Development of Intratumoral Drug Delivery Based Strategies for Antitumor Therapy

Zhimei Jiang1,3, Yuzhi Fu1,3, Hongxin Shen1,3

1Department of Pharmacy, West China Second University Hospital of Sichuan University, Chengdu, People’s Republic of China; 2Evidence-Based Pharmacy Center, West China Second University Hospital of Sichuan University, Chengdu, People’s Republic of China; 3Key Laboratory of Birth Defects and Related Diseases of Women and Children (Sichuan University), Ministry of Education, Chengdu, People’s Republic of China

Correspondence: Hongxin Shen, West China Second University Hospital, Sichuan University, No. 20, Third Section, Renmin South Road, Chengdu, Sichuan, 610041, People’s Republic of China, Email 1103747222@qq.com

Abstract: Research for tumor treatment with significant therapy effects and minimal side-effects has been widely carried over the past few decades. Different drug forms have received a lot of attention. However, systemic biodistribution induces efficacy and safety issues. Intratumoral delivery of agents might overcome these problems because of its abundant tumor accumulation and retention, thereby reducing side effects. Delivering hydrogels, nanoparticles, microneedles, and microspheres drug carriers directly to tumors can realize not only targeted tumor therapy but also low side-effects. Furthermore, intratumoral administration has been integrated with treatment strategies such as chemotherapy, enhancing radiotherapy, immunotherapy, phototherapy, magnetic fluid hyperthermia, and multimodal therapy. Some of these strategies are ongoing clinical trials or applied clinically. However, many barriers hinder it from being an ideal and widely used option, such as decreased drug penetration impeded by collagen fibers of a tumor, drug squeezed out by high density and high pressure, mature intratumoral injection technique. In this review, we systematically discuss intratumoral delivery of different drug carriers and current development of intratumoral therapy strategies.

Keywords: intratumoral drug delivery, drug carrier, treatment strategy, antitumor therapy

Introduction

At present, many kinds of strategies have been developed for the therapy of various tumors, such as surgery, chemotherapy, radiotherapy, immunotherapy, and photothermal therapy. Some early solid tumors are preferentially treated by surgical resection; however, advanced carcinomas at stage III–IV, inoperable regions, or elderly patients unable to undergo surgical treatment require improved systemic treatment strategies.1 Currently, there are still many challenges in cancer treatment. Traditional treatment methods such as radiation therapy and chemotherapy can inhibit tumor growth to a certain extent but are unlikely to completely kill tumor cells, resulting in significant side-effects, tumor recurrence, and metastasis. Therapeutic drug delivery systems have been widely investigated because of their anticancer potential to target tumors, enhance radiotherapy, or immunotherapy. However, challenges and limitations also urgently need to be solved. Drugs show the characteristics of non-specific delivery, causing various adverse reactions and inefficient treatment. Hopefully, the intratumoral delivery system which refers to drugs directly injected into tumors may provide a meaningful response. Its unique anticancer efficacy was associated with increased tumor distribution, prolonged retention of therapeutic drugs within the tumors, and decreased normal tissue uptake.2,3

Intratumoral therapy is different from systemic administration, because it directly delivers therapeutic substances into the tumor sites, increases the concentration and station of antitumor drugs, thereby enhancing the antitumor effect and reducing systemic toxicity.4 Recently, intratumoral delivery systems have been extensively explored. This mainly focused on nanoparticles,5 hydrogels,6 microneedles,7 microspheres,8 etc. For example, Chattopadhyay et al.3,9 employed the intratumoral injection of trastuzumab-gold nanoparticles to maximize tumor localization for X-ray radio sensitization and reduce uptake by the liver and spleen. It caused 25-fold higher tumor uptake, 10-fold lower spleen uptake of trastuzumab-gold nanoparticles than that post-intravenous injection at 48 hours. Its liver uptake was also modestly
decreased. In addition, the pharmacodynamics revealed that tumor size was decreased by 2-fold in a trastuzumab-gold nanoparticles and X-radiation group that in an X-radiation alone group.

However, for intratumoral drug delivery systems, the complex and chaotic vasculatures formed in and around the tumor, and the properties of their abnormal mesenchyme, hindered effective permeation of therapeutic drugs to tumor cells which reduced their anticancer activity. Moreover, uniform distribution within the tumor mass was another challenge which might determine the therapeutic efficacy. Promisingly, the smart formulations have higher potential, owing to their superior advantages, such as target delivery, easy tumor penetration, stimulus-responsive property, sustained drug release, as well as minimal systemic side-effects. The smart intratumoral delivery systems may be suitable to utilize drug carriers overcoming short retention times in tumors and the penetration barriers. In addition, further ligand modifications enable tumor targeted delivery to be possible. Stimulus-responsive natures such as pH, reactive oxygen species, light, or heat make drugs a good choice for local tumor-specific targeting. Currently, in order to achieve the desired antitumor effects with high safety, the intratumoral delivery systems have been intensively developed in the past decades. Fortunately, recent progress has revealed that intratumoral delivery systems enable tumor targeted delivery of not only chemotherapeutic drugs and immunotherapeutic agents, but also radiosensitizers, photothermal agents, or other sensitive substances to enhance its effects and reduce side-effects.

Intratumoral administration can be realized through direct injection or image-guided injection, which enables not only high concentrations of drugs in tumor tissue, but also reduces systemic side-effects. A suitable drug carrier may contribute to reasonable drug loading and release. Intratumoral administrations have been integrated with treatment strategies. Some of these strategies are ongoing clinical trials or applied clinically. This review focuses on the intratumoral delivery of well-studied drug carriers and current development of intratumoral therapy strategies.

Application of Intratumoral Delivery System with Different Drug Carriers for Antitumor Therapy

Hydrogels for Intratumoral Administration

Hydrogels are prepared by biocompatible materials and form a three-dimensional network structure, which is suitable for loading various antitumor drugs. Currently, in-situ forming hydrogels have attracted increasing attention because of their...
many advantages. Local delivery of therapeutic agents through hydrogels can directly provide continuous release and a high dose of therapeutic drugs in tumor tissues, which may prevent prolonged blood circulation and reduce side-effects among normal tissues. Stimulus responsive hydrogels are solutions which can easily flow during intratumoral administration, but rapidly change to gel phase once injected into tumor sites. Hence, the application of intratumoral injectable in-situ forming hydrogel is a promising route to local drug delivery.

Hydrogels can be prepared through in-situ cross-linking, of which chemical and physical based cross-linking are most commonly used. A mixture of polymer, cross-linker, and antitumor drugs will form in-situ hydrogels through chemical reaction. In addition, physical hydrogels are prepared by electrostatic interaction, hydrogen bonds, and entanglement of chains. Recent studies have revealed that smart hydrogels can also emerge intelligent sol-gel changes according to environmental stimuli of tumors such as light, temperature, pH, etc. Zhang et al explored an in-situ hydrogel system triggered by near-infrared (NIR) laser. It showed multi-mechanism antitumor therapy. A solution containing poly(ethylene glycol) double acrylates (PEGDA), Doxorubicin (DOX), and TiO$_2$ introduced by multi-walled carbon nanotubes (MWCNTs) substrate was injected into the tumor of mice, and then quickly gelled through photo-crosslinking triggered by a NIR laser. It allowed for convenient injection in vitro and sustained release in vivo. Similarly, the temperature sensitivity is another intelligent approach to form in-situ hydrogels. Wan et al synthesized D-PNA100 nanomedicines via acid-base neutralization of DOX and pAA100-bpNIPAM200-b-pAA100 (PNA100). Within a specific concentration range (5.0–10.0% of D-PNA100), the phase behavior transition of temperature sensitive sol-gel occurred. Temperature sensitive hydrogels were in a liquid state with good fluidity at room temperature and became in a gel state at 37°C with good retention. An in vitro release experiment of D-PNA100 hydrogels revealed a sustained DOX-release over ten days. Due to its excellent retention, intratumoral administration of temperature-sensitive D-PNA100 hydrogels achieved the highest antitumor activity among all treatment groups. Furthermore, Raza et al prepared a pH responsive hydrogel for the intratumoral delivery of paclitaxel to enhance the efficacy of antitumor chemotherapy. Since the acidic pH of tumor cell microenvironment is relatively lower than that of normal cell, the smart hydrogel is designed to be pH-sensitive. The pH responsive FER-8 peptide hydrogel is stable and rarely degradative at neutral pH 7.4, which can be explained by the uncharged basic amino acids, resulting in no repulsion and completely compound solubilized. However, a higher decomposition rate was observed in the acidic environment because of electrostatic repulsion among glutamic acid residues which facilitated drug release from hydrogels in a tumor microenvironment. It is indicated that environmental stimuli is promising to be explored as the nature of novel in-situ forming hydrogels for intratumoral administration against tumors.

### Nanoparticles for Intratumoral Administration

A lot of nanoparticles have been developed for antitumor therapy, but it is still difficult to achieve tumor high targeting or distribution of drugs though systemic administration. The rapid liver and spleen uptake upon i.v. administration led to reduced uptake of target tissues. It may be attributed to the basis of enhanced permeability and retention (EPR) effect which is seriously doubted in human bodies. Furthermore, the survival percentages of marketed nanoscale anticancer drugs (such as Doxil®, Abraxane®) are not higher than that of free drug solutions. Therefore, compared to the intratumoral therapy with free drugs, intratumoral delivery of drugs through nanoparticle formulations provides opportunities to realize higher encapsulate rates, lower systemic toxicity, target property, and sustained release.

As an alternative strategy for cancer treatment, intratumoral injecting nanoparticles directly into tumors to deliver therapeutic drugs to the tumor site has been widely developed. Reversed lipid-based nanoparticles (RLBN) have been explored as carriers for hydrophilic drugs which spread rapidly from injection sites to systemic circulation. RLBN had a polar core and a reversed lipophilic periphery, which made it soluble and stable in hydrophobic liquids and entrap hydrophilic drugs. In addition, DOX entrapped by RLBN after a single intratumoral injection in HepG2 tumor-bearing mice showed much more remarkable antitumor activity than that of free DOX. Furthermore, nanoparticles as drug carriers have also been used to improve short effective diffusion distance such as nitric oxide (NO) which possessed powerful cytotoxicity and lack of off-target side-effects. Lee et al prepared poly(lactic-co-glycolic acid) (PLGA)-conjugated linear polyethyleneimine diazeniumdiolate (LP/NO)-modified with albumin nanoparticles. It offered sufficient NO-loading as well as sustained NO-release. Moreover, surface coating with albumin changed the surface charge of...
nanoparticles, which enhanced tumor penetration by decreasing dense extracellular matrix (ECM) interaction. Enhanced antitumor activity was demonstrated by in vivo experiment in B16F10-tumor-bearing mice which indicated improved spatial distribution and matrix penetration in tumor tissue. Taken together, nanoparticles as carriers of intratumoral delivery drugs represent a promising modality for tumor treatment.

Even though a lot of effort has been made to increase total tumor accumulation of systemic administration of nanoparticles such as modified with monoclonal antibodies or targeting ligands, these approaches have been proved to be moderately successful. Studies revealed that tumor-targeting nanoparticles through intravenous administration did not improve accumulation in solid tumors, but improved internalization within tumor cells compared with non-targeting nanoparticles. This may be attributed to a large number of proteins in blood which can tightly bind to the surface of nanoparticles, thereby changing their physical and chemical characteristic and stability and hindering the specific binding of targeted molecules to the receptor. Furthermore, the uptake and clearance by liver and spleen hinder further delivery to tumor tissue and lead to toxicity of healthy tissues. High heterogeneity of enhanced permeability and retention effect in different tumor types and different regions of the same tumor is another challenge. However, intratumoral injection of targeted nanoparticles may be a feasible treatment option for cancer therapy because of blood sequestration, high tumor retention and tumor internalization, as well as reduced exposure of the liver and spleen.

**Microneedles for Intratumoral Administration**

Microneedles (MNs) are needle arrays of micrometer length, which establish hundreds of microchannels in the skin through almost painlessly direct piercing into the cuticula in a minimally invasive pattern and increased efficacy of transdermal administration. Recently, MNs have displayed great potential to facilitate intratumoral delivery of therapy drugs within the lesion site of tumors. MN patches can offer uniform drug delivery throughout the tumor tissue, which is critical for in-situ chemotherapy and immunotherapy. After administration, needle tips are dissolved by the environment fluid of the tumor tissue and gradually release encapsulated drugs or nanoparticles. Loading therapeutic agents into nanoparticles further leads to sustained release. In addition, the side-effects of normal tissues related to system circulation are remarkably reduced.

MNs can integrate with nanoparticles concentrated at needle tips, demonstrating superior synergistic antitumor effects compared with intravenous and intratumoral injection of nanoparticles. Furthermore, functionalized MNs with thermal-sensitive, pH-sensitive properties were prepared to achieve specific controlled release of the drug. Wu et al explored metal-organic frameworks (MOFs) constructed using MIL-88 as an internal core which is composed of iron ions and 2-aminoterephthalic acid and ZIF-8 as an external shell which consists of zinc ions and 2-methyl imidazole. Indocyanine green (ICG) was loaded into the internal core and DOX was encapsulated into the external shell, which formed MIL-88-ICG@ZIF-8-DOX nanoparticles. The above nanoparticles were further loaded into dissolving micro-needles. It was thermal-sensitive attributed to IGG converting light energy into heat and pH-sensitive because of ZIF-8 degradation triggered by tumor acid microenvironment. MNs could also be designed to delivery drugs deeper into the tumor tissues. This feature was realized though active MNs loaded with magnesium (Mg) microparticles, which exhibited improved overall survival as well as tumor growth inhibition compared to injection and passive MNs. Due to its ability to generate hydrogen gas bubbles, a Mg micromotor exerts a thrust on drug payloads penetrating deep into tumor tissues. Therefore, well-designed MNs are suitable for intratumoral delivery to enhance anti-tumor efficacy.

**Microspheres for Intratumoral Administration**

Microspheres (MS) have been increasingly developed as drug delivery systems for anticancer therapy. MS can serve as drug reservoirs, slowly release drugs by multiple mechanisms such as hydrolysis, self-diffusion, transport, and erosion. The sustained release characteristics are profit to increasing drug retention at the tumor site. Furthermore, MS can protect drugs from chemical degradation and transformation, thereby improving the drug stability. Especially the biocompatible and biodegradable poly(lactic-co-glycolic-acid) (PLGA) material has been widely used. Nowadays, intratumoral delivery of MS results in high drug concentration in tumors, minor leakage in plasma, and good therapeutic effect.
Despite its efficient cancer therapy, the core-shell MS was prepared to obtain high drug loading capacity. Ni et al explored PLGA microspheres, in which the polydopamine (PDA) nanoparticles as photothermal therapeutic agent and DOX as chemotherapy agent were loaded in the interior hollow space with the PLGA as the outer shell layer. NIR irradiation at the tumor site triggered a hyperthermia effect through PDA nanoparticles, while releasing DOX and diffusing into tumor cells. Noteworthy, drug accumulation in the tumor was shown to be 6-fold higher in intratumoral delivery of the microspheres group than that in intravenous injection of the DOX group. The above description further demonstrates the advantages of intratumoral administration compared with systemic delivery.

Additionally, the morphology of MS plays an important role in solubility, encapsulation efficiency, and drug release behavior, which are affected by oil phase viscosity, stirring rate, and organic solvent evaporation. The above characteristics further affect antitumor activity of MS preparations. Zhang et al prepared smooth paclitaxel (PTX) PLGA-MS by a characteristic of internal sporadic porosity and rough PTX-PLGA-MS with porous internal structures and microporous surface. The above different morphological types were explored to study the difference in pharmacodynamics. Finally, the rough MS group after a single injection achieved higher antitumor activity (tumor inhibition rate ¼ 58.33%) than that in the free PTX and smooth MS group. It might be attributed to sustained drug release and adhering throughout tumor tissues of rough MS. Decreased drug administration frequency and reduced drug distribution in normal tissues alleviated the adverse reactions. Microspheres with the characteristic of biodegradability, biocompatibility, and minimal systemic toxicity are an important strategy for the delivery of short serum half-life, dose limiting side-effect drugs. As drug carriers, they serve as drug reservoirs allowing sustained drug release which improves therapeutic efficiency.

Strategies Based on Intratumoral Drug Delivery System for Antitumor Therapy

Design Intratumoral Delivery System for Chemotherapy

Chemotherapy is one of the most classic antitumor treatments and widely applied in clinical practice. An intratumoral delivery system has been demonstrated to be effective in delivering chemotherapeutic agents to tumors. Merdan et al prepared curcumin-loaded nanoparticles with particle size about 169 ± 4.8 nm which significantly decreased the tumor size (from 66.6 to 34.9 mm$^2$) after intratumoral injection compared with intravenous administration on glioblastoma. It was demonstrated that the intratumoral administration of nanoparticles with chemotherapeutics was a preferred method in brain tumors by offering target drug accumulation at the tumor site. Furthermore, drug carriers could be functionalized by surface modification to enhance target activity. Farokhzad et al reported docetaxel-PLGA-b-PEG nanoparticles conjugated with A10 2'-fluoropyrimidine RNA aptamers which recognized prostate-specific membrane antigen (PSMA). PSMA was usually expressed on the surface of prostate cancer cells which enabled increasing cell absorption leading to significantly enhanced cellular toxicity compared with nontargeted nanoparticles. Consistently, complete tumor reduction in five of seven and 100% of animals surviving for 109-days were shown in targeted nanoparticles, whereas complete tumor reduction in two of seven and 57% of survivability were shown for nontargeted nanoparticles.

Traditional chemotherapy against malignant tumors was usually accompanied with metastasis. Therefore, it is meaningful to enhance antitumor and anti-metastasis activity simultaneously. Yang et al explored an intratumoral injectable phospholipids-based phase separation gel encapsulated with 5-fluorouracil and magnesium oxide (5FU + MgO-PPSG). It exhibited sustained release 5-fluorouracil to achieve anti-tumor efficacy and alkaline substance neutralizing tumor acidic microenvironment as well as magnesium oxide to reach anti-metastasis activity. Systemic toxicity is another important consideration factor for chemotherapy. DOX-loaded polymer-lipid hybrid nanoparticles were prepared for intratumoral delivery. It was demonstrated that the unwanted normal tissue toxicity was very low for intratumoral treatment. In conclusion, intratumoral delivery systems have great potential to improve chemotherapy efficacy.

Design Intratumoral Delivery System to Enhance Radiotherapy

Radiotherapy (RT) is the mainstay of cancer therapy. Unfortunately, it generally results in significant side-effects to normal tissues and organs due to the lack of selectivity and the following high dose. Therefore, research has been
conducted to achieve safe and effective radiation therapy. An active area focused on developing radiosensitizers which made tumor cells easier to kill for radiotherapy.\textsuperscript{17} Gold nanoparticles could improve the biological effective dose of radiation.\textsuperscript{55} Targeted gold nanoparticles by attaching trastuzumab was prepared to target human epidermal growth factor receptor-2 (HER-2) positive tumors.\textsuperscript{9} This acted as a radiosensitizer and has demonstrated that systemically administration of targeted nanoparticles had a fast clearance from blood, while intratumoral injection led to high tumor retention and low systemic exposure. Intratumoral delivery of radio sensitizers represents an attractive delivery strategy.

To extend the short half-life and increase the antitumor effect of radiosensitizers, their delivery modes and cancer cell targeting features could be incorporated. Tang et al explored cytarabine hyaluronic acid-tyrmine (Are-HA-Tyr) hydrogel to sensitize tumor cells to RT. The combination of RT and Are-HA-Tyr hydrogel increased survival and prolonged the tumor growth delay compared to either monotherapy in a Lewis lung cancer xenograft model. Furthermore, the combination therapy could significantly induce cell cycle arrest in the G2/M phase, decrease the proliferation index, and increase apoptosis. In addition, lower toxicity was also observed in intratumoral delivery of Are-HA-Tyr hydrogel and RT group which was consistent with improved targeting and lower toxicity.\textsuperscript{56} The radiosensitizing effect of different doses was reasonably evaluated to suggest the maximally effective dose. Liu et al investigated the radiosensitizing effect of silver nanoparticles (AgNPs) with 10/20 μg/10 μL on glioma cells. Most importantly, the combination of AgNPs and radiation significantly enhanced survival time compared with irradiation alone (P<0.001). However, the average survival time and cure rate of glioma-bearing rats observed in 10 and 20 μg of AgNPs and 6-MV X-irradiation group were equal, indicating that 10 μg of AgNPs represents the maximum effective dose. The negligible accumulation in normal tissues around the tumor was quantitatively determined, which further indicated the clinical potential of AgNPs combined with radiotherapy.

Intratumoral administration of radioisotopes was investigated to overcome the lack of selectivity by external therapy which led to a high systemic radiation dose and damage of normal tissues. Furthermore, a carrier system for radioisotope was applied to remain long enough at the tumor site enhancing the treatment effect and reducing side-effects to normal tissues. Several studies have already reported a prolonged retention time in tumor site and low radioactive concentration in blood for 8 days.\textsuperscript{57,58} Therefore, radiotherapy using a carrier with radioisotopes when injected directly into the tumor could provide a promising modality for antitumor treatment.

**Design Intratumoral Delivery System for Immunotherapy**

Cancer immunotherapy is gaining increasing attention due to its ability to stimulate immune cells to recognize and attack tumor cells with high specificity, effectiveness, and persistence. Currently, its main design is to deliverantigen vaccines, immune checkpoint inhibitors, genetic vaccines, antibodies, or cytokines to treat tumors.\textsuperscript{59} Additionally, the route of administration affects behavior and therapeutic efficacy. Most current applications are based on systemic administration and corresponding immune activation.\textsuperscript{60} A growing amount of evidence suggests that intratumoral delivery of oncolytic circus or innate immune stimulators exhibits therapeutic potential against tumors.\textsuperscript{51,62} The in-situ vaccination was able to alter the tumor microenvironment to anti-tumor state.\textsuperscript{63} Furthermore, it was discovered that intratumoral injection of nanovaccine achieved a more significant antitumor effect than subcutaneous delivery with the same dose.\textsuperscript{64} Mohsen et al\textsuperscript{65} explored intratumoral injections of microcrystalline tyrosine decorated with cucumber mosaic virus-like particles to activate tumor antigen-specific T cells. This “immune-enhancer” turned immunologically cold tumors into hot tumors, and inhibited tumor growth locally and systemically.

Cytokines as the important immune-therapeutics fail to achieve adequate concentrations in tumors via systemic administration due to dose-limiting toxicity.\textsuperscript{66} However, intratumoral delivery may improve the therapeutic effect, because it can deliver cytokines to tumors as immunostimulants. Liu et al\textsuperscript{67} used novel lipid nanoparticles for efficient intratumoral delivery of cytokine mRNAs. Dual IL-12 and IL-27 delivery showed the most potent inhibition of tumor growth due to enhanced influence of immune effectors on the tumor vascular system and activating the secretion of downstream anti-tumor signaling molecules by effector cells. In addition, sustained tumor inhibition after treatment stops might suggest that immune memory responses had been established among the treated mice. Severe adverse side-effects for cytokine-based cancer therapy were not induced, which was speculatively attributed to the intratumoral injection approach with low systemic release of cytokine and rapid cytokine utilized by nearby immune cells. Therefore, the
intratumoral delivery of appropriate cytokines loaded by nanoparticles might be an appropriate modality for current immunotherapy.

Furthermore, the intratumoral delivery system could be explored as the administration of immunotherapeutic agents, with encouraging clinical data to improve their degradation and specificity, such as synthetic non-methylated cytosine-phosphate-guanine (CpG).\textsuperscript{68} CpG has been extensively studied for its efficacy in tumor prevention and regression.\textsuperscript{69} It was susceptible to nuclease-mediated degradation and lacked specificity to target tumor cells after systemic administration. Zhang et al\textsuperscript{70} designed 3-aminopropyltriethoxysilane (APTES)-modified Fe\textsubscript{3}O\textsubscript{4} nanoparticles loaded with CpG (FeNP/CpG), and the particle size was approximately 50 nm. Compared with free CpG, FeNP/CpG showed improved cellular uptake of CpG in vitro in bone marrow-derived dendritic cells and enhanced antitumor efficacy through stimulating better humoral as well as cellular immune responses in vivo.

**Design Intratumoral Delivery System for Phototherapy**

Phototherapy is one of the most promising cancer therapy methods, as it has many advantages such as negligible drug resistance, noninvasive features, high spatial selectivity, low side-effects, and manual control through light radiation.\textsuperscript{71,72} It mainly includes photodynamic therapy (PDT) and photothermal therapy (PTT). PDT is a cancer treatment strategy that uses light and a photosensitizer (PS) to produce cytotoxic singlet oxygen (\(1^1O_2\)) as well as reactive oxygen species (ROS) to kill surrounding cancer cells.\textsuperscript{73} PTT is based on a photo absorbing agent, which converts absorbed light energy into heat to destroy cancer cells.\textsuperscript{74,75} However, limited success has been achieved in vivo because of the major uptake by the reticuloendothelial system after systemic injection, especially the liver, kidney, and spleen.\textsuperscript{76} Therefore, high tumor distribution or targeting may be difficult to realize. Currently, a lot of research has focused on intratumoral injection, which could be able to increase accumulation of drugs for PDT and PTT.\textsuperscript{74,77} This may be a potential method of drug administration for phototherapy.

Due to the light absorption and scattering by tissues, the limited penetration depth of most traditional photosensitizers results in poor therapeutic efficacy on large or internal tumors. Therefore, the promising carrier of photosensitizer should enhance penetration depth such as upconversion nanoparticles (UCNPs). Due to the ideal choice of near-infrared light as optical tissue penetration for phototherapy, UCNPs could convert NIR light to visible photons for activating a photosensitizer to generate ROS.\textsuperscript{78} Moreover, environmentally controlled PDT could be realized through pH-responsive UCNPs, which are negatively charged in alkaline and neutral surroundings and switched to positively charged under slightly acidic environments. The smart feature facilitates their interaction with cell membranes to improve cellular uptake. Wang et al obtained charge-reversible UCNPs through coating with dimethyl maleic acid groups and polyethylene glycol chains, which remarkably improved intracellular uptake and increased tumor retention. In addition, to prevent local pain and postoperative complications caused by frequent intratumoral injections, hydrogels provided a novel means of PDT strategies to promote multiple rounds of therapy after a single injection.\textsuperscript{79}

In PTT, efficient delivery of a photo absorbing agent to the whole tumor is crucial for minimizing tumor recurrence by achieving a cytotoxic temperature anywhere within the tumor.\textsuperscript{80} Cell-mediated delivery systems were widely applied because they could cross the almost impermeable biological barriers.\textsuperscript{81,82} Li et al\textsuperscript{83} explored bovine serum albumin (BSA)-coated Au nanorods (7 nm)-laden-macrophages. They showed significant improvement in photothermal conversion nearly anywhere in the tumor. After intratumoral injection, this resulted in minimized tumor recurrence rates compared with non-macrophages laden preparations in vivo. Hence, it is highly desirable to enhance tumor coverage in PTT. Besides, multi-modal imaging-guided PTT has attracted increasing attention recently as it can offer comprehensive information for intensive therapy. Sun et al\textsuperscript{84} successfully prepared melanin-manganese nanoparticles for magnetic resonance/photoacoustic dual-modal imaging guided PTT. In vivo results revealed that nanoparticles after intratumoral injection began to diffuse and spread throughout the entire tumor sites at 3 hours, indicating the optimal treatment time. Melanin-manganese nanoparticles for PTT in vivo showed efficient tumor ablation, no recurrence, and negligible side-effects.
Design Intratumoral Delivery System for Magnetic Fluid Hyperthermia

Magnetic fluid hyperthermia (MFH) is the process of introducing magnetic fluid into the tumor area through a certain method and placing it in an alternating magnetic field. Magnetic particles are heated up under the action of the alternating magnetic field by relaxing the rearrangement of the magnetic vector in the magnetic field, thereby achieving the temperature required to kill tumor cells. However, it has high targeting and specificity due to the non-distribution of magnetic fluids and unremarkable heating in normal tissues around tumors. It renders MFH as an attractive new approach for deep tumors hyperthermia. Therefore, intratumoral delivery of magnetic fluids could realize tumor deposition, allowing for a series of MFH therapies without repeated administration and tumor inhibition.

Johanssen et al treated an orthotopic prostate tumor model of rat with two MFH therapies following a single intratumoral injection of magnetic fluid. The mean iron content in the prostate of rats was 82.5% of the injected dose, while the iron content in the liver, spleen, and lungs was 5.3%, 0.5%, and 1.0%, respectively. Furthermore, compared to the control group, the stable tumor deposition resulted in 44–51% tumor inhibition. Kettering et al demonstrated that magnetic nanoparticles accumulation was not affected by magnetic heating and remained within the intratumoral injection side for 7 days, thereby not affecting healthy tissue. Chauhan et al explored chitosan-coated Fe₃O₄ nanoparticles to evaluate the antitumor response in an ectopic tumor model of Ce glioblastoma for MHT. The magnetic nanoparticles showed high heating efficiency, rapidly inhibiting 69.4% of tumors within 8 days, and completely inhibiting within 32 days after intratumoral injection on the first and seventh day. What is more, no recurrence was observed over a 5-month follow-up. Studies were also conducted on magnetic nanoparticles mediated hyperthermia in animal models of melanoma and breast tumors. Furthermore, the first clinical experiences with hyperthermia using magnetic nanoparticles in prostate carcinoma had been published.

Design Intratumoral Delivery System for Multimodal Therapy

Development of combination therapy plays a crucial role in enhancing antitumor activity and inhibiting tumor recurrence. Multifunctional formulations have been widely studied for cancer therapy, such as nanomaterials. Nanocarriers have been prepared as chemo-photo-thermal therapy, chemo-immunotherapy, chemo-radiotherapy, radio-immunotherapy, thermo-radiotherapy agents, and even two or more synergistic therapies. Hashimoto et al combined DOX-loaded functional dendrimers and gold nanorods to achieve chemo-photothermal therapy. Under laser irradiation, the formulation showed almost complete tumor growth inhibition, especially through intratumoral injection. Kossatz et al explored superparamagnetic iron oxide nanoparticles functionalized with DOX and Nucant multivalent pseudo peptide (MF66-N6LDOX) for magnetic hyperthermia and chemotherapy. MF66-N6LDOX showed increased nanoparticle uptake in tumor cells and greater cytotoxicity for breast cancer cells than both individual free ligands. Furthermore, it exhibited significant tumor growth suppression after intratumoral injection in vivo, which took advantage of the direct deposition at the target site for magnetic nanoparticles. Wang et al developed an ultrasound based drug delivery strategy for the treatment of prostate cancer which had poor response to routine immunotherapy because of the tumor immunosuppressive microenvironment. To generate effective antitumor immunity, exosomes were encapsulated with immune adjuvant R848 and sonosensitizer Chlorin e6 and accumulated at the tumor site through intratumoral injection. Ultrasonic irradiation and engineered exosomes synergistically improved R848-mediated maturation of dendritic cells and reprogrammed the phenotype of macrophages from immunosuppressive M2-like to antitumor M1-like, further reverting the immunosuppressive microenvironment. Therefore, the ultrasound-based immunotherapy showed obvious tumor growth and stable body weights of mice. More types of intratumoral delivery systems for multimodal cancer therapy are summarized in Table 1.

Summary and Perspective

To realize further improvements in quality-of-life and survival, numerous efforts have been made in the fight against cancer, starting from understanding the mechanism of cancer to patient treatment. Various treatment strategies for cancer treatment are being developed. Intratumoral injection may provide a meaningful response due to directly delivering therapeutic substances into tumor tissues. This still needs to go through a series of complex screenings to become
Table 1 Summary of Different Types of Intratumoral Drug Delivery Based Multimodal Therapy

<table>
<thead>
<tr>
<th>Reference</th>
<th>Multimodal Therapy Type</th>
<th>Formulation</th>
<th>Cell Line</th>
<th>Antitumor Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li et al (2016)</td>
<td>Chemoradiotherapy</td>
<td>Pluronic (R) F127-based thermosensitive hydrogel loading Au NPs and DOX</td>
<td>Melanoma (B16) and Human hepatocellular liver carcinoma (HepG2) cell lines</td>
<td>The tumor growth suppression was significantly increased in the co-loaded hydrogel group compared to the control group.</td>
</tr>
<tr>
<td>Wang et al (2023)</td>
<td>Thermoradiotherapy</td>
<td>Sorafenib loaded-PLGA hydrogel (SPH)</td>
<td>4T1 cell lines</td>
<td>SPH and microwave hyperthermia could achieve double RT sensitive effect, significant tumor growth inhibition, absence of significant systemic toxicity.</td>
</tr>
<tr>
<td>Zeng et al (2021)</td>
<td>Thermoradiotherapy</td>
<td>SFO-based nanozymes hydrogel</td>
<td>4T1 cell lines</td>
<td>Hyperthermia through single intratumoral administration of magnetic liposomes reduced tumor growth by 2.5 fold compared to the control group. There was no remarkable leaching or drainage of magnetic liposomes from the tumor site to other vital organs.</td>
</tr>
<tr>
<td>Gogoi et al (2017)</td>
<td>Magnetic fluid hyperthermia and chemotherapy</td>
<td>Magnetically responsive hybrid nanoparticles containing paclitaxel and a biphase suspension of La0.75Sr0.25 MnO2 and Fe3O4 nanoparticles</td>
<td>Fibrosarcoma (HT-1080) cell line</td>
<td>Enhanced RT regimen through Au NPs significantly increased the response to anti PD-L1 antibody for immunotherapy in 4T1Luc Triple Negative Breast Cancer mouse model, which was measured by tumor growth delay and animal survival increase.</td>
</tr>
<tr>
<td>Jovic et al (2022)</td>
<td>Immuno-radiotherapy</td>
<td>Anti-PD-L1 antibody and AuNPs</td>
<td>4T1Luc tumor cell line</td>
<td>The combined therapy exhibited an additive effect. Magnetic fluid hyperthermia therapy and radiation with 20 Gy were equally effective for tumor growth inhibition as radiation alone with 60 Gy, and reduced tumor growth by 87.5–89.2% compared with controls.</td>
</tr>
<tr>
<td>Benstols et al (2022)</td>
<td>Immuno-chemotherapy</td>
<td>DOX, resiquemd and macrophage inflammatory protein-3 loaded ROS –responsive dual targeted nanosystem</td>
<td>4T1 tumor cell line</td>
<td>Intratumoral injection of the nanosystem induced significant regression of cancer.</td>
</tr>
<tr>
<td>Gorgizadeh et al (2020)</td>
<td>Photothermal and sonodynamic therapy</td>
<td>Nanocomposite comprising MnFe2O4 and carbon</td>
<td>B16/F10 tumor cell line</td>
<td>Hyperthermia through single intratumoral administration of magnetic liposomes reduced tumor growth by 2.5 fold compared to the control group. There was no remarkable leaching or drainage of magnetic liposomes from the tumor site to other vital organs.</td>
</tr>
<tr>
<td>Yata et al (2017)</td>
<td>Photothermal immunotherapy</td>
<td>Composite-type Au NPs-DNA hydrogels</td>
<td>EG7-OVA cell line</td>
<td>Combined therapy improved local temperature and mRNA expression of heat shock protein 70 of EG7-OVA tumor which increased specific IgG levels and contributed specific interferon-gamma production from splenocytes of tumor associated antigen. It significantly delayed tumor growth and prolonged survival of tumor-bearing mice.</td>
</tr>
<tr>
<td>Qin et al (2020)</td>
<td>Photothermal chemotherapy</td>
<td>Photothermal agent IR-780 and paclitaxel loaded solid lipid nanoparticles and further concentrated in the tips of dissolving microneedles</td>
<td>B16 cell line</td>
<td>The curable rate of complete eradication for primary tumor was 100% in 30 days and after 100 days of therapy the survival rate was up to 66.67%.</td>
</tr>
<tr>
<td>Yang et al (2022)</td>
<td>Sonodynamic-immunotherapy</td>
<td>Fluorinated covalent conjugate polymers loading sonosensitizer and anti-CD47 mAb</td>
<td>CT26 cell line</td>
<td>Intratumoral injection of E1B gene-deleted adenovirus combined with chemotherapeutic agents achieved a significant reduction in tumor growth and improved survival rates.</td>
</tr>
</tbody>
</table>

a suitable candidate anticancer drug, such as cytotoxic screening through cell lines or animal models to evaluate the effect of drugs on cell viability, cell proliferation, and analysis applied to evaluate the apoptosis inducing effect of drugs, the cell cycle analysis, animal models to evaluate the effects of tumor growth and migration in vivo, pharmacokinetic and toxicological evaluations, and clinical trials. The safety of intratumoral injection has been proved by numerous researchers, and this technique has been used in clinics. Intratumoral injection of E1B gene-deleted adenovirus combined with chemotherapy and immunotherapy has shown promising results in preclinical studies.
with cisplatin-based chemotherapy has been conducted for Phase III randomized clinical trials. Furthermore, if the tumor is not visible on the skin, drugs can be administered by image or endoscope guided intervention. Endobronchial ultrasound-guided intratumoral administration of chemotherapeutic agents was clinically applied in lung cancer treatment. Intratumoral administration is a preferred method integrated with other modalities to minimize systemic side-effects such as the targeted delivery of cytokines IL-2 and IFN for cancer immunotherapy. Delivery of oncolytic virus through intratumoral injection has been clinically approved such as talimogene laherparepvec for intratumoral injection of melanoma. Furthermore, intratumoral delivery systems have been extensively studied based on different formulations, involving hydrogels, nanoparticles, microneedles, microspheres, etc. These drug carriers enhance tumor targeting and retention, thereby improving treatment efficacy and reducing distribution of other organs to cope with mild systemic adverse reactions. Furthermore, physical methods were also applied to enhance the effect of intratumoral drug delivery such as cryotherapy and electroporation. Cryotherapy induced tissue necrosis at low temperature to achieve tumor lysis which was demonstrated to further stimulate local immune response. Electroporation was high voltage electrical pulses transmitted to the tumor through electrodes causing membrane rupture which could promote the entry of anticancer drugs into cancer cells.

Despite its enormous potential, there are still challenges for intratumoral delivery systems in successful tumor therapy. For example, thermo-sensitive hydrogel has been widely investigated to overcome drug squeezing out of pinprick by high pressure difference. Furthermore, the high pressure of dense tumor tissues may result in poor penetration of the formulation. Multipoint injection and combined local treatment with active substances were studied in an effort to improve uniform drug distribution within tumors. Losartan potassium as an anti-fibrotic agent was able to improve intratumoral penetration by reducing collagens in tumors. Combined drug release delivery systems, such as microsphere-based hydrogel and nanoparticles contained hydrogel, have been evaluated for improved drug delivery and drug loading property as well as controlled drug release to prolong antitumor efficiency. Novel preparations in-depth research for optimization of intratumoral therapy must be conducted to further select injection form, location, number, and amount, and accelerate successful translation for clinical use. Especially, the efficacy of intratumoral administration might be operator-dependent because of the quality of in-situ delivery. A serious concern for clinically applied intratumoral therapies was the technical aspect, especially when considering multi-center phase III registration trials. Therefore, it is essential to develop mate intratumoral puncture techniques.

In summary, the combination therapy with enhanced antitumor efficiency and reduced systemic adverse reactions represents a high-potential cancer treatment strategy, of which an intratumoral delivery system will play an integral role.

**Disclosure**

The authors report no conflicts of interest in this work.

**References**


