small molecule compounds as the targeting moieties, in contrast to ADCs, provide a new, less established approach to targeted delivery. However, SMDCs have several advantages, including 1) a non-immunogenic nature, 2) a much more manageable synthesis, and 3) lower molecular weights, all of which contribute to a high potential for cell penetration in solid tumours (Zhuang et al., 2019). SMDCs have been successfully targeted against Folate Receptor, Prostate Specific Membrane Antigen, Somatostatin Receptor, and Carbonic Anhydrase IX (Ghiasikhou et al., 2019). More recently, other receptors (such as biotin receptor, bombesin receptor, Eph receptor) have gained attention as they can be potentially targeted with small molecules (Rana and Bhatnagar, 2021). Among them, the folate receptor (Iannazzo et al.) has received most of the attention and several compounds primarily developed by Endocyte have entered clinical trials (Zhuang et al., 2019). The first compounds that used folate as a targeting moiety were used for imaging. Etarfolatide was one of the first products in its class to make it to the clinic (Patel et al., 2021). A SMDC most commonly used as a delivery vehicle for folic acid is vintafolide, a conjugate containing desacetylvinblastin hydrazide (DAVLBH). Developed at Endocyte and later licensed to Merck in a $1 billion deal, this drug reached phase III clinical trials for platinum-resistant ovarian cancer. However, the results of the clinical trials, reported shortly after the European Medicines Agency (EMA) had recommended the drug approval, halted its development (Vlahov et al., 2017; Reddy et al., 2018). An innovative compound in the clinic, the peptide based SMDC 177Lu-DOTATATE, has been approved by the USFDA and EMA for

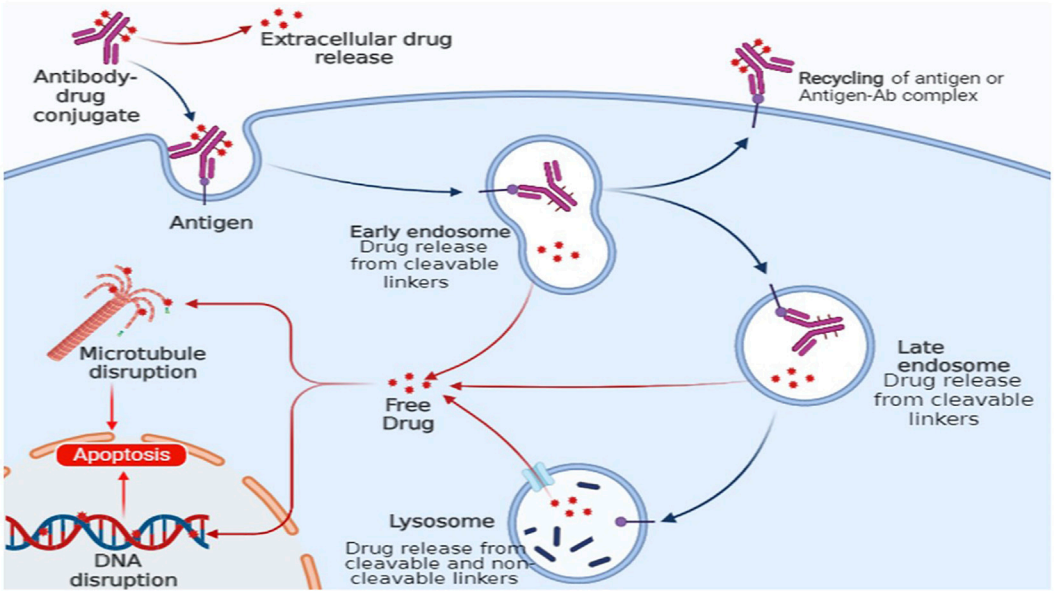

FIGURE 9
The general mechanism of action of an antibody-drug conjugate (ADC).

the treatment of gastroenteropancreatic neuroendocrine tumors (Curtis et al., 2014). Several recent preclinical studies demonstrated the striking potency of various chemotherapeutic agents such as PEN-866, EC145, AEZS-108, NGR-TNF (Asn-Gly-Arg-TNFa), and EC0225 in xenograft models of solid tumours including breast, pancreatic, and small cell lung cancer in xenograft models of solid tumours including breast, pancreatic, and small cell lung cancer (SCLC) (Patel et al., 2021). In theory, SMDCs can deliver cytotoxic agents to target cells that overexpress specific receptors such as FR, PSMA, and others by targeting ligand to the receptors and allowing it to be internalised *via* receptor-mediated endocytosis (Leamon and Jackman, 2008). Once the SMDC–receptor complex is internalised, it travels from the endosome to the lysosome, where the cytotoxic drug is released from the SMDCs *via* deconjugation (cleaving the linker) in intracellular compartments, resulting in cell death (Patel et al., 2021). SMDCs targeting cancer endocytosis, heat shock protein 90 (HSP90), BCR/ABL fusion protein, PSMA, GLUT1, LRP1, aminopeptidase N (APN), and somatostatin receptor are all in clinical trials (SSTR). All of them are currently undergoing clinical trials in various stages (Table 2). The SMDC approach, on the other hand, has been widely used in the fields of radiotherapy and cancer diagnosis. The efficacy of ligand-targeted compounds used for cancer imaging has been demonstrated in clinical trials by the identification and localization of tumours (Srinivasarao et al., 2015; Srinivasarao and Low, 2017; Banerji et al., 2018; Patel et al., 2021; Rana and Bhatnagar, 2021).

5.5 Aptamers based SDDSs

An aptamer is a simple, small, single-stranded deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) that folds into a three-dimensional conformation just like an antibody for binding to target molecules (Nimjee et al., 2017). Aptamers can typically bind to various molecules, such as overexpressed receptors, for diagnostic and therapeutic purposes using an *in vitro* iterative selection method known as SELEX (Systematic Evolution of Ligands by Exponential Enrichment) (Maghsoudi et al., 2019). Aptamers are more beneficial, less toxic, and easier to modify and synthesise in the lab than antibodies. Furthermore, aptamers were chosen as a new family of cancer therapeutics because of their numerous advantages over recent cancer therapies such as monoclonal antibodies. Their promising affinity for specific tumour cell lines, higher robustness than antibodies, fast *in vitro* selection, low immunogenicity, and better penetration into solid tumour tissue are just a few of these advantages (Hori et al., 2018). Antisoma developed AS1411, a 26-nt guanosine-rich G-quadruplex DNA oligonucleotide that was the first aptamer to enter clinical trials for cancer treatment. AS1411 was discovered in a screen for antiproliferative DNA oligonucleotides, not by SELEX (Hori et al., 2018). Aptamer–drug conjugates are particularly useful in the treatment of chemotherapeutic agents with systemic side effects. Doxorubicin (Dox) has been used as a model agent for cell-specific aptamer conjugation. Dox is a traditional chemotherapeutic agent

TABLE 2 Some medicines for cancer treatment based on SDDSs that are in clinical trials or already commercialized.

Product	Targeting	Smart Carrier	Drug Payload	Stimuli	Indication	Clinical status	Identifier
Doxil	Passive	PEGylated liposome	Doxorubicin	****	Ovarian cancer, AIDS-related Kaposi's sarcoma, breast cancer, myeloma	Approved 1995 by FDA	****
DaunoXome	Passive	Liposome	Daunorubicin	****	HIV-associated Kaposi's sarcoma	Approved 1996 by FDA	****
Myocet	Passive	Non-PEGylated liposomal	Doxorubicin	****	Metastatic breast cancer	Approved 2000 by EMEA	****
Lipusu	Passive	Liposome	Paclitaxel	****	Ovarian cancer, non-small cell lung cancer	Approved 2003 by CFDA	****
Nanoxel	Passive	Polymeric micelle	Paclitaxel	****	Breast cancer, non-small-cell lung cancer, and ovarian cancer	Approved 2006 by CDSCO	****
Marqibo	Passive	Liposome	Vincristine Sulfate	****	Philadelphia chromosome-negative acute lymphoblastic leukemia	Approved 2012 by FDA	****
Kadcyla (ado-trastuzumab Emtansine)	Active	Anti-HER2 tumor cell specific antigen	Maytansinoid DM1	pH	Early Breast Cancer	Approved 2013 by FDA	****
Gemtuzumab ozogamicin	Active	Anti-CD33 tumor cell specific antigen	Calicheamicin	pH	Acute myeloid lymphoma	Approved 2017 by FDA	****
Besponsa (Inotuzumab ozogamicin)	Active	Anti-CD22 tumor cell specific antigen	Calicheamicin	pH	Relapsed acute lymphoblastic Leukemia	Approved 2017 by FDA	
Brentuximab vedotin	Active	Anti-CD30 tumor cell specific antigen	Monomethyl Auristatin E (MMAE)	Enzyme	Relapsed Hodgkin's lymphoma and anaplastic large cell lymphoma	Approved 2022 by FDA	****
Depatuxizumab mafodotin	Active	Anti-EGFR tumor cell specific antigen	Monomethyl Auristatin E (MMAE)	****	Glioblastoma	Phase III	NCT02573324
Enfortumab vedotin	Active	Anti-EGFR tumor cell specific antigen	Monomethyl Auristatin E (MMAE)	Enzyme	Advanced urothelial cancer	Phase III	NCT04136808
Vintafolide (EC145)	Active	Folic acid	Desacetylvinblastine	pH	Solid tumors,	Phase I (completed)	NCT01002924
					Recurrent or refractory solid tumors,	Phase II (completed)	NCT00308269
					Platinum resistant ovarian cancer	Phase II (completed)	NCT00722592
					FR (++) second line non-small cell lung cancer	Phase II (completed)	NCT01577654

(Continued on following page)

10.3389/fchem.2022.1095598

TABLE 2 (*Continued*) Some medicines for cancer treatment based on SDDSs that are in clinical trials or already commercialized.

Product	Targeting	Smart Carrier	Drug Payload	Stimuli	Indication	Clinical status	Identifier
Vintafolide (EC145) + Etrafolide (EC 20)	Active	Folic acid	Desacetylvinblastine	pH	Ovarian cancer, endometrial cancer	Phase II (completed)	NCT00507741
					Adenocarcinoma of lungs	Phase II (completed)	NCT00511485
EC1456 and EC20	Active	Folic acid	Tubulysin	pH	Solid tumors, non-small cell lung carcinoma	Phase I (completed)	NCT01999738
Glufosfamide	Active	Glucose	Fluorouracil	****	Second line metastatic pancreatic cancer	Phase III (recruiting)	NCT01954992
MAGNABLATE I	Passive	Iron oxide magnetite	Doxorubicin	Magnetic	Prostate cancer	Phase I	NCT02033447
NC6300	Passive	Polymeric micelles	Epirubicin	pH	Solid tumor, soft tissue sarcoma, metastatic sarcoma, sarcoma	Phase I and II	NCT03168061
(MTC-DOX)	Passive	Iron and carbon	Doxorubicin	Magnetic	Unresectable hepatocellular carcinoma	Phase II and III	NCT00034333
					Hepatocellular carcinoma	Phase I and II	NCT00054951
					Liver metastasis	Phase I and II	NCT00041808
ThermoDox	Passive	Liposome	Doxorubicin	Temperature	Recurrent regional breast cancer	Phase I and II	NCT00826085
					Liver tumor	Phase I	NCT02181075
					Pediatric refractory solid tumor	Phase I	NCT02536183
			Doxorubicin combined with high Intensity focused ultrasound (HIFU)	Temperature	Painful bone metastasis, breast carcinoma, non-small cell lung cancer, small cell lung cancer, adenocarcinoma	Phase II	NCT01640847

10.3389/fchem.2022.1095598

that induces cancer cell death by intercalating into DNA. Dox can non-covalently conjugate to aptamers *via* intercalation into their GC-rich regions for delivery into specific cells, according to some studies (Bagalkot et al., 2006; Hu et al., 2012; Subramanian et al., 2012). Several other groups have reported novel types of aptamer–Dox conjugates in the recent years. Wen et al. isolated a CD38-targeting DNA aptamer and used CG-cargo to non-covalently conjugate Dox to it in a CG-repeat structure (Wen et al., 2016). The aptamer-Dox conjugate was formed with a 1:5 M ratio of aptamer to Dox using the CG-repeat structure. It specifically released Dox in tumour cells when systemically administered to multiple myeloma-bearing mice, inhibiting tumour growth and improving mouse survival rates (Wen et al., 2016). Trinh et al. developed AS1411-Dox, a drug-DNA adduct, by crosslinking Dox and AS1411 with formaldehyde overnight at 10°. AS1411-Dox inhibited tumour growth in hepatocellular carcinoma-bearing mice without causing severe toxicity in non-tumor tissues when given systemically (Trinh et al., 2015). Covalent conjugation to aptamers has also been utilized to target other chemotherapy agents to cancer cells. For example, Zhao et al. developed a cell-specific aptamer—methotrexate (MTX) conjugate to specifically inhibit AML (Zhao et al., 2015). In the first step, they isolated a DNA aptamer that targets CD117, an antigen that is highly expressed on AML cells. MTX was covalently conjugated with DNA aptamers with G-quadruplex structures using N-hydroxysuccinimide (NHS). The CD117 aptamer-MTX conjugate specifically inhibited the cell growth in AML (Zhao et al., 2015).

6 challenges and the future perspectives

It is inevitable that every opportunity will come with some challenges. There is no exception to this rule with SDDSs. In order for SDDSs to succeed, they must overcome the toxicity of nanocarriers in the human body, the cost-effectiveness of the system, the heterogeneity and diversity of cancers, and the lack of specific regulatory guidelines (Shi et al., 2017).

To kill cancer cells, smart carriers needs to transport and release anti-cancer drugs at the targeted sites. In nanocarrier delivery, the biggest challenge is the toxicity of nanocarriers, which will need to be studied further in the future, as well as the limitation between their use in small animals and their clinical effectiveness. Depending on the chemical composition, size, shape, specific surface area, surface charge, as well as the presence or absence of a shell around the nanocarrier, conventional nanocarriers can accumulate in different vital organs including the lungs, spleen, kidneys, liver, and heart. Similarly, in case of ADCs, the high cost of production, limited penetration into solid tumor masses, and premature drug release the main concerns (Lo et al., 2022).

The challenges associated with ligands include selection of an appropriate ligand, developing conjugation strategies, and characterizing the release of the drug from the ligand (selection of a linker). A carrier-based challenge involves carrier selection and carrier physicochemical and pharmacokinetic characterization. SDDSs formulation requires additional steps in chemical synthesis and purification. Furthermore, there are additional quality control and regulatory steps, increased costs, and longer timeframes. The majority of these carriers have been designed and tested in small animal models, with excellent therapeutic results; however, the translation of animal results into human success has been limited. In order to fully comprehend the advantages and disadvantages of these vehicles, more clinical data is needed.

Another challenge that limits application of SDDSs is functional group complementarity as well as release of drug in active form in cellular melieu. pH-responsive delivery can be accomplished by the controlled protonation of the functional groups in the linker of SDDS and pH-responsive bond cleavage. Similarly, pH-induced bond cleavage can release drugs directly or by breaking up the carrier's topological structure. Chemical bonds that can be cleaved by pH-responsive materials include hydrazine, oxime, amide, imine, ketal or acetal, orthoester, and phenyl vinyl ether. When drugs are linked to the carrier by these bonds, their cleavage in an acidic environment leads to their release. For the redox-responsive system, commonly used linkers include disulfide and diselenide linkages which will be broken with significant increases in the level of surrounding reducing agents such as GSH. On similar lines, it is necessary for enzyme-responsive SDDSs to tolerate specific conditions of pH and presence of other ions in cellular mileiu that may interfere with enzyme activity. Besides in the DDS the substrate should mimic and also be complementary to the binding pocket of target enzyme for the targeted enzyme to act, the actions of the targeted enzyme must alter the properties of the linkers used in SDDS as well. It is also worth mentioning that the long-term effects of associated with toxicity due to accumulation of nanocarriers or other SDDDs in patients is a necessity that requires investigation. All the above are formidable challenges for medicinal chemists; however, the potential of SDDSs in translational medicine cannot be denied.

In the future, SDDS will combine diagnosis and targeted therapy into one, centralized treatment system. A novel theranostic strategy has the potential to facilitate highly selective, effective, and relatively sensitive treatments of cancer and other chronic diseases, leading to personalized chemotherapy with improved outcomes for patients. All smart drug delivery systems all share the same goal: to benefit patients. Future research on smart DDSs for controlled drug delivery should concentrate on clinical translation so that more stimulus-sensitive nanomedicine may be employed in clinical settings.

7 Conclusion

The pharmaceutical and biotechnology industries are undergoing a significant transformation. Although the last decade has seen significant advances in drug delivery yet challenges remain. Smart drug delivery systems have the potential to overcome the limitations of traditional drug delivery methods. The development of smart drug delivery systems holds a lot of promise for pathology-specific medication design and delivery techniques that are tailored as per therapeutic needs.

Smart drug delivery systems incorporate several benefits, which includes i) a long shelf life and is not readily degraded or cleared by the reticuloendothelial system (RES) during blood circulation, ii) efficient intracellular drug delivery at the tumour targeted region or location that meets iii) drug pharmacodynamics of kinetics and spatial control, and iv) tolerability.

Another class of SDDSs include stimuli-responsive nanocarriers that can be used to deliver diagnostic and therapeutic substances to specific locations. Many improvements in stimulus sensitive delivery systems have been made in the last few years. In this review, literature studies of internal stimuli such as pH, redox, and enzyme demonstrate a superior property of controlling and adjusting the location and time of drug release without the use of any other external remote apparatus, leading in increased therapeutic drug internalisation in target cells and external stimuli such as light, ultrasound, and magnetic fields can also be utilised to initiate or increase drug release at disease sites. Smart Nanocarriers, a marvel of modern technology, are critical in the delivery of anti-cancer drugs. Because of their outstanding characteristics for cancer therapy, organic and inorganic based smart nanomaterials have recovered a lot of interest.

Changes will be made in clinical trials to allow for the specific targeting of cancer cells, which will enhance cancer patients' quality of life by minimising the side effects of chemotherapeutic drugs and improving overall survival. Liposomes, nano-suspension, polymer nanoparticles, nanocapsule, micelles, doxil, and other nanocarriers have been authorised in clinical trials (Mi, 2020).

SDDS has a bright future and offers many opportunities for improving quality of life and patient compliance, and it could become the future of Translational Medicine.

Author contributions

AR wrote the manuscript with support from MA. All the diagrams were drawn by AR. AR, MA, and PS collected references. SB and BCD supervised the whole work and edited the manuscript. All the authors approved this manuscript.

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Conflict of interest

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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Chemiluminescent polymeric nanoprobes for tumor diagnosis: A mini review

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Chemiluminescence (CL), a distinct luminescent process by taking advantage of chemical reactions rather than external light source, has recently attracted considerable research interests due to its high sensitivity and low background signal. The sensitivity and specificity of chemiluminescent signals in complex tumor microenvironment provide a sound basis for accurate detection of tumors. Various chemiluminescent nanoprobes with superior performance have been obtained by structural modification of chemiluminescent units or introduction of fluorescent dyes. In this review, we focused on the recent progress of chemiluminescent polymeric systems based on various chromophore substrates, including luminol, peroxyoxalates, 1, 2-dioxetanes and their derivatives for tumor detecting. And we also emphasized the design strategies, mechanisms and diagnostic applications of representative chemiluminescent polymeric nanoprobes. Finally, the critical challenges and perspectives of chemiluminescent systems usage in tumor diagnosis were also discussed.

KEYWORDS

chemiluminescence, tumor diagnosis, luminol, peroxyoxalates, 1,2-dioxetanes

Introduction

Optical imaging plays a vital role in early diagnosis and treatment of diseases. It not only visualizes the detection of lesion sites, but also improves the accuracy of disease treatment (Thomas, 2015). However, due to tissue self-illumination, low tissue penetration depth and less noise interference, conventional optical imaging limits its application in living organisms (Li and Pu, 2019). Chemiluminescence refers to the process in which luminescence is generated through chemical reactions without external light source or other energy. In brief, chemical substances are oxidized into unstable high-energy intermediates, which subsequently disintegrate to emit light or transfer energy to surrounding fluorophores (Vacher et al., 2018). Chemiluminescence possesses the advantages of high sensitivity, deep tissue penetration depth, without external light source required, and high signal-to-background ratio, which provides new methods and ideas for the further development of optical imaging technology (Gnaim et al., 2018).

Chemiluminescence can be classified into two types, namely direct chemiluminescence and indirect chemiluminescence according to the energy conversion principle of luminescence (Yan et al., 2019). Direct chemiluminescence refers to the oxidation of a chemiluminescent substrate to form an excited-state intermediate with high energy, which then returns to the ground state to release photons, followed by the emission of light. The most representative is the luminol chemiluminescent system (Li et al., 2022). Indirect chemiluminescence generally involes chemiluminescence resonance energy transfer (CRET) process. 1, 2-dioxetane derivatives or

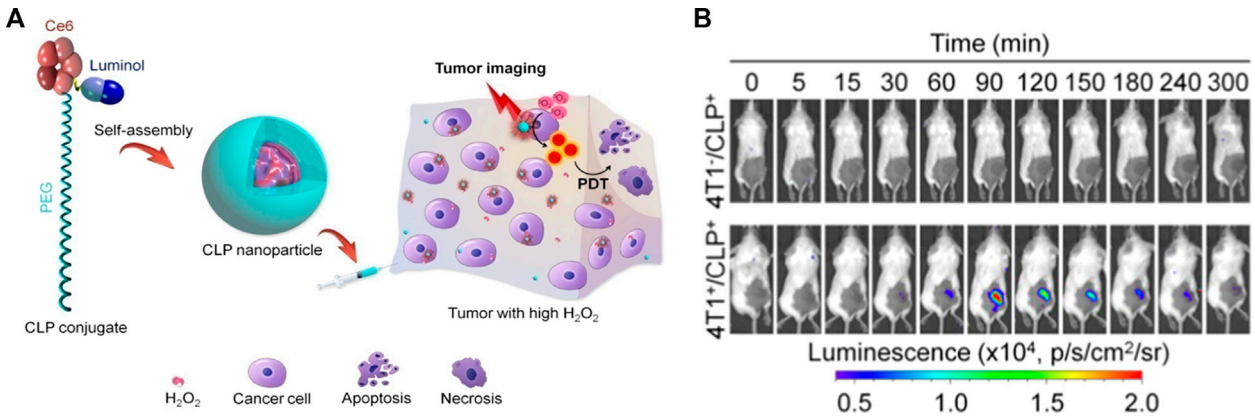


FIGURE 1
(A) Schematic illustration of CL imaging of tumors with high H_2O_2 and H_2O_2-mediated photodynamic therapy using CLP nanoparticles. **(B)** Time-dependent *in vivo* CL imaging of healthy mice and 4T1 tumor-bearing mice after local injection of CLP nanoparticles (An et al., 2020).

peroxyoxalates are widely used as typical indirect CL systems for biomedical assays (Su et al., 2019; Tzani et al., 2021).

Despite the rapid development of chemiluminescence, the application of chemiluminescent substrate in biomedical fields is still limited compared to fluorescence due to its hydrophobicity, weak signal, and fast decay (Miao and Pu, 2018). To overcome these shortcomings, chemiluminescence systems combined with nanotechnology or different modifications have emerged and aroused the wide interest (Tiwari and Dhoble, 2018). In recent years, polymeric nanoparticles have attracted extensive attention due to their high brightness and high quantum yield, as well as their low toxicity, good biocompatibility and various synthesis methods (Kaeser and Schenning, 2010). This review aims to sum up recent advances in chemiluminescent polymeric nanoprobes based on three chromophore substrates, including luminol, peroxyoxalates, and 1, 2-dioxetanes. Moreover, the design strategies and luminescence mechanisms of different chemiluminescent polymeric nanoprobes are discussed, and their applications in tumor diagnosis are further elaborated.

Luminol-based chemiluminescent polymeric nanoprobes

Luminol (5-amino-2, 3-dihydrophthalazine-1, 4-dione) and its derivatives are currently the most classical chemiluminescent reagents. In 1928, Albrecht (1928) discovered that luminol could emit CL when reacting with oxidizing agents such as hydrogen peroxide (H_2O_2) in alkaline media. Despite its low detection limit and good selectivity, the luminol chemiluminescent probe still suffers from a short emission wavelength (~425 nm) and limited tissue penetration depth, hindering the application of this system in bioimaging *in vivo* (Zhang et al., 2013). The chemiluminescent properties of luminol itself are susceptible to external factors, therefore, nanomaterials with different catalytic properties are often used to modulate the performance of this chemiluminescent system. Graphene oxide was utilized to amplify the CL signal of luminol (Yang et al., 2014). Huang et al. (2006) demonstrated effective CRET between luminol and CdTe quantum dots and the red shift of the luminescence wavelength to the quantum dots in the luminol/H_2O_2 CL system. In addition, the researchers obtained luminol-based chemiluminescenct polymeric systems with improved properties by modifying luminol or linking it with different fluorescent dyes.

Reactive oxygen species are critical for cancer development, progression, and metastasis, thus early detection of ROS within cancer cells is crucial for the monitoring and management of cancers. Abnormal superabundant generation of H_2O_2, a major ROS, has been closely associated with cancer development (Trachootham et al., 2009). An et al. (2020) utilized luminol and poly (ethylene glycol) to simultaneously couple chlorin e6 to form an amphiphilic conjugate (defined as CLP), followed by the self-assembly of CLP to form luminescent nanoparticles. These nanoparticles with core-shell nanostructures could be activated by H_2O_2, leading to CL imaging and *in situ* photodynamic therapy (PDT) of tumors with high expression of H_2O_2, shown in Figures 1A, B. Nan et al. (2019) designed a polycarbonate copolymer (PMPC-ONA) micelle decorated with benzyl alcohol and then luminol, fluorophore and heme were encapsulated into the micelle to form the L/H/S@PMPC-ONA nanoprobe for H_2O_2 imaging *in vivo*. Once H_2O_2 encountered the heme in the nanoparticles, ROS were generated, and then luminol would be excited to trigger chemiluminescence, while the stability of the nanoparticles decreases, thus releasing the fluorescent indicator to detect H_2O_2. Chemiluminescence exhibits the potential for ROS detection since no excitation light source needs to be involved and the relationship between light emission and analyte concentration is explicit. Lee et al. (2016) designed hydrogen peroxide responsive hybrid nanoparticles (HNPs) consisting of the PEGylated QDs and a luminol derivative (L012) as the CL agent. The energy transduction owning to the reaction between L012 and H_2O_2 enabled HNPs to achieve light production. Furthermore, as a promising diagnostic agent for cancer, HNPs displayed non-invasive near-infrared (NIR) imaging of H_2O_2 *in vivo* without background interference. Endogenous photoactivation is also of great importance for the diagnosis and treatment of tumors. Mao et al. (2021) constructed

two gold nanoparticles (tAuNP and makuNP) by modifying 2, 5-diphenyltetrazole and methacrylic acid on the surface of gold nanoparticles for tumor imaging and therapy. The mAuNPs were absorbed with luminol to form self-illuminating mAuNP/Lu nanoparticles. Owning to the tetrazole/alkene cycloaddition, H_2O_2-initiated mAuNP/Lu nanoparticles could specifically crosslink with tAuNP nanoparticles to generate large particle aggregates. Subsequently, these aggregates contributed to strong CL effect catalyzed by H_2O_2 in tumor microenvironment, leading to strengthened uptake and retention of AuNPs.

The specific identification of tumor markers in the blood and tissues of cancer patients is significant for early cancer diagnosis (Freedland, 2011). Yan et al. (2022) constructed a dual-carrier CL sensor that recognized tumor markers through an enzyme digestion CL signal amplification strategy. This sensor underwent aggregation and identified target markers, and then strong CL signals were collected under luminol catalysis reaction. Zhang et al. (2016) designed a novel signal enhancement strategy in which CRET occurred between reduced graphene oxide (rGO) as an energy acceptor and luminol as a donor. And the probe provided a more sensitive signal amplification strategy. Additionally, Ag-C_3N_4 nanosheet loaded with luminol capped AuNPs nanocomposite was developed as the electrochemiluminescence (ECL) signal nanoprobe for early tumor diagnosis (Liu et al., 2021). The CL signal could be obviously enhanced by cells in luminol-H_2O_2 system, and the signal intensity and the cell concentration were positively correlated. Based on the above phenomena, Ding et al. (2020) designed a new cell-assisted enhanced CL strategy to identify tumor cells rapidly. The above probes provided highly accurate and quantitative analysis of tumor markers in complex biological samples, showing great potential for clinical application.

Peroxyoxalate-based chemiluminescent polymeric nanoprobes

The reaction of anthracene, 9, 10-diphenylanthracene and N-methylacridine with hydrogen peroxide and oxalyl chloride can produce a "blue-white light," which is known as peroxyoxalate chemiluminescence (PO-CL), firstly discovered by Chandross (1963). PO-CL modulates the emission wavelength by adding different fluorophores to the reaction system, rather than modifying the CL molecule. PO-CL systems belong to indirect chemiluminescence, generally consists of oxalate, oxidant (usually hydrogen peroxide) and suitable dye. The formation of high-energy 1, 2-dioxadione intermediates is a key step leading to chemiluminescence emission (Delafresnaye et al., 2020). The three most commonly used oxalate eaters are TCPO (bis (2, 4, 6-trichlorophenyl) oxalate), CPPO (bis (2, 4, 5-trichlorophenyl-6-carbopentoxyphenyl) oxalate) and DNPO (bis (2, 4-dinitrophenyl) oxalate) (Lee et al., 2012). The detection of H_2O_2 in water, microorganisms in food, and blood samples in crime scenes all rely on the PO-CL reaction (García and Baeyens, 2000). Thanks to its high sensitivity, high quantum yield and long luminescence lifetime, peroxyoxalate-based chemiluminescence systems have been widely used in bioimaging and tumor therapy (Yang and Zhang, 2021).

PO-CL systems are usually designed as nanoparticles for *in vivo* bioimaging because of their susceptibility to decomposition when exposed to water. Shuhendler et al. (2014) developed a nanoparticle based on a dual-channel imaging function for imaging liver injury in mice, i.e., CRET-based peroxyoxalate chemiluminescence for H_2O_2 detection and fluorescence resonance energy transfer (FRET)-based fluorescence for $ONOO^-$ and hypochlorite detection. The probe allowed for rapid and real-time direct assessment of acute hepatotoxicity. Zhen et al. (2016) constructed a H_2O_2-responsive chemiluminescent semiconducting polymer nanoparticle (SPN) using TCPO and applied it to the detection of endogenous H_2O_2 in peritonitis and neuroinflammation. With high brightness and proper near-infrared window, the SPN achieved ultrasensitive detection of H_2O_2. A PO-CL system based on CPPO with cascaded CRET and FRET processes was designed to permit near-infrared region II (NIR-II) chemiluminescence imaging in arthritic mice (Yang et al., 2020). The strategy avoided the loss of energy transfer as much as possible through the rational design of the probe.

Chemiluminescent polymeric nanoprobes that react with H_2O_2 within the tumor microenvironment may achieve accurate imaging of tumors. Yu et al. (2022) combined pluronic F-127 and polymer containing oxalate ester (POE) to form nanoparticles by means of hydrophilicity and hydrophobicity. The nanoparticles loaded anti-tumor drug could realize the tumor tracking by H_2O_2-related chemiluminescence and the tumor therapy by drug releasing, which possessed great potential for precise localization and efficient treatment of tumor. In addition, since tumor cells consume more glucose than normal tissues, the glucose level of tumor tissues ware also utilized to enable tumor detection and imaging (Li et al., 2019).

Apart from disease diagnosis related to hydrogen peroxide overproduction, the CL platform can be used to perform PDT, avoiding the limitations of external light sources and light penetration depth (Yuan et al., 2012). Excessive hydrogen peroxide is an intrinsic feature of tumor cells. Andrey et al. took advantage of this feature to designed and synthesised a dispersion composed of polyoxalate and tetramethylhematoporphyrin (TMHP). In the presence of TMHP, singlet oxygen ($^1O_2$) formation by reaction with endogenous H_2O_2 through PO-CL leaded to tumor cell elimination (Romanyuk et al., 2017). Wu et al. (2019) came up with a self-luminescing nanoreactor by coencapsulating CPPO, poly [(9,9′-dioctyl-2,7-divinylene-fluorenylene)-alt-2-methoxy-5-(2-ethyl-hexyloxy)-1,4-phenylene] (PFPV), and the photosensitizer tetraphenylporphyrin with polyethylene glycol-polycaprolactone (PEG-PCL) and folate-PEG-cholesterol. This novel system could achieve self-luminescence emission and singlet oxygen ($^1O_2$) production, which had important implications for imaging and treatment of cancer. Most conventional photosensitizers usually suffer from aggregation-caused quenching (ACQ) effects, resulting in reduced luminescence intensity and ROS generation (Li et al., 2021). The emergence of aggregation-induced emission luminogens (AIEgens) exhibiting enhanced fluorescence intensity and ROS production largely solves the above problems. Mao et al. developed a novel nanoparticle (C-TBD NPs) with chemiexcited far-red/NIR emission and $^1O_2$ production capability using pluronic F127 and soybean oil co-loaded with CPPO and TBD, a photosensitizer with AIE properties. C-TBD NPs could emit chemiluminescence in response to hydrogen peroxide at the tumor site, thus enabling precise tumor tracking *in vivo*. In addition, C-TBD NPs also produced effective $^1O_2$ to induce apoptosis of tumor cells (Mao et al., 2017).

Chemodynamic therapy (CDT), a new class of oncology therapeutic techniques based on the iron-based Fenton reaction, offers an alternative opportunity for cancer treatment (Yang et al., 2019). In order to enhance therapeutic efficiency and minimize side

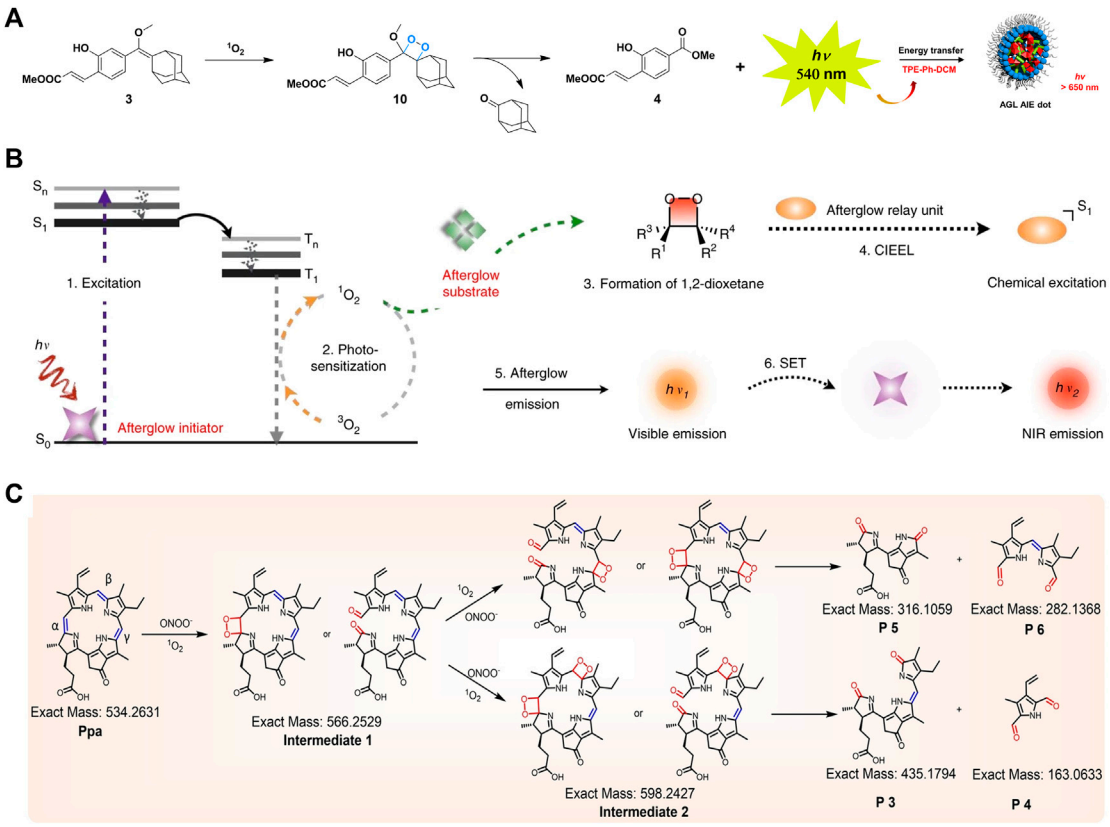


FIGURE 2
(A) Schematic diagram of the mechanism for NIR afterglow of the AGL AIE Dot (Ni et al., 2018). **(B)** Schematic diagram of the mechanism for intraparticle afterglow luminescence of ALNPs (Jiang et al., 2019). **(C)** Proposed mechanism for persistent luminescence of pheophorbide A (Duan et al., 2022).

effects, the real-time monitoring of ROS production during CDT is extremely essential. However, CDT reagents that can emit ROS-associated signals are rare. Wang et al. (2019) constructed a semiconducting polymer nanoplatform containing CPPO, hemin and glucose oxidase (GOD). This ROS-dependent chemiluminescence of SPN allowed optical monitoring of intra-tumor ROS production during CDT. This nanoplatform represented the first intelligent strategy to enable chemiluminescence imaging for monitoring CDT, demonstrating great potential in assessing treatment responsiveness and predicting early treatment outcomes.

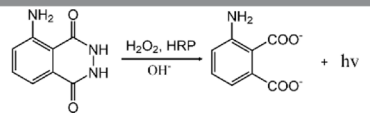
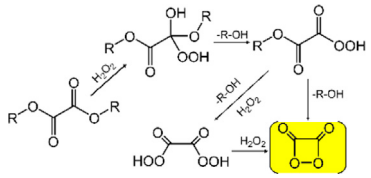
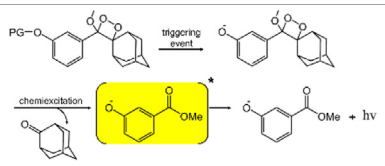
1, 2-dioxetane-based chemiluminescent polymeric nanoprobes

Chemiluminescent systems based on luminol and peroxyoxalates require the involvement of oxidants, making them often used for the detection of active species, which limits their scope of application. 1, 2-dioxetanes do not require the involvement of additional oxidants (hydrogen peroxide, oxygen, potassium permanganate, etc.), which simplifies the analytical process, improves detection sensitivity, and expands the field of chemiluminescence applications (Kopecky and Mumford, 1969; Schaap et al., 1989). Schaap's group found that the deprotonation of the phenolic hydroxyl substituent could transform 1, 2-dioxetane into a superior luminescent intermediate in 1982 (Schaap and Gagnon, 1982). Subsequently, Schaap's group further improved the thermal stability of 1, 2-dioxetane derivatives by introducing adamantane substituents (Schaap et al., 1987). However, previous 1, 2-dioxetane CL systems had a great tendency to be quenched by water, resulting in short emission wavelength and low luminescence intensity, which made it difficult to be applied *in vivo*.

To enhance the chemiluminescence intensity, the researchers have made numerous attempts, i.e., simple or complex modifications of the dioxetane scaffold: 1) the processing of dioxetane units into polymer monomers (Gnaim and Shabat, 2017). 2) the covalent binding of fluorescent dyes with higher fluorescence efficiency to Schaap's 1, 2 dioxetanes (Matsumoto, 2004; Hananya et al., 2016). 3) the introduction of electron-withdrawing groups in the neighboring positions of phenoxy 1, 2-dioxetane (Eilon et al., 2018). Based on the above basic research, the researchers designed and constructed various chemiluminescent polymeric systems based on 1, 2-dioxetanes, which showed excellent performance in cancer detection.

Afterglow luminescence is a process of persistent luminescence after the cessation of light excitation, and afterglow imaging holds great promise in the biomedical fields. Ni et al. constructed a NIR afterglow luminescent nanoparticle (AGL AIE dots) with AIE characteristics by encapsulating the NIR emissive AIE molecule (TPE-Ph-DCM) and the enol ether precursor of Schaap's 1, 2-dioxetane with Lipid-PEG_{2000} through nanoprecipitation. The AGL AIE dots finally emitted NIR afterglow luminescence through the generation of $^1O_2$ by TPE-Ph-DCM, the formation and decomposition

TABLE 1 Overview of three chemiluminescent substrates.

Platform	Mechanism	Advantages	Disadvantages
Luminol		Good water solubility; Stable properties; High CL efficiency; Simple synthesis	Slow reaction rate; Short CL emission wavelength (425 nm); Shallow tissue penetration depths
Peroxyoxalate		High sensitivity; High quantum efficiency (30% or higher for certain oxalate phenyl systems); Long luminescence lifetime	Poor compatibility with aqueous systems
1,2-dioxetane		Deep penetration; Less light scattering; High sensitivity; Powerful luminescence intensity; Long half-lives	Complex synthesis; Short emission wavelength; Light instability

of dioxetane, the release of chemical energy, and the energy transfer to TPE-Ph-DCM, which lasted for more than 10 days after single light excitation through self-cycling luminescence mechanism. The afterglow luminescent signal of AGL AIE dots was extremely quenched in the liver, therefore, AGL AIE dots had great prospects in the application of accurate image-guided tumor surgery, shown in Figure 2A (Ni et al., 2018). Immunotherapy, an emerging cancer treatment in recent years, offers new treatment options for cancer patients, yet the response rate of patients in clinical applications has not been significant (Sambi et al., 2019). In order to improve the efficacy of immunotherapy, monitoring the immune responses of patients is inevitable. Cui et al. (2020) constructed semiconducting polymeric nanoreporters (SPNRs) by means of combining the semiconducting polymer and the dioxetane derivative. As the first reporter that could release chemiluminescent signals activated by superoxide anions, SPNRs could sensitively distinguish immune cells containing high O_2-levels from other cells including cancer cells and normal cells, enabling real-time imaging of immune activation during cancer immunotherapy *in vivo*. The aforementioned PDT is a promising strategy for cancer therapy. Fan et al. (2021) designed nanoparticles that specifically respond to alkaline phosphatase overexpressed on hepatocellular carcinoma cells, ultimately producing $^1O_2$ and NIR fluorescence for tumor diagnosis and treatment.

In addition to the direct addition of dioxetane units, some chemiluminescence systems can also emit chemiluminescence by forming unstable dioxetane intermediates in response to the tumor microenvironment. Miao et al. (2017) applied PEG-*b*-PPG-*b*-PEG coated poly (2methoxy-5-(2-ethylhexyloxy)-1, 4-phenylenevinylene) (MEHPPV) to prepare SPNs to amplify and redshift the afterglow effectively. Under the oxidation of PPV, SPNs generated unstable dioxolane intermediates, which slowly degraded into PPV aldehyde and emitted afterglow. This strategy could be applied to the detection of lymph nodes and tumors in living mice. Jiang et al. (2019) demonstrated an excellent method for converting ordinary fluorescent agents into afterglow luminescent nanoparticles (ALNPs) by forming unstable 1, 2-dioxetanes intermediates in order to detect tumors *in vivo* rapidly. This method consisted of the following steps: the photosensitizer absorbed light and converted it into $^1O_2$, then the reactive molecule reacted with $^1O_2$ to generate unstable 1, 2-dioxetane, and event ually the luminescence emitted by receiving the energy from 1, 2-dioxetane through semiconducting polymer, shown in Figure 2B. Lu et al. (2020) developed a novel chemiluminescent polymeric system (ultrathin MnOx-SPNs) with both CDT and pH responsiveness. The $^1O_2$ produced by MnOx in response to the acidic tumor microenvironment reacted with SP, followed by forming thiophene-dioxetane intermediates and transferring energy to SPNs, leading to real-time monitoring of $^1O_2$ generation and ratiometric CL/FL imaging for guiding cancer therapy. Duan et al. (2022) demonstrated persistent luminescence was detected from porphyrins after stopping the excitation light or reacting with peroxynitrite, verifying the successive oxidation of vinylene bonds may form unstable dioxetane intermediates. Such supramolecular probes could realize the light-triggered function conversion from photoacoustic imaging to persistent luminescence imaging, resulting in the successful implementation of image-guided tumor surgery, shown in Figure 2C. The mechanisms, advantages and disadvantages of the three chemiluminescent substrates were shown in Table 1.

Discussion

This article briefly reviewed recent developments of chemiluminescent polymeric nanosystems based on the chemiluminescence substrates including luminol, peroxyoxalates and 1, 2-dioxetanes. Moreover, luminescence mechanisms, design strategies and applications in biosensing and tumor diagnosis of chemiluminescent polymeric nanosystems were described in detail. An increasing number of studies have been performed to modify these three chemiluminescent substrates by direct or indirect means to improve luminescence efficiency and intensity for highly sensitive and

precise quantitative analysis of solid tumors and even tumor markers. However, despite the remarkable progress in a wide range of fields, there still exist some problems that need to be solved and broken through: 1) Chemiluminescent systems based on 1, 2-dioxetanes have limited their biological applications due to their complex synthetic routes and short emission wavelengths. 2) Similar to water-soluble and lipid-soluble fluorescent dyes, the released dye tends to diffuse from the reaction site when a chemiluminescent probe is activated, making it more difficult to provide *in situ* information for imaging. The introduction of a second near-infrared (NIR-II) window (1,000–1,700 nm) into chemiluminescence imaging can achieve deeper penetration depths and higher signal-to-noise ratios, which is certainly helpful to solve the above issues. In addition, finding more stable fluorophores with higher antioxidant capacity and increasing the fluorescence quantum yield of fluorophores can maintain good efficiency of chemiluminescence, and the choice of enzymes-initiated chemiluminescence systems to avoid ROS/RNS oxidation is also an effective means. Over the past few years, chemiluminescent polymeric nanoprobes have developed significantly as imaging analytical tools for tumor diagnosis. In addition to tumor tissue imaging, the chemical flexibility of chemiluminescent polymeric nanoprobes holds great promise for their application in multifunctional therapeutic platforms.

Author contributions

XZ designed and wrote the manuscript. CL, WC, and GW searched articles relating to the subject. HZ and HL supervised the whole work. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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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ROS-responsive ADPH nanoparticles for image-guided surgery

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In recent years, organic fluorescent probes with tumor microenvironment (TME)-responsive fluorescence turn-on properties have been increasingly used in imaging-guided tumor resection due to their higher signal-to-noise ratio for tumor imaging compared to non-responsive fluorescent probes. However, although researchers have developed many organic fluorescent nanoprobes responsive to pH, GSH, and other TME, few probes that respond to high levels of reactive oxygen species (ROS) in the TME have been reported in imaging-guided surgery applications. In this work, we prepared Amplex® Red (ADHP) with excellent ROS response performance as an ROS-responsive nanoprobe and studied its application in image-guided tumor resection for the first time. To confirm whether the nanoprobe can be used as an effective biological indicator to distinguish tumor sites, we first detected 4T1 cells with the ADHP nanoprobe, demonstrating that the probe can utilize ROS in tumor cells for responsive real-time imaging. Furthermore, we conducted fluorescence imaging *in vivo* in 4T1 tumor-bearing mice, and the ADHP probe can rapidly oxidize to form resorufin in response to ROS, which can effectively reduce the background fluorescence signal compared with the single resorufin probe. Finally, we successfully carried out image-guided surgery of 4T1 abdominal tumors under the guidance of fluorescence signals. This work provides a new idea for developing more TME-responsive fluorescent probes and exploring their application in image-guided surgery.

KEYWORDS

nanoparticle, reactive oxygen species, image-guided, surgery, breast cancer

1 Introduction

Breast cancer is the prevalent malignancy in women worldwide, and in 2020 with an estimated 2.3 million new patients and 685,000 mortality cases (Sung et al., 2021), accounting for 30% of new female tumors in 2021 (Siegel et al., 2021). It is a serious threat to women's health and an important cause of female death. The preferred treatment for breast cancer is surgical excision combined with radiotherapy and chemotherapy (Ma et al., 2020b). The surgical outcome and patient prognosis depend largely on the complete resection rate of the tumor. Postoperative tumor residue was associated with poor prognosis, high recurrence rate, and low survival rate. The higher the tumor resection rate is, the longer the overall survival of patients (Kimbrough et al., 2013; McCann et al., 2013; Tummala et al., 2013). At present, the extent of surgical resection still relies heavily on the surgeon's experience. However, the visual and tactile distinction between tumor and healthy tissue is not effective, making it difficult to determine the surgical margins and leaving tiny lesions that can lead to recurrence and

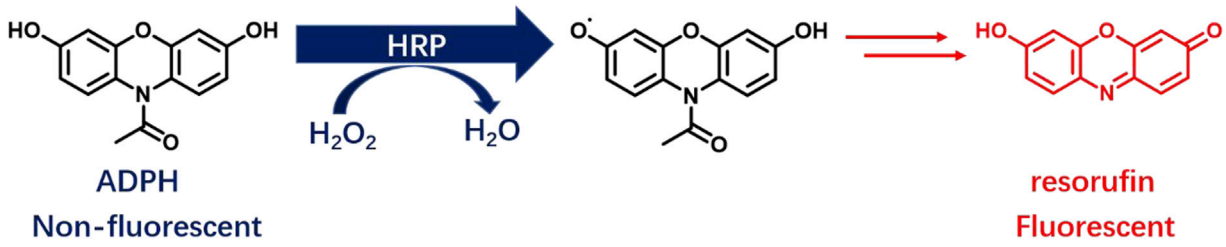

FIGURE 1
Scheme of the oxidative transformation of ADPH to resorufin.

spread after surgery, while the quality of life of patients is seriously affected if the extent of surgical excision is excessively extended. Therefore, the key to the success of surgery is how to accurately locate and image tumors and their microscopic lesions intraoperatively and how to maximize the removal of tumors while protecting healthy tissues as much as possible. Recently, molecular imaging techniques has rapidly advancing in the field of bioimaging due to its advantages of great sensitivity, speedy and immediate imaging, biological safety, ease of detection and low cost, etc. Fluorescence imaging-guided surgery brings hope to solving the abovementioned challenges (Antaris et al., 2016; Zheng et al., 2017; Qi et al., 2018).

Molecular imaging techniques allows non-invasive real-time tumor diagnosis and imaging-guided surgery, assisting surgeons in detecting and resecting tiny tumors sensitively and accurately, significantly improving the therapeutic outcome of tumor surgery. Fluorescent probes could improve the signal-to-background ratio (SBR) by increasing the target signal or decreasing the background signal intensity to enhance the imaging sensitivity and specificity (Owens et al., 2016). Fluorescent probes can generally be divided into two categories: "Always ON" probes and "Turn ON" probes (responsive probes) (Lou et al., 2015; Liu et al., 2017; Jiao et al., 2018; Li and Pu, 2019; Zhang et al., 2019; Xu et al., 2022). "Always ON" probes emit fluorescence continuously under all conditions (whether they reach the target), which increases the background signal and reduces the SBR. In contrast, "Turn ON" probes change the fluorescence signal from "off" to "on" in response to the target (e.g., pH, ROS, or bioenzyme), maximizing the target signal while minimizing the background signal, thus maximizing the SBR, improving the sensitivity and resolution of biosensing (Tang et al., 2019).

Among them, organic fluorescent probes with the tumor microenvironment (TME)-responsive fluorescence-on properties have attracted our interest. The breast TME is a complex ecological environment. In addition to hypoxia, acidosis, elevated levels of lactic acid and adenosine (Cassim and Pouyssegur, 2020), and reactive oxygen species (ROS) levels are much higher than those in normal tissues (Xu et al., 2017; Ma et al., 2020a; Malla et al., 2021; Zhang et al., 2021). Although many organic fluorescent nanoprobes, such as pondus hydrogenii (pH), glutathione (GSH), and other TME responses, have been developed by researchers and applied to imaging-guided surgery, fluorescence probes that respond to high levels of ROS in the TME have rarely been reported. We hypothesized that high levels of ROS in the TME could be used as a bioindicator to identify solid tumors and thus use ROS-responsive fluorescent probes to accurately identify tumor tissue and perform image-guided surgery. In this work, the 10-acetyl-3, 7-dihydroxyphenoxazine (Amplex® Red, ADPH) with excellent ROS responsiveness was prepared as a ROS-responsive nanoprobe, and the performance of ROS-responsive fluorescence imaging was first assessed at the cellular level. Then, its fluorescence imaging sensitivity in complex biological environment *in vivo* was validated in mice models, and its application in image-guided tumor resection was investigated. The results show that the ROS-responsive fluorescent nanoprobe can perform effective fluorescence imaging of tumors and their microscopic lesions, providing a new theoretical basis for the application of responsive fluorescent probes in the surgical navigation of breast cancer.

2 Experimental section

2.1 Materials and methods

All chemicals and reagents were acquired from chemical sources and applied as received. ADPH (Jinming Biotechnology), resorufin (Macklin), 1, 2- distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy- (polyethylene glycol)-2000] (DSPE-PEG_{2000}) (Energy Chemical), hydrogen peroxide (H_2O_2, Enokai Technology), 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT, Sigma), horseradish peroxidase (HRP, Sigma), buthionine sulfoximine (BSO, Macklin). The murine 4T1 breast cancer cell lines were obtained from American Type Culture Collection (ATCC). The absorbance and fluorescence spectra were obtained using a PerkinElmer Lambda 365 spectrophotometer and a HITACHI F-4,700 fluorescence spectrophotometer. Investigation of dynamic light scattering (DLS) using a 90 plus particle size analyzer. The *in vivo* fluorescence imaging was taken by an IVIS Lumina II (Xenogen).

2.2 Preparation of NPs

A compound of fluorescent probes (1 mg), DSPE-PEG_{2000} (4 mg), and dimethyl sulfoxide (DMSO) (1 mL) were sonicated to obtain a clear solution by complete dissolution. The solution was then speedily injected into 10 mL of distilled water over 4 min using a microtip probe ultrasound generator (XL2000, Misonix Consolidated, NY). The compound was then shifted into a permeation bag (molecular weight

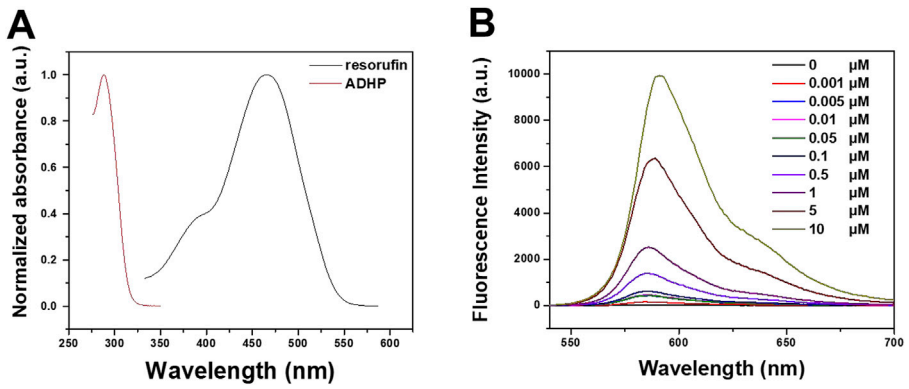

FIGURE 2
(A) Absorption spectra of ADHP and resorufin in DMOS. **(B)** Fluorescence intensity variation of ADPH in response to different concentrations of H_2O_2.

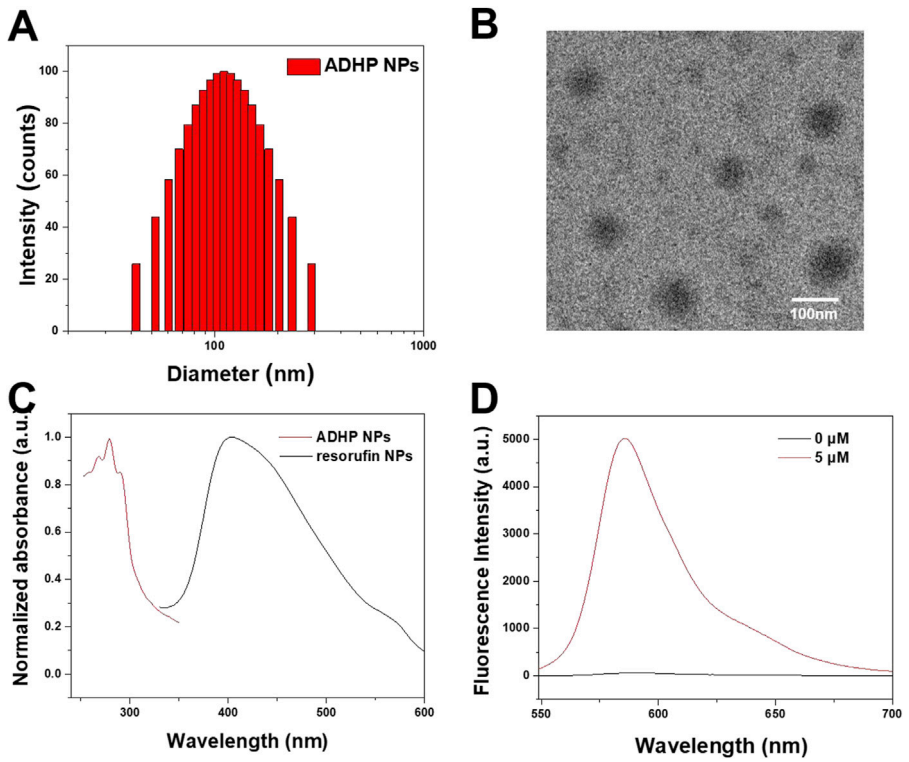

FIGURE 3
(A) DLS profile of ADHP NPs. **(B)** Representative transmission electron microscopy images of the ADHP NPs. **(C)** Absorbance spectra of ADHP NPs and resorufin NPs. **(D)** Fluorescence spectra of ADHP NPs (concentration 0 and 5 μM) after responding with H_2O_2.

cutoff (MWCO) = 5,000 Da), permeated in distilled water for 24 h, ultrafiltered to 1 mL by ultrafiltration (MWCO = 10,000 Da) and filtered through a 0.2 μm syringe filter before use.

2.3 Cell culture

The 4T1 breast cancer cell lines were grown and incubated in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum and 1% penicillin/streptomycin at 37°C, 5% CO_2, saturated humidity in a cell culture incubator.

2.4 *In vitro* cytotoxicity study

4T1 breast cancer cells were inoculated in 96-well plates maintaining a density of 5,000 cells per well and the MTT assay was performed after 24 h of adhesion. The cells were then

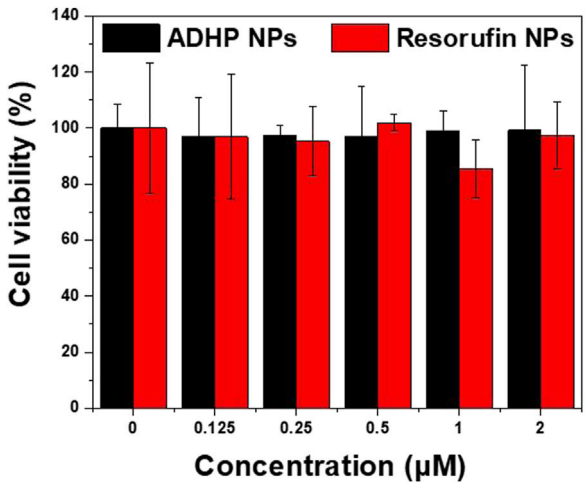

FIGURE 4
The viability of 4T1 cells after incubation with various concentrations of ADHP NPs and resorufin NPs protected from light for 24 h.

incubated with various concentrations of ADHP and resorufin NPs for 24 h. Then 10 μL of freshly made MTT solution (medium concentration of 5 mg/mL) was added to each well. After a total incubation of 4 h, the supernatant was discarded and 100 μL of DMSO was added to dissolve the precipitate, which was gently shaken on a shaker. The absorbance of MTT at 490 nm was measured by an enzymatic standard (GENios Tecan). The absorbance of cells incubated with NPs was expressed as the ratio of the absorbance of cells incubated with NPs to the absorbance of cells incubated in medium only.

2.5 Tumor-bearing mouse model

Following the guidelines of the Tianjin Experimental Animal Use and Care Committee, the overall project protocol was approved by the Animal Ethics Committee of Nankai University. All animal studies were conducted using 6-week-old female BALB/c mice, purchased from the Experimental Animal Centre of the Chinese Academy of Military Medical Sciences. To establish the mice transplantation models for breast cancer, 4T1 cells (5×10^5) were taken and mixed with 100 μL of phosphate buffered saline (PBS) and injected into the peritoneal cavity of mice. Tumors were grown for approximately 7 days followed by fluorescence imaging or surgical treatment experiments.

2.6 *In vivo* fluorescence imaging

Fluorescence imaging of ADHP NPs and resorufin NPs in 4T1 subcutaneous tumor-bearing mice was performed. Mice were intravenous with ADHP NPs or resorufin NPs (200 μL, 30 μM based on ADHP or resorufin) and sacrificed after 24 h, and the major organs (heart, liver, spleen, lungs, kidneys, tumours) were obtained and imaged using IVIS Lumina II.

2.7 Fluorescence imaging-guided tumor surgery

The mice models of peritoneal metastasis of luciferase-expressing 4T1 were intravenous with 200 μL of ADHP NPs (30 μM based on ADHP). 24 h later, the mice were anesthetized with 2% isoflurane, the abdominal cavities were dissected, bioluminescence imaging and fluorescence imaging of the peritoneal metastases were performed, and the metastases and residual microscopic tumor nodules were excised following fluorescence imaging guidance.

2.8 Histological study

Histological analysis was carried out on the tumors excised in the aforementioned fluorescence guided-image surgery. Simply, tumors were immobilized in 4% paraformaldehyde, embedded into wax blocks, and sectioned to a thickness of 5 μm, followed by hematoxylin-eosin (H&E) staining. Pathological sections were ured by a digital microscope (Leica QWin).

3 Results and discussion

3.1 Photophysical properties of molecules

Mammalian cells can produce ROS through a variety of mechanisms, of which H_2O_2 is one of the main types and plays various essential roles in cellular physiological and pathological processes (Cheung and Vousden, 2022). ADPH, catalyzed by HRP, can react with ROS (mainly H_2O_2) to produce resorufin, which has red fluorescence (Zhou et al., 1997) (Figure 1).

The photophysical properties of ADPH and resorufin in DMSO were first investigated. Figure 2A showed the absorption spectra of ADPH in DMSO with a peak at 288 nm, then resorufin with an absorption peak at 467 nm and an emission peak at 570 nm (Supplementary Figure S1). Further the fluorescence intensity variation of ADPH in response to various concentrations of H_2O_2 was studied. The results showed that different concentrations of H_2O_2 (from 0 to 10 μM) reacted with 2 μM ADPH under 0.1 U/mL HRP catalysis, resulting in a concentration-dependent increase in the fluorescence emission intensity of the reaction product at 590 nm (Figure 2B). These results indicate that ADPH can effectively respond to ROS stimulation and turn on the fluorescence signal, which provides the possibility of a responsive tracer.

3.2 Photophysical properties of nanoparticles (NPs)

To increase the aqueous dispersion of ADPH and resorufin molecules, a biocompatible amphiphilic polymer, DSPE-PEG_{2000}, was used as an encapsulation matrix to encapsulate the hydrophobic pores of NPs by nanoprecipitation (Li et al., 2018). The ADPH NPs and resorufin NPs could be uniformly dispersed in water to form pale-red and orange–red solutions with

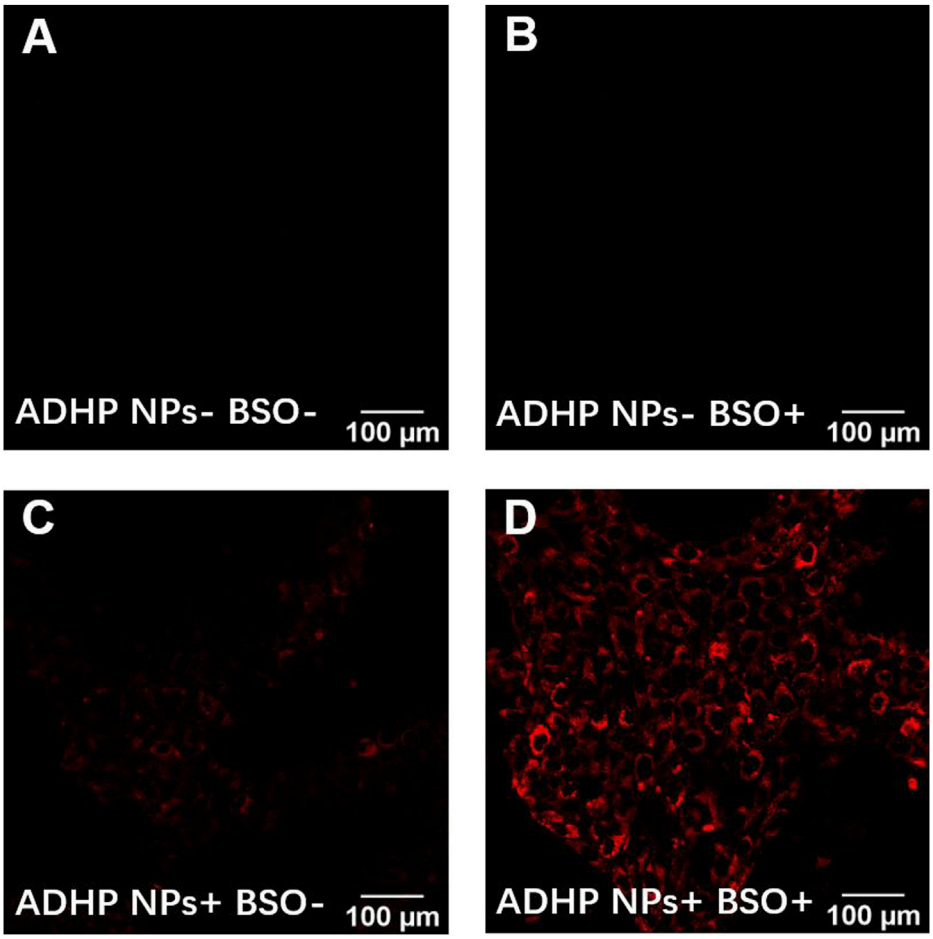

FIGURE 5
Imaging under fluorescence microscope of ADHP NPs *in vitro*: 4T1 cells **(A)** incubated with PBS for 7 h, **(B)** preincubated with PBS for 4 h, later were exposed to BSO (50 mM) for another 3 h, **(C)** incubated with ADHP NPs(2 μM) for 7 h, **(D)**preincubated with ADHP NPs (2 μM) for 4 h, later were added BSO (50 mM) and incubated for another 3 h.

high transparency, respectively. The DLS outcomes indicated that the average hydrodynamic diameters of the ADHP NPs and resorufin NPs were 110.7 nm and 141.43 nm, respectively (Figure 3A; Supplementary Figure S2A), which allowed the NPs to passively target tumor tissue with enhanced permeation and retention (EPR) effects (Cheng et al., 2013). The morphology of the ADHP NPs was characterized by transmission electron microscopy (TEM), showing a homogeneous spherical structure with an average diameter of about 110 nm (Figure 3B). The slightly larger diameter measured by DLS relative to the TEM results may be due to the shrinkage of nanoparticles during TEM sample preparation. Both NPs and resorufin NPs showed good colloidal stability in 10% serum aqueous solution (Supplementary Figure S3), and the nanoprobe solution remained clear and transparent for a week. Furthermore, the UV absorption and fluorescence spectra of the nanoprobes were investigated in an aqueous solution. The maximum absorption peak of ADHP NPs was located at 281 nm, and the emission peak was located at 585 nm after responding with H_2O_2 (Figures 3C, D). The maximum absorption and emission peaks of resorufin NPs were located at 404 nm (Figure 3C) and 594 nm (Supplementary Figure S2B), respectively.

3.3 Biocompatibility of NPs

Biocompatibility is key to the application of nanoprobes. Prior to the *in vivo* study, the cytotoxicity of ADHP NPs and resorufin NPs *in vitro* were first examined. As shown in Figure 4, after coincubation with 4T1 breast cancer cell lines for 24 h with different concentrations of NPs, the cell survival rate was higher than 95% in each group, and no significant cytotoxicity was observed. The cellular uptake of the nanoprobes was subsequently examined. Confocal microscopy showed that resorufin NPs could be effectively internalized and localized in the cytoplasm after coincubation with 4T1 cells for 4 h (Supplementary Figure S4). These results suggest that the NPs are biocompatible and can be effectively taken up by breast cancer cells.

3.4 Fluorescence imaging of ADHP NPs *in vitro*

ROS play a crucial role in the breast TME and are associated with a range of pathophysiological processes, including regulating cell proliferation, activating oncogenes, mediating genomic instability, inducing inflammation, initiating metabolic reprogramming, and

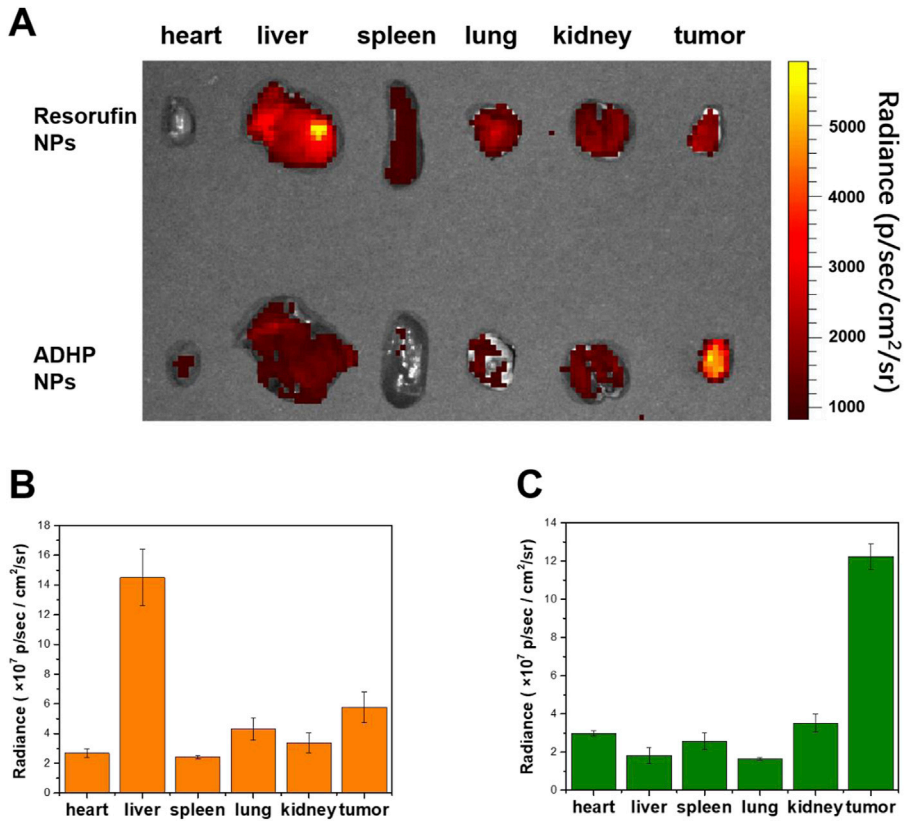

FIGURE 6
(A) *Ex vivo* fluorescence imaging of separate tissues of 4T1 subcutaneous tumor-bearing mice injected intravenously of 200 μL of ADHP NPs or resorufin NPs (30 μM based on each molecule) for 24 h. **(B)** Mean fluorescent signals of dissimilar tissues based on the images in the resorufin NPs group. **(C)** The average fluorescent signals in dissimilar tissues based on the images in the ADHP NPs group.

promoting metastasis (Kalyanaraman et al., 2018; Weinberg et al., 2019). Many studies have confirmed that ROS levels in the breast TME are higher than those in normal tissues (Malla et al., 2021). To validate the ROS response of ADHP NPs and the ability to image cells *in vitro*, the BSO, a glutamylcysteine synthase inhibitor, was used to increase intracellular H_2O_2 levels. Confocal fluorescence microscopy results showed that in the two groups without the addition of ADHP NPs, no fluorescence signal was detected in the group with or without BSO (Figures 5A, B). In the other two groups, after co-incubation with ADHP NPs for 4 h, one group added 50 mM BSO and incubated for another 3 h. As shown in Figure 5C, there was a weak fluorescence signal in the BSO (−) group, indicating a low level of ROS in 4T1 cells cultured *in vitro*, while the fluorescence brightness in the BSO (+) group was significantly enhanced (Figure 5D). It is suggested that the addition of BSO can significantly increase the level of ROS in tumor cells, and ADHP NPs as a responsive fluorescent probe can fully respond and turn on the fluorescent signal for imaging.

3.5 Fluorescence imaging of tumors

In vitro experiments confirmed that ADHP NPs can respond to ROS in tumor cells to switch on the fluorescent signal; therefore, the *in vivo* performance of ROS-responsive fluorescent probes for fluorescence imaging in tumor-bearing mice was further assessed by calculating the tumor-liver signal ratio. ADHP NPs and resorufin NPs were intravenously injected into 4T1 subcutaneous tumor-bearing mice, respectively. The mice were sacrificed 24 h later, and the tumors and major organs were removed for fluorescence imaging at the same time (Figure 6A). *Ex vivo* fluorescence imaging showed that the liver, lung, and tumor in the resorufin NPs group displayed fluorescence, of which the liver had the strongest fluorescence intensity. The fluorescence ratio of the tumor to the liver was only 0.4 (Figure 6B), suggesting that the tumor-to-liver signal ratio of the "Always ON" fluorescent probe was low. Due to the strong liver fluorescence signal background during *in vivo* imaging, it is difficult to distinguish the tumor fluorescence signal. In contrast, in the ADHP NPs group, although the liver, lung, and tumor showed fluorescence signals, the fluorescence ratio of the tumor to the liver was as high as 6.67 (Figure 6C). These results suggest that because of the higher ROS levels in tumor tissue than in normal tissue, ROS-responsive ADHP NPs can respond adequately to them and switch on the fluorescent signal. Thus, the interference of background signals in fluorescence imaging can be minimized. More importantly, the fluorescence signal of the liver is negligible, which improves the sensitivity and resolution of fluorescence

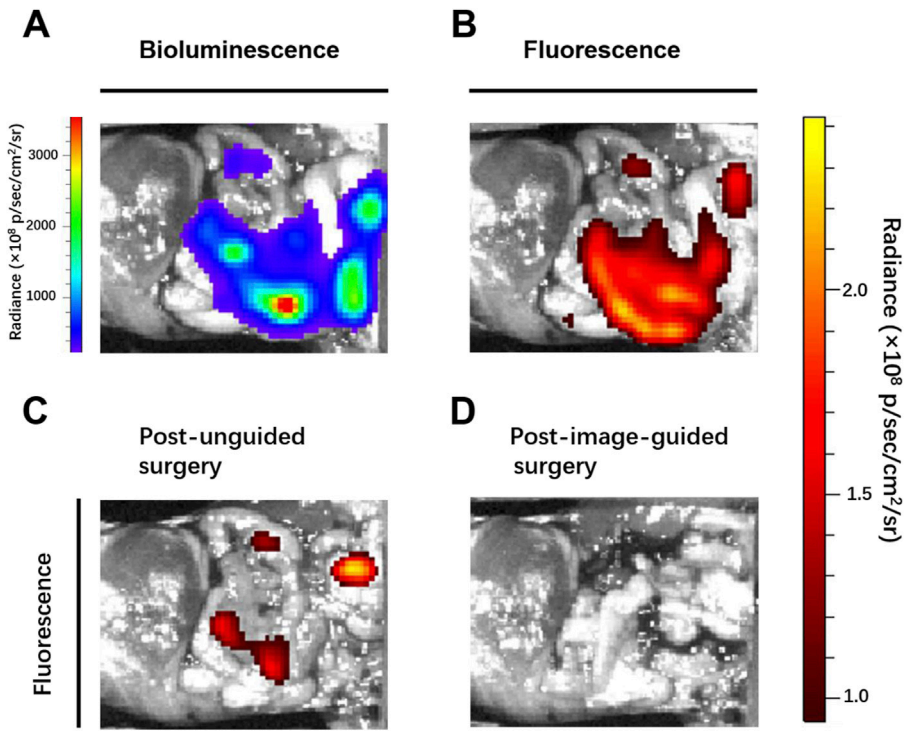

FIGURE 7
Mice with peritoneal metastases were intravenously injected with 200 μL of ADPH NPs (30 μM based on ADHP) and operated on 24 h after injection: **(A)** bioluminescence and **(B)** fluorescence images of the peritoneal cavity of mice before tumor resection. Fluorescent images typical of mice **(C)** post unguided surgery and **(D)** post second surgery with guidance of the fluorescence of ADHP NPs.

imaging and shows great advantages in precise image-guided tumor surgery.

3.6 Fluorescence imaging-guided tumor surgery

Breast cancer is the commonest malignancy in women and is prone to distant metastases in the lung, liver, bone and brain, and is a major cause of death (Ahmad, 2019; Slamon et al., 2019). Patients with distant metastases have a poor prognosis, the five-year survival rate was only 27% (DeSantis et al., 2019). In clinical surgical oncology, intraoperative imaging to precisely locate tumor nodes, detect and completely remove all tumor lesions can greatly improve the success rate of surgery and avoid tumor recurrence. After abdominal metastasis of breast cancer, many tiny tumor nodules are scattered in the peritoneal cavity, leading to further metastasis, and spread of the tumor, and the survival rate of patients is significantly reduced. Therefore, accurate preoperative evaluation and intraoperative real-time imaging are necessary. Thus, we established mice models of peritoneal metastasis of 4T1 breast cancer cells to assess the ability of ADHP NPs as an ROS-responsive nanoprobe to identify microscopic tumor nodules *in vivo*. To detect the tumor distribution, we selected luciferase-expressing 4T1 breast cancer cell lines, which showed bioluminescence after injection of luciferin. After intravenously injecting ADHP NPs (200 μL, 30 μM based on ADHP) into a mouse for 24 h, the mouse's abdominal cavity was dissected for bioluminescence imaging and fluorescence imaging. As a result of the EPR effect of tumors (Cheng et al., 2013), ADPH NPs can be enriched in the tumor site and the fluorescence signal of ADHP NPs completely coincided with the bioluminescence signal of fluorescein in the peritoneal cavity. It is proved that ADHP NPs can turn on the fluorescence signal in response to the high concentration of ROS in TME and accurately locate the tumor lesions. (Figures 7A, B).

Clinically, surgeons mainly rely on the naked eye to distinguish which tissues need to be removed and retained. Although sizeable tumors (>1 mm) were excised by the surgeon (the First Affiliated Hospital of Nanjing Medical University) after naked eye resolution, there were still some tiny (submillimeter level) unidentifiable tumor nodules remaining. With the help of the fluorescence signal of the high SBR of AHDP NPs, the residual tiny metastases in the peritoneal cavity of the tumor-bearing mice were clearly visible. Therefore, a second surgical resection was performed under the guidance of fluorescence imaging signals, and the tumors were observed to be excised postoperatively (Figures 7C, D). The operation took approximately 15 min. The bioluminescence signals and fluorescence signals of all resected tumor nodules completely overlapped (Figures 8A, B). Histological staining confirmed that the resected tissues were tumors (Supplementary Figure S5). In this study, by ROS responsive fluorescence signal of ADHP NPs, we maximized the fluorescence signal intensity in tumor tissue, while minimizing the background fluorescence signal intensity, providing high resolution real-time fluorescence images

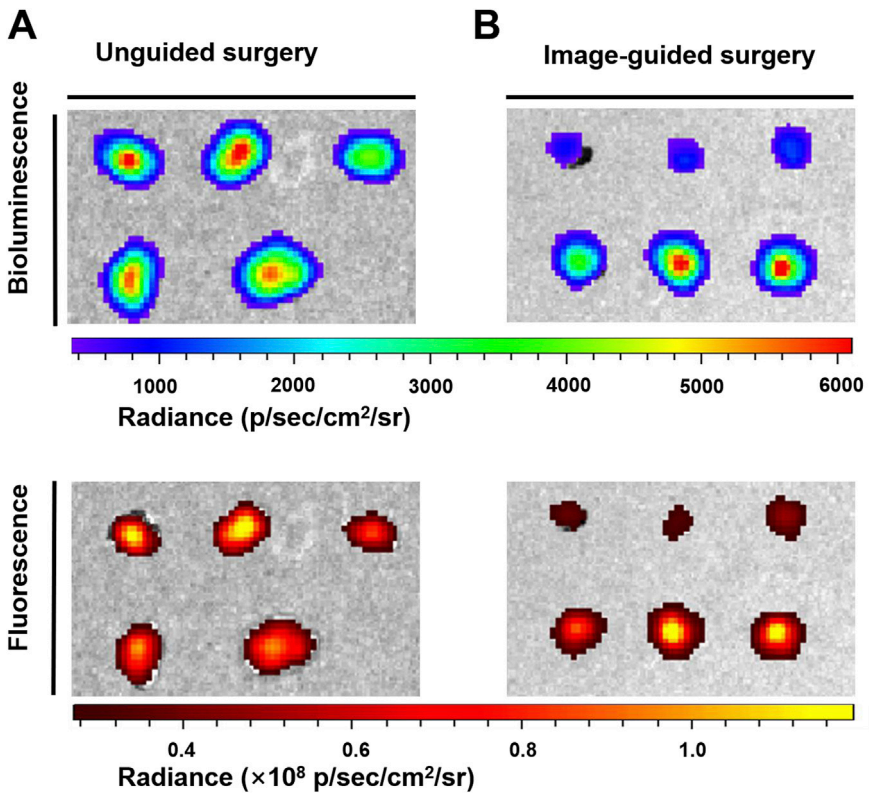

FIGURE 8
The peritoneal carcinomatosis-bearing mice were intravenously injected with 200 μL of ADPH NPs (30 μM based on ADHP) followed by the operation of the abdomen at 24 h after injection: bioluminescence and fluorescence images typical of resected tumor nodules **(A)** post unguided surgery and **(B)** post the second surgery with guidance of fluorescence imaging of ADPH NPs.

about the tumor tissue during surgery, improving the detection rate of microscopic lesions, assisting surgeons in pinpointing tumor lesions, improving the efficacy of surgical resection, and greatly reducing the risk of tumor recurrence.

4 Conclusion

In conclusion, we prepared a ROS-responsive fluorescent probe based on ADHP, which has ROS-responsive properties. *In vitro* studies have shown that ADHP NPs can effectively respond to ROS in tumors to turn on the fluorescent signal for imaging and have good biocompatibility. Using resorufin NPs as the contrast agent, the tumor-to-liver signal ratio of ADHP NPs was first measured *in vivo*, demonstrating that ROS-responsive fluorescent probes can effectively reduce the background signal interference in fluorescence imaging. Subsequently, under the real-time fluorescence signals guidance of ADHP NPs, peritoneal metastases and their boundaries with normal tissues were clearly displayed, and the tiny tumor lesions were accurately located and resected. The ROS-responsive fluorescence probes can minimize the background signal in fluorescence imaging and provide accurate intraoperative visualization of submillimeter tumor lesions with higher sensitivity and resolution, ensuring complete tumor removal. The ROS-responsive fluorescence probe offers more possibilities for the future use of responsive fluorescence probes in surgical navigation.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was reviewed and approved by the Animal Ethics Committee of Nankai University.

Author contributions

KS and RX designed and conducted a series of experiments. BX analyzed the experimental data. PL and JB proofread the manuscript and the methods. YT and XL supervised the experiments and reviewed the manuscript. QT provided funding and supervised the experiments. All the authors read and approved the final manuscript.

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Conflict of interest

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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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fchem.2023.1121957/full#supplementary-material

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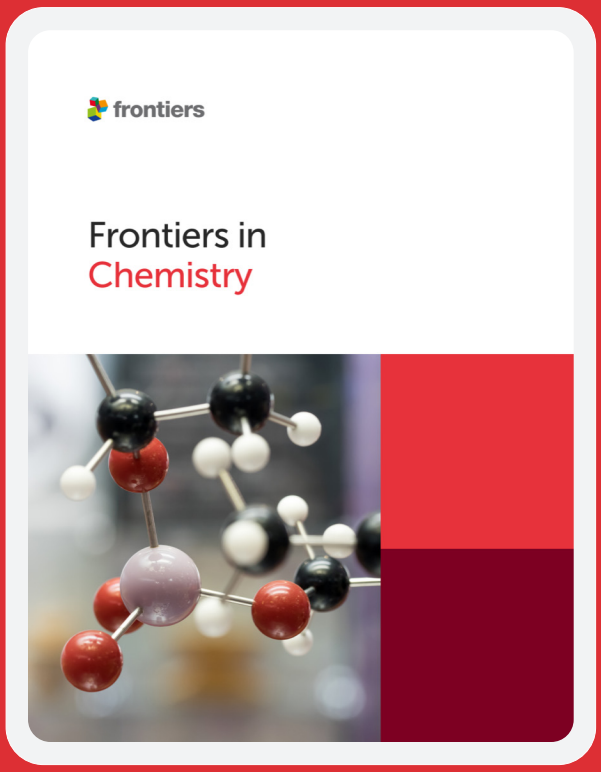

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