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Review Article

Polymer Nanoparticle-Based Chemotherapy for Spinal Malignancies

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Malignant spinal tumors, categorized into primary and metastatic ones, are one of the most serious diseases due to their high morbidity and mortality rates. Common primary spinal tumors include chordoma, chondrosarcoma, osteosarcoma, Ewing's sarcoma, and multiple myeloma. Spinal malignancies are not only locally invasive and destructive to adjacent structures, such as bone, neural, and vascular structures, but also disruptive to distant organs (e.g., lung). Current treatments for spinal malignancies, including wide resection, radiotherapy, and chemotherapy, have made significant progress like improving patients' quality of life. Among them, chemotherapy plays an important role, but its potential for clinical application is limited by severe side effects and drug resistance. To ameliorate the current situation, various polymer nanoparticles have been developed as promising excipients to facilitate the effective treatment of spinal malignancies by utilizing their potent advantages, for example, targeting, stimuli response, and synergetic effect. This review overviews the development of polymer nanoparticles for antineoplastic delivery in the treatment of spinal malignancies and discusses future prospects of polymer nanoparticle-based treatment methods.

1. Introduction

Malignant spinal tumors are classified as primary or metastatic ones. Although the morbidity rates of primary malignant spinal tumors (e.g., chordoma, chondrosarcoma, osteosarcoma, Ewing's sarcoma, and multiple myeloma) are low—less than 5% out of all osseous neoplasms and 0.2% out of all cancers [1]—the malignancy rate is strikingly high. Additionally, as the occurrence of metastatic disease becomes much more common, patients are increasingly susceptible to a large number of metastatic tumors from lung, breast, prostate, renal cell, gastrointestinal neoplasms, and so forth that can pervade the spine [2]. According to statistics, 5 to 14% of cancer patients are afflicted with spinal metastatic tumors, which have a high morbidity rate [2, 3]. Currently, the mainstays of spinal tumor treatment include corticosteroids, bisphosphonates, radiotherapy, surgery, and chemotherapy [4]. Despite the fact that comprehensive treatments can effectively improve survival rates, many concomitant shortcomings still exist. With the development of cancer nanotechnology and pharmaceutical nanotechnology, new directions of research and improvements to malignant spinal tumor treatment may be discovered.

1.1. Epidemiology and Pathogenesis. Primary malignant spinal tumors are far less common compared to metastatic spinal tumors, which account for less than 0.2% of all neoplasms [1]. However, primary malignant spinal tumors not

only are difficult to identify and cure but can also easily recur [5]. They arise from bones and soft tissues around spinal columns, presenting a challenge to diagnosis and treatment [6]. The early symptoms of primary malignant spinal tumors are pain, spinal instability, and compression of the spinal canal with ensuing neurological deficits [7]. Given that neurological symptoms are observed in approximately 52% of primary tumors, which often lead to poor prognoses [8, 9], patients should be wary upon discovering solitary osseous lesions associated with back pain. Although en bloc spondylectomy, radiotherapy, and neoadjuvant chemotherapy have made great progress and prolonged survival rates to a certain extent, issues, such as surgery-induced tumor cell dissemination [10] and spinal radiation sickness still remain [1]. Therefore, it is urgent to explore more safe and effective treatments for primary spinal tumors.

According to reports by the International Agency for Research on Cancer (IARC), prostate cancer in men and breast cancer in women possess the highest incidence rates among the myriad cancers in the world. These two types of cancer, along with lung cancer, cause 15–20% of all secondary metastatic spinal tumors [11], with non-Hodgkin's lymphoma, renal cell cancer, and multiple myeloma accounting for an additional 5–10% and colorectal cancer, sarcoma, neuroblastoma, Hodgkin's disease, and germ cell tumor accounting for the rest [4]. Like primary spinal tumors, metastatic spinal tumors have three main symptoms—pain, spinal instability, and neurological deficits [12, 13]. It is worth mentioning that metastatic epidural spinal cord compression (MESCC) is the most exigent symptom of metastatic spinal tumors because it may cause patients to become paraplegic if not properly treated in time [14]. Due to the axial skeleton containing a higher percentage of red bone marrow, it is a more common target site, to which tumors metastasize as compared with the appendicular skeleton. The main afflicted areas include the ribs, pelvis, and spine, with partial metastasis through the Batson venous plexus, which bypasses the lung circulation [15]. The most critical form of metastasis, observed in 85% of patients, occurs via an indirect route, through which an initial haematogenous metastasis traverses to the vertebral body [16]. Due to the high rate of incidence and recurrence of metastatic spinal tumors, more effective treatment methods (e.g., targeted nanomedicine) are urgently needed.

1.2. Construction of Animal Models. In order to better investigate spinal malignancies, researchers have developed and established various models of spinal metastasis. Early on, Mantha et al. developed an intraosseous spinal tumor model in rats [17], which soon became a classic model that was popularized by many researchers. Briefly, CRL-1666 breast adenocarcinoma was intraperitoneally implanted into the L-6 vertebral body of rat, after which the authors used functional and histological analysis methods to evaluate tumor proliferation and spinal cord compression. With further development of experimental techniques, more advanced animal models that enable the laboratory study of human spinal metastasis became an urgent need. Tatsui and coworkers developed an orthotopic model of spinal metastasis by using a transperitoneal surgical approach to implant PC-14 human lung tumors into the L-3 vertebral body of nude mice [18]. Furthermore, at the 2012 Spine Section Meeting, Zadnik and collaborators presented a novel animal model of human breast cancer metastasis to the spine that uses intracardiac injection and luciferase-expressing MDA-231 human breast cancer cells [19]. This new model has served as a powerful tool for studying the effect of human cancer metastasis on spinal behavior, thereby greatly benefiting patients with breast cancer.

However, these methods require complex surgical procedures, making it difficult to ensure the reproducibility and consistency of the models. To address this issue, Ziblys' group used a dorsal approach to intraosseously implant CRL-1666 adenocarcinoma tissue obtained from other subcutaneous tumor-bearing rats [20]. This improved rat model simplifies the surgical procedure, shortens the operation time, and reduces mortality. More importantly, it ensures consistent and reproducible tumor growth, resulting in spinal cord compression and related neurological symptoms. Apart from this, Wang and colleagues created a murine model of renal cell carcinoma (RCC) spinal metastasis that enables the testing of targeted therapies for RCC with spinal involvement [21]. Also, Liang et al. established a model of human breast cancer metastasis through intraosseous injection of tumor cells [22]. The aforementioned studies provide us with a basis for studying spinal malignancies and developing targeted therapies for their effective treatment.

1.3. Therapy Status. Treatment of spinal malignancies is quite difficult and complicated. Existing methods of treatment that are generally accepted include surgery, radiotherapy, and chemotherapy. The development of surgical techniques and radiosurgical technology has significantly improved the efficacy of spinal malignancy treatment [23]. Currently, one of the most popular treatments is wide spinal resection using either en bloc laminectomy [24] or hemilaminectomy [25], followed by en bloc corpectomy and dorsoventral stabilization [10]. On the other hand, Patchell et al. found that direct decompressive surgery combined with postoperative radiotherapy is more effective than radiotherapy alone for treating metastatic cancer-induced spinal cord compression [26]. For example, proton beam radiotherapy (PBRT), which utilizes ionizing radiation with reduced scatter in surrounding tissues, can be used to cure unresected or partially resected primary tumors, such as chordoma of the cervical spine, mobile spine, and sacrum [27]. Subsequently, stereotactic radiosurgery (SRS) was developed to reduce radiation damage of surrounding tissues [1], but it did not achieve the desired clinical effect. Nevertheless, this multidisciplinary approach shows great promise of offering patients who undergo treatment for metastatic and primary tumors of the spinal column the best chance of long-term survival.

Excitingly, advances in the past few decades have given rise to promising alternatives for treating malignant spinal tumors in the form of nanotherapeutics and nanodiagnostics, many of which have been commercialized or have reached clinical trials [28]. Among these advances, the most important is the development of polymer nanoparticles with antineoplastic drugs adsorbed onto them [29]. As depicted in

Figure 1, compared with traditional chemotherapeutic drugs, antineoplastic drug-loaded polymer nanoparticles have obvious advantages in several applications: (a) promotion of stimuli-responsive release: drugs in the nanocarriers can be released slowly or controllably through endogenous intracellular stimuli (e.g., pH, reduction, reactive oxygen species, or specific enzymes) [30–45] or exogenous excitations (e.g., light, temperature, or voltage) [46–48]; (b) synergetic therapy: different drugs encapsulated in the same polymer nanoparticles can be smartly released to achieve synergistic and joint effects [49, 50]; (c) crossing of biological barriers: antineoplastic drug-loaded polymer nanoparticles can be delivered orally or across the blood-brain barrier [51] as well as escape from intracellular autophagy; (d) targeted therapy: nanoparticles can be used to identify various tumors *via* targeting ligands conjugated onto their surfaces [52, 53]; (e) enhanced tumor accumulation: the nanosized platforms can facilitate the localization of drugs to tumor tissue through the enhanced permeability and retention (EPR) effect [54, 55]; (f) prolonged circulation time: high-molecular-weight polymer nanoparticles can increase the half-life of encapsulated drugs in the blood, effectively prolonging the drug retention time in the lesion [56, 57]. Due to the aforementioned advantages, polymer nanoparticles can greatly improve the efficacy of treatment and reduce the risks associated with radiosurgery. With advances in research and developing applications of polymer nanoparticles, the treatment of malignant spinal tumors has reached a new turning point.

2. Drug-Encapsulated Polymer Nanoparticles for Chemotherapy of Spinal Malignancies

In this part, we will review polymer nanoparticle-based delivery systems for spinal malignancy treatment by the type of therapeutics that is employed (Figure 1). Specifically, single-agent systems and multidrug synergistic formulations will be discussed in detail.

2.1. Single-Agent Platforms. Doxorubicin (DOX) as a traditional chemotherapeutic drug is widely used in cancer treatment, particularly for spinal tumors. However, the dose-dependent cardiotoxicity, myelosuppression, nephrotoxicity, and development of multidrug resistance associated with unformulated DOX limit its therapeutic efficacy [58]. Previously, research efforts were undertaken to improve this situation through the use of polymer nanoparticles. The nanoparticulate system is based on biodegradable, biocompatible, and Food and Drug Administration- (FDA-) approved components, so it possesses reduced systemic side effects. In another case, Subia and collaborators designed a silk fibroin-based cytocompatible 3D scaffold as an *in vitro* 3D distribution model, with which the efficiency of DOX-loaded, folic acid-conjugated silk fibroin nanoparticles as a drug delivery system for the treatment of human breast adenocarcinoma was evaluated. Their experimental results demonstrated that the drug-loaded folate-conjugated nanoparticles can effectively recognize cancer cells in the 3D *in vitro* bone metastasis model, suggesting that these polymer nanoparticles can be used as potential therapeutic agents in the treatment of breast cancer bone metastasis, especially spinal metastasis [59]. The two studies described above indicate that the DOX-loaded nanoparticles can potentially be used to treat spinal malignancies, including primary (e.g., fibrosarcoma) and metastatic (e.g., breast cancer) ones.

Additionally, Ding's group synthesized three poly(ethylene glycol)-polyleucine (PEG-PLeu) di- or triblock copolymers through ring-opening polymerization (ROP) of leucine *N*-carboxyanhydride (Leu NCA) with amino-terminated PEG as a macroinitiator [60, 61]. DOX was loaded into micelles through a nanoprecipitation technique. The copolymers could spontaneously self-assemble into micelles in PBS at pH 7.4 (Figure 2(a)). The DOX-loaded micelles could be efficiently taken up through endocytosis and exhibited effective drug release (Figure 2(d)) in both MG63 and Saos-2 cells, which are two types of human osteosarcoma cell lines. Notably, the DOX-loaded micelles improved the antiosteosarcoma efficiency (Figures 2(b) and 2(c)). Overall, the chirality-mediated polypeptide micelles based on triblock PEG-PLeu copolymers, with enhanced chemotherapeutic efficacies and reduced side effects, show great potential for application in the treatment of osteosarcoma, which is one of the most severe primary malignant spinal tumors with high incidence and low survival rates.

Stemming from the need for a form of cancer treatment that maximizes drug exposure to the diseased tissues while minimizing off-target side effects, targeted therapy has been proposed as a promising alternative to current treatment options, which are suboptimal and have low efficacies, for treating spinal malignancies, such as osteosarcoma, chordoma, and bone metastatic cancer. For instance, Morton and coworkers used layer-by-layer assembly to generate tissue-specific functional drug carriers for treating primary osteosarcoma [62]. Specifically, this was accomplished *via* surface modification of drug-loaded nanoparticles with an aqueous polyelectrolyte, poly(acrylic acid) (PAA) that was side-chain-functionalized with alendronate. The results showed that the DOX-loaded liposomal nanoparticles accumulated in subcutaneous 143B osteosarcoma xenografts, significantly attenuated the tumor burden, and prolonged animal survival time. More significantly, the authors demonstrated that these functional nanoparticles are also highly promising for future research on the treatment of bone-localized metastases of invasive cancer cell types, such as breast and lung cancers.

Nanotechnology has played an important role in improving the efficiency of a variety of chemotherapeutic drugs, one of which is paclitaxel (PTX), a representative microtubule-stabilizing chemotherapy drug that can be used to treat ovarian cancer, breast cancer, lung cancer, multiple myeloma, and other forms of cancer [63, 64]. Previous PTX-based therapies have shown limited therapeutic efficacies because the poor water solubility and low permeability of PTX often cause severe allergic reactions. Recently, nanoparticles and micelles have been developed as PTX delivery vehicles to improve its water solubility.

Methotrexate (MTX), an antifolate and antineoplastic agent, suppresses tumor cell growth and reproduction by inhibiting dihydrofolate reductase (DHFR) to terminate DNA biosynthesis of tumor cells and can be used to treat

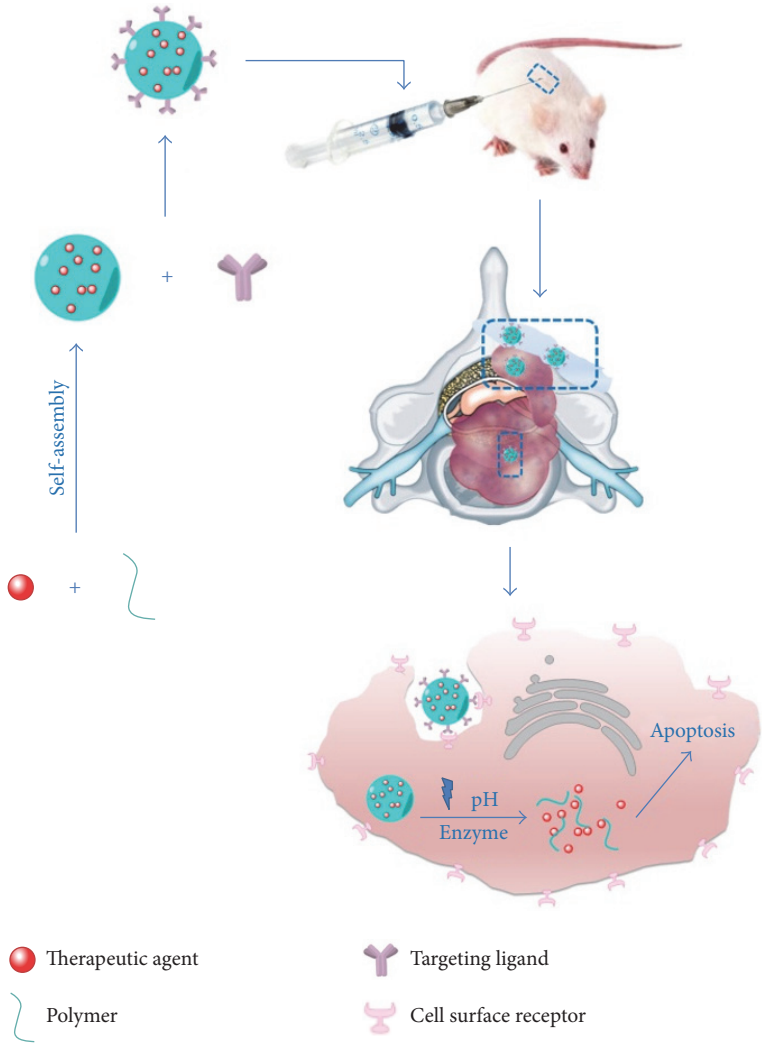

Figure 1: Schematic illustration for formation of polymer nanoparticle, *in situ* administration, slow and sustained release *in vivo*, accumulation in tumor tissue, and controlled intracellular drug release of polymer nanoformulation. Upon entering the tumor cells, the nanoparticle releases the therapeutic agents when subjected to stimuli, such as light activation, enzyme, and lower pH (pH < 6.8), thereby inducing cell death.

osteosarcoma, chondrosarcoma, rheumatoid arthritis, giant cell tumor, and other diseases. Recently, a few works have reported that a combination of MTX and nanomaterials was utilized to effectively inhibit bone tumor formation. In one such study, Li et al. designed a new implant that uses an intermediate layer of anionic nanoparticles, comprising poly(L-lysine) (PLL) and heparin (Hep), that is sandwiched between chitosan/methotrexate (CS/MTX) layers and dopamine-Ti (DA-Ti) substrates [65]. They used various functionalized Ti substrates to culture osteoclastoma cells and investigated cell adhesion, cytoskeleton, proliferation, cytotoxicity, and apoptosis. Moreover, they assayed the growth of *Staphylococcus aureus* in the different Ti substrates (Figure 3) and discovered that CH-MTX-Ti substrates not only effectively inhibited cell proliferation and induced apoptosis, but also resisted adhesion and bacteria growth.

Ifosfamide (IFS), a broad-spectrum and cell cycle nonspecific antitumor drug, can not only cross-link with DNA and hinder DNA synthesis, but also interfere with the function of RNA. It has been reported that IFS not only significantly improved event-free survival and overall survival, but also increased good histologic response rate in patients with osteosarcoma [66], making it an effective antineoplastic agent for the treatment of osteosarcoma. In one study, Chen and coworkers designed and prepared acid-sensitive IFS-loaded poly(lactic-co-glycolic acid-) (PLGA-) dextran polymer nanoparticles (PD/IFS) to inhibit MG63 and SaOS-2 cancer cells [67]. First, PLGA-dextran formed self-assembled polymer micelles in the aqueous medium, and the particle size of PD/IFS was observed to be 124 ± 3.45 nm (Figure 4(a)) with an excellent dispersity index of 0.124 (PDI). Second, IFS was effectively entrapped in the nanoparticles with a loading and encapsulation efficiency of (20.15 ± 3.5)% and (89 ± 1.95)%, respectively. They found that these drug-loaded core-shell nanocarriers promoted sustained drug release at pH 7.4 and induced accelerated release at pH 5.0 (Figure 4(b)).

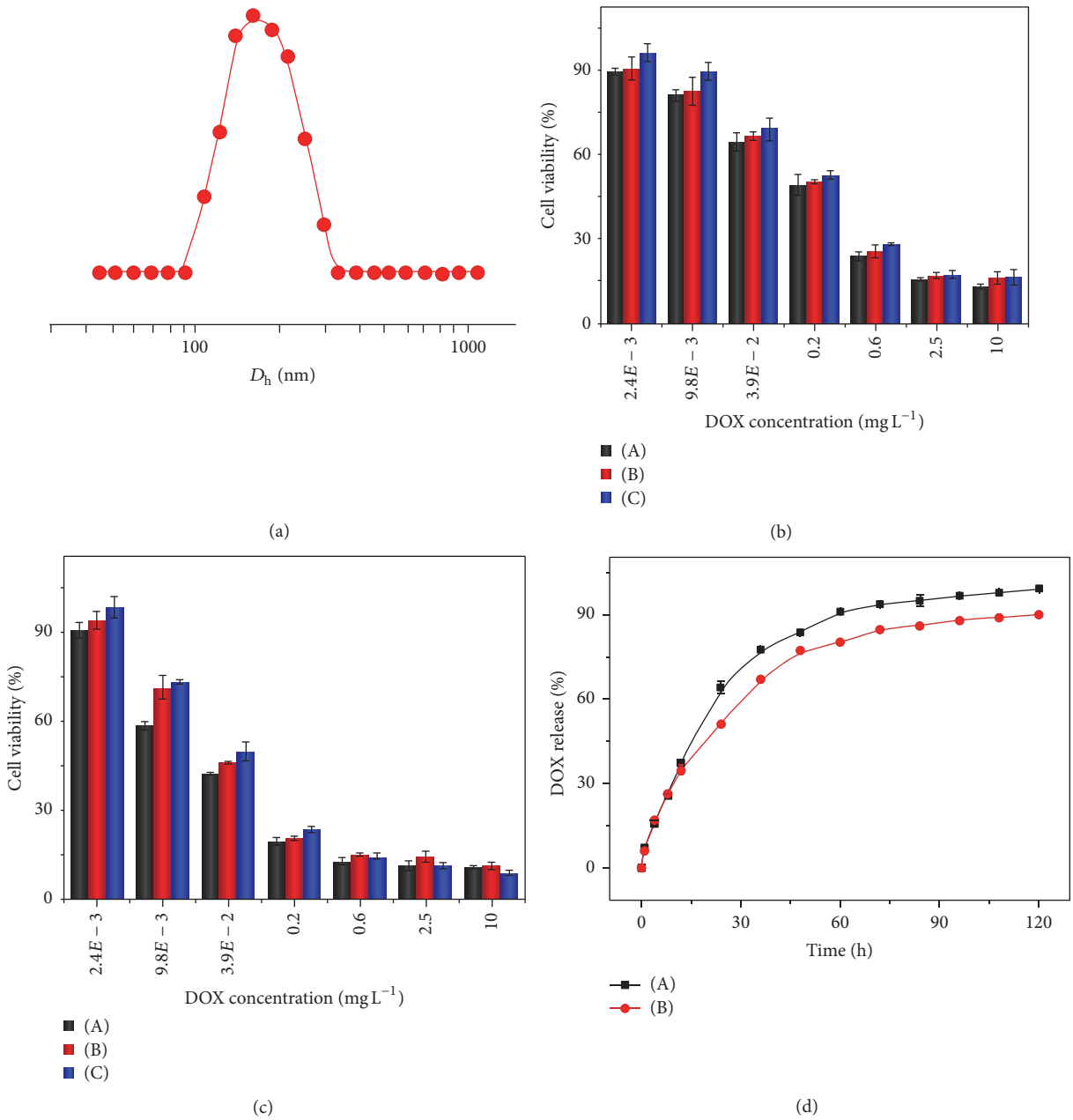

Figure 2: (a) Typical D_h of P(D,L-Leu)-*b*-PEG-*b*-P(D,L-Leu) (PDL) micelle. *In vitro* cytotoxicities of (A) DOX-loaded P(L-Leu)-*b*-PEG-*b*-P(L-Leu) (PL), (B) PDL micelles, and (C) free DOX against (b) MG63 and (c) SaOS-2 cells after incubation for 72 h. Data are presented as mean ± standard deviation (SD; $n = 3$). (d) Release plots of DOX from DOX-loaded PL (A) and PDL micelles (B) in PBS. Data are presented as mean ± SD ($n = 3$).

Analysis of *in vitro* MG63 and Saos-2 anticancer activity corroborated that PD/IFS nanoparticles demonstrated higher antitumor activity and greater induction of apoptosis than using free IFS (Figures 4(c) and 4(d)). This study suggests that nanoparticulate encapsulation of the antitumor agent increases the therapeutic efficacy and may be a promising method for treating malignant spinal tumors.

Multiple myeloma, another common primary tumor of the spine, is incurable and gives rise to complications that include extensive osteolytic bone destruction, renal failure, anemia, and hyperkalemia [68, 69]. In their work on improving the efficacy of targeted therapy for multiple myeloma, Pan and coworkers proposed the new concept of integrating Sn2 lipase-labile phospholipid prodrugs with contact-facilitated drug delivery. Their drug targets the b-HLHZ ip transcription factor c-Myc(MYC), which is a powerful oncogene that activates the development of myeloma [70]. An index compound (10058-F4) was synthesized and modified into the Sn2 prodrug form, c-Myc-inhibitor-1 prodrug (MI1-PD) [71]. The Sn2 phospholipid prodrug MI1-PD significantly increased

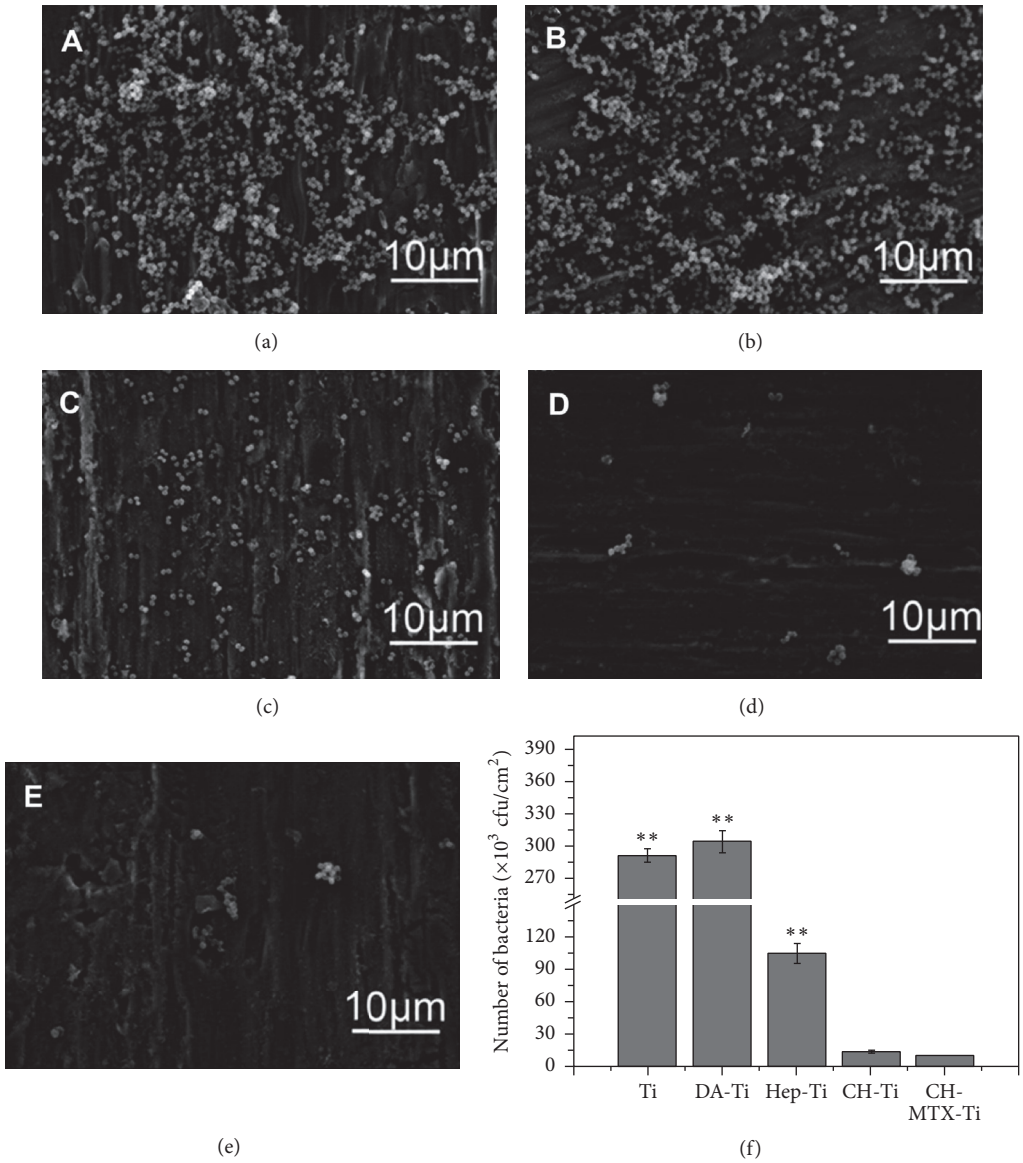

FIGURE 3: SEM images of differently functionalized Ti substrates after exposure to the *S. aureus* suspension (10^7 cfu mL^{-1}) in PBS for 8 h: (a) pristine Ti, (b) DA-Ti, (c) PLL/Hep-Ti, (d) CH-Ti, and (e) CH-MTX-Ti. (f) The number of *S. aureus* (cfu) in culture normalized to the area of the substrates after the bacterial cell culture interacted with various Ti substrates for 24 h. ** denotes significant differences compared to substrates modified with the CH-MTX-Ti group. The error bars represent the standard deviations calculated from three independent experiments.

drug potency against human (H929 and U266) and mouse (5TGM1) myeloma cells in cytotoxicity tests. They also found in an orthotopic mouse model of disseminated myeloma that administration of VLA-4-targeted MI1-PD nanoparticles significantly reduced the tumor burden (as reflected by serum immunoglobulin) and increased the survival rate. In another work, Ashley and collaborators designed VLA-4-targeted liposomal carfilzomib (CFZ) nanoparticle (TNP[CFZ]) that targeted VLA-4-expressing multiple myeloma cells. The liposomal CFZ nanoparticle (NP[CFZ]) was prepared by incorporating CFZ into the liposome using a passive loading technique (Figure 5(a)) [72]. Compared to free CFZ, both NP[CFZ] and TNP[CFZ] demonstrated increased cytotoxicity *in vitro*, induced apoptosis, and maintained significant tumor growth inhibition *in vivo* while reducing systemic toxicities. Moreover, when TNP[CFZ] was administered in combination with free DOX, significant synergism was observed in multiple myeloma.1S (combination index: 0.533) and NCI-H929 cells (combination index: 0.583) (Figures 5(b) and 5(c)). These studies indicated that both first-generation liposomal CFZ nanoparticles and Sn2 lipase-labile phospholipid prodrugs, when combined with contact-facilitated drug delivery, are effective treatments of multiple myeloma capable of improving patient prognosis.

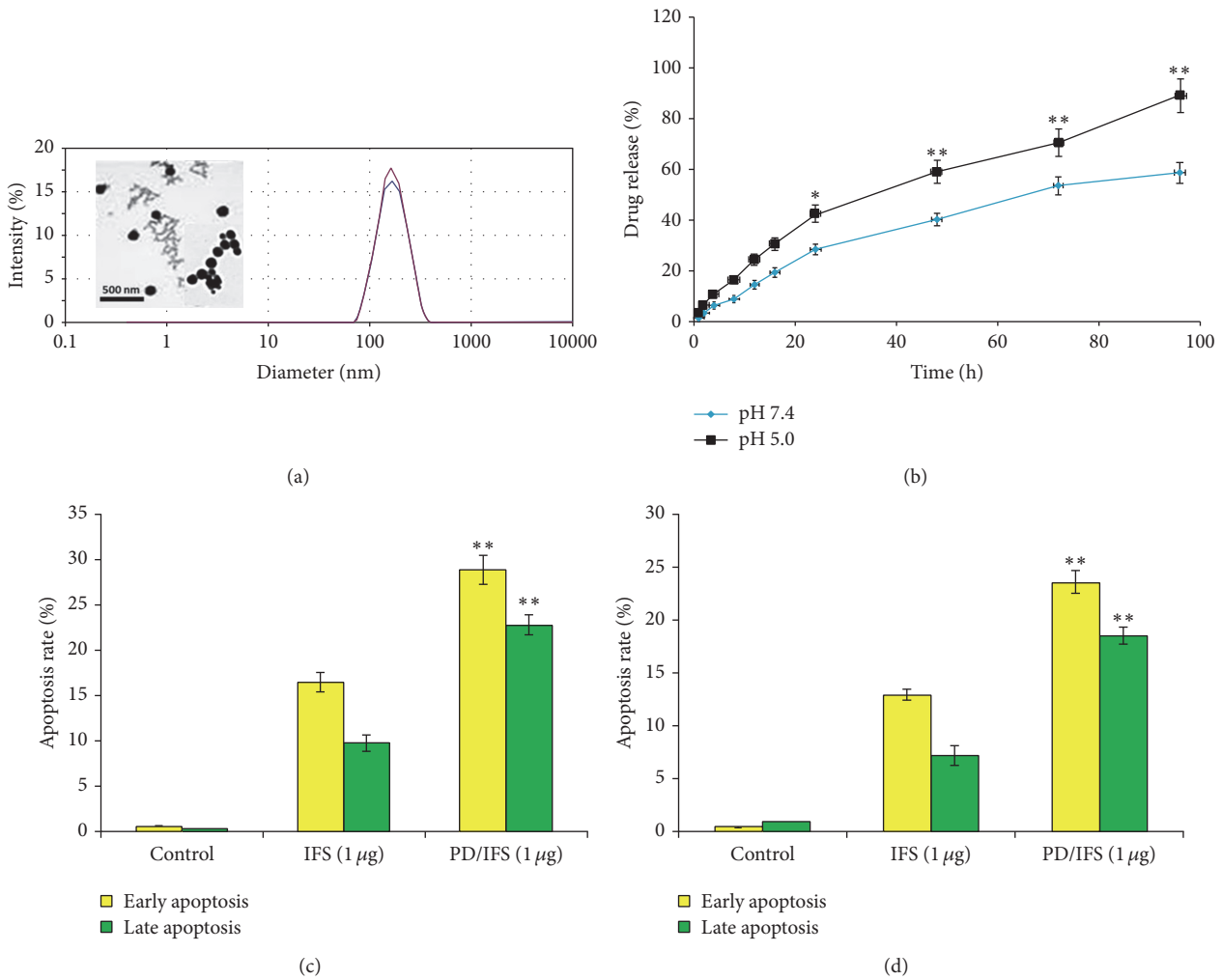

FIGURE 4: (a) Particle size distribution of IFS-loaded PLGA-dextran (PD/IFS) nanoparticles and TEM image of PD/IFS. (b) The release profile of IFS from the PLGA-dextran nanoparticulate system. The release study was performed in phosphate-buffered saline and acetate-buffered saline. The study was carried out for 96 h. $^{*}p < 0.05$ and $^{**}p < 0.01$ are the statistical difference between the pH 7.4 and pH 5.5 release media. Apoptosis analysis was detected by Annexin-V/PI staining. Apoptosis of (c) MG63 and (d) Saos-2 cancer cells. The respective cell percentages in early and late apoptosis for different time periods are presented in the bar graph. $^{**}p < 0.01$ is the statistical difference in apoptosis between IFS and PD/IFS for both cancer cells.

Additionally, research has shown that the tumor microenvironment contains cancer-associated fibroblasts as well as extracellular matrices (ECMs) that consist of fibrous structural proteins (collagen and elastin), fibrous adhesive proteins, and proteoglycans (PGs) [73]. Taking advantage of the fact that chondrosarcomas are rich in PG, Miot-Noirault and coworkers developed a PG-targeting strategy that uses a quaternary ammonium (QA), which acts as a carrier to selectively deliver therapeutic drugs or imaging agents to ECM-rich tissues, such as cartilage and chondrosarcoma [74]. They synthesized gadolinium-based small rigid platforms (SRP) that were functionalized with QA and radiolabeled with $^{(111)}$Indium (^{111}In-SRP@QA) [75]. Then, they evaluated the biodistribution of ^{111}In-SRP@QA in two experimental models and found that tumor accumulation and retention of ^{111}In-SRP@QA were increased by 40% compared to that of nonfunctionalized SRP in a swarm rat chondrosarcoma (SRC) orthotopic model. These results indicated that ^{111}In-SRP@QA may offer a promising radiobiological approach for treating highly radioresistant PG-rich tumors like chondrosarcoma.

2.2. Multidrug Synergistic Formulations. Combinational chemotherapy is widely used to prevent drug resistance in tumors [76, 77]. The combination of two drugs is believed to not only reduce drug-induced mutations and drug resistance, but also synergistically enhance the therapeutic efficacy while reducing side effects. Different drugs can target different stages of the cell proliferation cycle to induce apoptosis, requiring us to carefully consider their mechanism of action and dose-effect ratio to minimize side effects.

Based on the advantages mentioned above, many fascinating synergistic components with different effects can be incorporated into nanoparticles and simultaneously endow

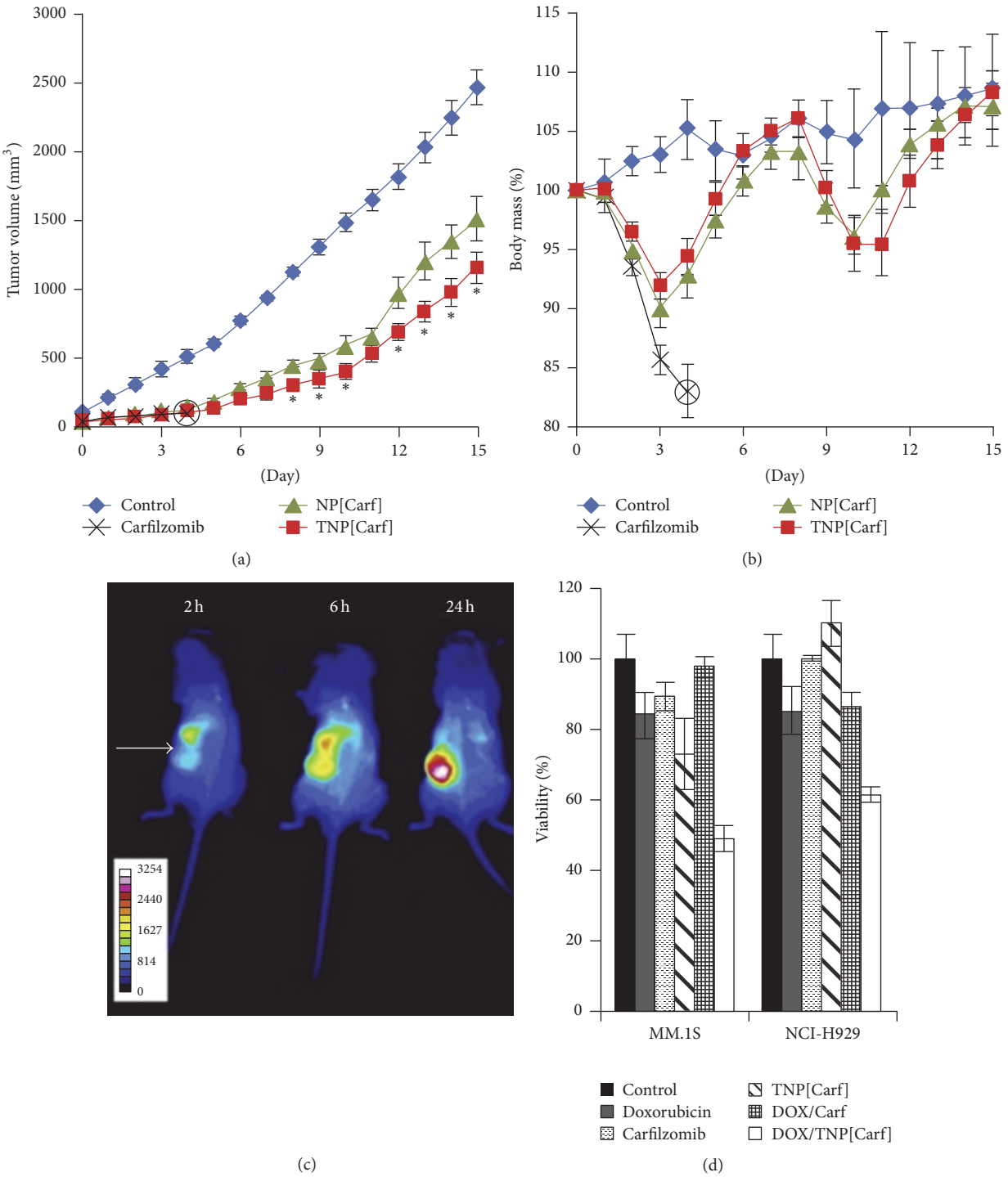

FIGURE 5: Liposomal CFZ nanoparticles preferentially accumulate in the tumor, inhibit tumor growth, and reduce systemic toxicities *in vivo*. Tumor-bearing SCID mice were injected intravenously on days 1, 2, 8, and 9 with NP[CFZ], TNP[CFZ], free CFZ, and PBS at a dose of 5 mg/kg CFZ equivalence. (a) Tumor growth inhibition was measured via calipers. The mice in the free CFZ group lost significant amounts of body mass (>15%) by day 4 and demonstrated moribundity. They were subsequently sacrificed on day 4 (black circles). TNP[CFZ] was significantly more efficacious than NP[CFZ] with $^{*}p < 0.05$. (b) Percentage of body weight of the animals was used as a measure of systemic toxicity. Mice in the CFZ group were sacrificed on day 4 due to drug-associated toxicities. Results showed that TNP[CFZ] significantly inhibited tumor growth while reducing the overall systemic toxicity. Data are shown as means ± SD of $n = 8$–10 per treatment group. (c) *In vivo* images of near-infrared dye-loaded targeted nanoparticles in tumor-bearing mice. Images were taken for all mice at $t = 2$, 6, and 24 h using noninvasive methods. The representative images show the accumulation of the nanoparticles in the tumor (white arrow) over time. (d) Liposomal CFZ nanoparticles demonstrate synergism with free DOX. MM.1S and NCI-H929 cells were cultured in the presence of either PBS (control), DOX, CFZ, TNP[CFZ], CFZ and DOX, or TNP[CFZ] and DOX at 50 nM DOX and/or 2 nM CFZ equivalent concentrations. Results showed that TNP[CFZ] exhibited a greater synergistic effect with DOX compared to free CFZ. Cell viability was assessed using Cell Counting Kit-8, and data represent means of triplicate cultures. $*$ represents statistical significance of TNP[CFZ] *versus* NP[CFZ] ($p < 0.05$).

them with intelligence and biological activity, forming multidrug synergistic systems that can deliver therapeutic biologics through these smart nanoparticles. Rejinold and coworkers designed such a system based on 5-fluorouracil (5-FU) and megestrol acetate (MEG). 5-FU, as a pyrimidine analogue, inhibits thymidylate synthase as well as DNA synthesis, which may lead to cell cycle arrest and apoptosis, respectively. As a progestin, MEG can interfere with the normal estrogen cycle and act as a novel drug for the treatment of estrogen-dependent tumors. The authors used 5-FU and MEG-loaded fibrinogen-*graft*-poly(*N*-vinyl caprolactam) nanogels (5-FU/MEG-fib-*graft*-PNVCL NGs) as targeted nanomedicines toward $\alpha_5\beta_1$-integrin receptors overexpressed in breast cancer cells as well as models for analyzing *in vitro* multidrug synergism [50]. The 5-FU/MEG-fib-*graft*-PNVCL NGs had good biocompatibility and thermoresponse. The lower critical solution temperature (LCST) could be regulated according to PNVCL/fibrinogen composition. The multidrug-loaded fib-*graft*-PNVCL NGs better enhanced cytotoxicity, apoptosis, and uptake by breast cancer (MCF-7) cells than using individual doses above their lower critical solution temperature, which could be modulated by tuning the PNVCL/fibrinogen composition. The 5-FU/MEG-fib-*graft*-PNVCL NGs, with diameters of 150–170 nm, could specifically target breast cancer cells as well as significantly enhance multidrug synergism and improve treatment efficacy.

In another work, Xu and colleagues successfully prepared multifunctional mesoporous silica nanoparticles (MSNs) *via* layer-by-layer assembly of polyamidoamine (PAMAM) dendrimer and chondroitin sulfate (CS) (Figure 6(a)) [78]. This multifunctional drug carrier possesses the requisite degree of smartness to enhance their therapeutic performance for applications in the treatment of cancer treatment. Utilizing layer-by-layer assembly, the authors loaded DOX and curcumin (CUR) onto the nanoparticles. Then, they discovered through *in vitro* release tests that DOX and CUR were more easily released within 28 hours under acidic conditions (Figures 6(b) and 6(c)). After implementing hemolysis assays and MTT assays, the authors observed the nanoparticles by confocal fluorescence microscopy and found that they exhibited good blood compatibility, fluorescent imaging ability, and high cytotoxicity toward A549 lung tumor cells, which are highly capable of spinal metastasis and invasion.

Chen and collaborators designed the RGD-functionalized and reconstituted high-density lipoprotein (HDL) nanoparticles [79]. These multifunctional tumor-targeting nanoparticles can be used as multimodal imaging probes in disease diagnosis, especially for Ewing's sarcoma. In the present study, HDL can be used as an endogenous multimodal nanoparticulate platform to deliver different targeting moieties and diagnostic/therapeutic agents. It endows them with miraculous abilities for tumor targeting and imaging. Chen et al. reconstituted HDL (rHDL) with amphiphilic gadolinium chelates and fluorescent dyes. Moreover, in order to make the rHDL nanoparticles specifically target angiogenic endothelial cells, they rerouted the rHDL nanoparticles through conjugation with $\alpha_v\beta_3$-integrin-specific RGD (rHDL-RGD). Afterwards, they utilized NIR imaging to evaluate the optical imaging efficacy as well as to investigate the binding/accumulation kinetics of the nanoparticles in tumors from a human sarcoma xenograft model (EW7 Ewing's sarcoma). Subsequently, the authors found that enriching rHDL with RGD accelerates *in vivo* tumor binding/accumulation, indicating that these nanoparticles may be used as an advanced diagnostic treatment platform for Ewing's sarcoma.

3. Clinical Research on Nanomedicines for Malignant Spinal Tumors

With the rapid development of nanotechnology, nanomedicine has been transforming traditional cancer chemotherapy. As of April 2016, there are about 125,000 articles on "nanoparticle" reported in PubMed, most of which were published in recent years, indicating that research in this field is growing rapidly. Even more exciting is that a large number of nanotherapeutics and nanodiagnostics have been commercialized or have reached clinical trials [28]. Importantly, since Cheng et al. described the lessons learned from first-generation nanomedicines, such as DOXIL® and Abraxane®, which have been approved for commercialization, several targeted nanoparticles for the treatment of metastatic or solid tumors, including spinal malignancies, have shown great promise for entry into clinical trials [80].

One of the first-generation commercial chemotherapeutic products that have been approved by the FDA is Nab-PTX (ABI-007, Abraxane1), which is a solvent-free, 130 nM albumin particle form of PTX used in the treatment of various solid tumors. In Houghton's group, nab-PTX was used to evaluate the antitumor effect of a limited series of Pediatric Preclinical Testing Program (PPTP) solid tumors. They observed that 5 out of 8 Ewing sarcoma xenograft models and 6 out of 8 rhabdomyosarcomas exhibited complete responses (CR) or maintained CR after treatment. There were no objective regressions in either neuroblastoma ($n = 2$) or osteosarcoma ($n = 2$) xenograft panels [81]. These results indicate that nab-PTX may be a potential therapeutic for the treatment of relapsed/refractory primary spinal tumors in children [81, 82].

4. Discussion

Spinal malignancy remains one of the most serious diseases that plague many people and seriously affect their quality of life [4]. The classic course of spinal tumor progression starts with pain, spinal instability, and compression of the spinal canal with concomitant neurological deficits associated with spinal tumor occurrence, followed by the development of complications that render the patient bedridden and paralyzed, and finally ends with death. Although surgery, radiotherapy, and neoadjuvant chemotherapy have gradually been improving this situation, they still cannot completely alleviate patient suffering, improve prognoses, or increase survival rates [83]. Malignant spinal tumors, which include primary and metastatic spinal tumors (with the latter accounting for the majority), are very common in clinical

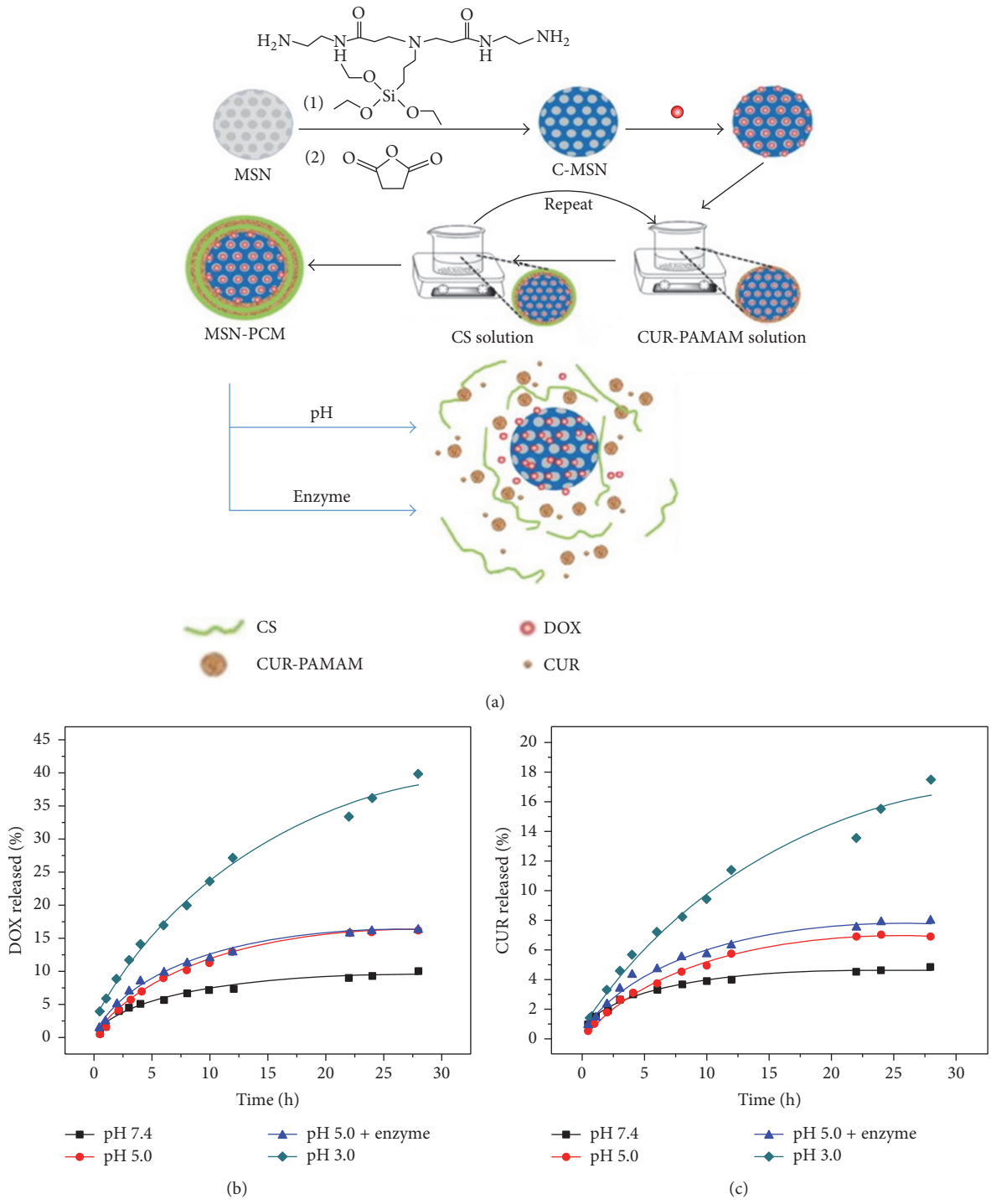

FIGURE 6: (a) Schematic representation of the preparation process of MSN-PCM and the pH- or enzyme-triggered release of DOX and CUR from MSN-PCM. Release profiles of (b) DOX and (c) CUR from MSN-PCM at different pHs with or without enzyme.

practice. Due to the limitations of traditional treatment regimens that limit clinical applications, animal models and nanomedicines are continuously being developed and improved upon to enrich the treatments for spinal malignancies.

The ideal nanoparticle should meet the following criteria: (1) favorable biocompatibility, (2) stability and good dispersion, (3) stimuli-responsive release, (4) target recognition, (5) synergistic and combinatory effects, (6) ability to traverse biological barriers and evade macrophage phagocytosis, (7) long circulation time to avoid liver inactivation, and so on. Due to their myriad of advantages in cancer therapy [29], polymer nanoparticles have emerged as attractive candidates for delivering a variety of payloads to their

targets. More researchers will focus on the design and development of multifunctional nanoparticles [80, 84] to achieve precisely timed, localized, synergistic, and smart release of diagnostic/therapeutic agents. The exciting news is that several polymer nanomedicines have been approved to enter clinical trials and even clinical application [85]. With more in-depth study in the future, nanomedicines for spinal tumor treatment may open up a new research direction.

5. Conclusions and Perspectives

The current mainstays of treatment for primary and metastatic spinal malignancies include surgery, radiotherapy, and chemotherapy. Although chemotherapy is the most traditional and effective strategy, high doses of chemotherapeutic drugs often lead to serious side effects, prompting an urgent need for advanced chemotherapeutic formulations that can enhance treatment efficacy and reduce complications. A promising strategy is to use polymer nanoparticles, which can increase the intracellular accumulation of anticancer drugs and reduce systemic toxicity. Due to various intrinsic advantages of polymer nanoparticles, an increasing number of research works in recent years have applied polymer nanoparticles to the diagnosis and treatment of spinal malignancies.

To date, however, polymer nanoparticles have not been extensively studied for the treatment of spinal malignancies, with extremely few clinically tested for further application. In the future, more research efforts are needed to explore the application of polymer nanoparticles to the development of versatile and smart theranostic systems. With further research advances, we believe that multimodal nanoparticles will be developed, which may be achieved by coencapsulation of multiple diagnostic agents and therapeutic agents into a targeted nanomedicine platform [86]. With sustained endeavors toward the development of versatile and intelligent polymer nanoparticles, polymer nanoparticle-based delivery systems are expected to play a more important role in the treatment of spinal malignancies.

Competing Interests

The authors declare no competing interests regarding the publication of this paper.

Authors' Contributions

Hongyun Ma and Weiqian Jiang contributed equally to this work.

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