Review

Drug Delivery Strategies for Platinum Based Chemotherapy

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Drug Delivery Strategies for Platinum Based Chemotherapy

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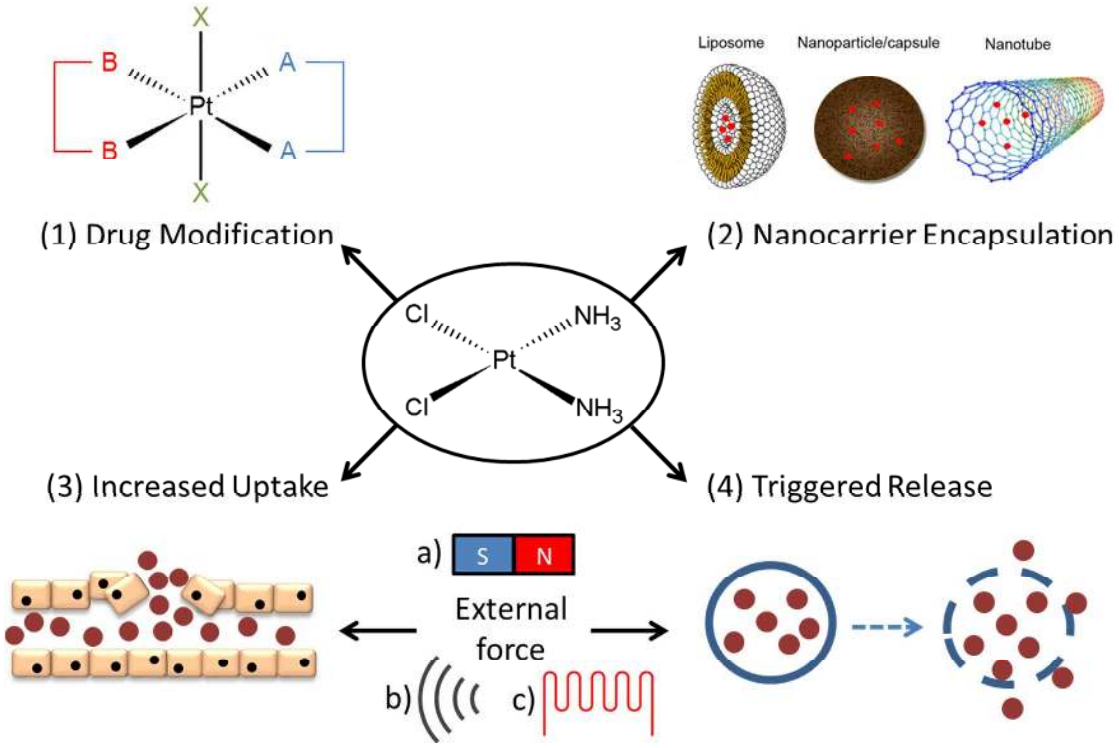
Abstract:

Few chemotherapeutics have had such an impact on cancer management as *cis*-diamminedichloridoplatinum(II) (CDDP), also known as cisplatin. The first member of the platinum based drug family, CDDP's potent toxicity in disrupting DNA replication has led to its widespread use in multi-drug therapies, with particular benefit in patients with testicular cancers. However, CDDP also produces significant side effects that limit the maximum systemic dose. Various strategies have been developed to address this challenge including encapsulation within micro- or nanocarriers and the use of external stimuli such as ultrasound to promote uptake and release. The aim of this article is to look at these strategies and recent scientific and clinical developments.

Graphical Abstract:



Alternative methods of delivery for cisplatin. (1) Cisplatin modifications reduce toxicity, enable binding to nanocarriers and provide sites of enzymatic or environmental action. (2) Nanocarrier encapsulation can reduce systemic toxicity and potentially improve retention at a tumor site by the enhanced permeability and retention (EPR) effect. (3) Tumor uptake of these nanocarriers can be further improved using external, physical force methods, for example a) magnetism, b) ultrasound and/or, c) heat. (4) Finally, these physical force methods, among others, can be used to trigger cisplatin release from nanocarriers to improve site specific delivery.

Vocabulary:

Nanocarrier – a particulate agent capable of encapsulating or conjugating to a drug; for instance a liposome, polymer nanoparticle, micelle, *etc.*, ranging in size from 1 nm to 500 nm.

Liposome – a lipid bilayer coated particle with an internal aqueous volume.

Polymeric nanoparticle – a polymer based particle that may be solid throughout or contain internal aqueous volumes, and can consist of multiple polymer components.

Micelle – a self-assembling particle that can be formed of lipids, ionic surfactants or amphiphilic block copolymers.

Enhanced permeability and retention (EPR) effect – an effect by which blood circulating nanocarriers extravasate into and are retained in the extracellular space in areas of the vasculature exhibiting abnormally large fenestrations between cells, such as in tumors.

Cisplatin (CDDP) – the earliest of the platinum based antineoplastic family of chemotherapeutics, consisting of a cis-arrangement of chloride and amine irons around a platinum (II) core.

Hyperthermia – an increase above the normal temperature range of the environment; in the human body ~37^{o}C. For most tissues, sub-lethal temperatures below 45^{o}C can be held for an extended duration with minimal cell death. Ablative hyperthermia above 60^{o}C causes irreversible denaturation of proteins and cell death.

The discovery of cisplatin and subsequent expansion of the platinum based chemotherapy drug family has revolutionized the treatment of certain cancers, and these drugs now account for almost 50% of clinically used anticancer therapeutic agents.[1] Initially discovered as an anti-bacterial agent over 50 years ago, cisplatin was found to have potent inhibitory effects on cancer.[2] This led to its use against a wide range of tumors, including head and neck, cervical, bladder and ovarian.[3] Of particular note is the use of cisplatin in testicular cancer. Its introduction to the combined drug therapy of disseminated germ cell tumors in testicular cancer raised the chemotherapy cure rate from 5% to approximately 80%.[4] Cisplatin is now used in a variety of different drug combinations and forms the cornerstone for a number of chemotherapy treatments.[5]

Despite its widespread clinical use, the side effects associated with the toxicity of cisplatin are significant and limit the maximum dose that can be administered.[6] Additionally, cisplatin resistance is a major concern for long term drug use. Thus, there has been great interest in developing strategies to reduce the systemic toxicity of cisplatin and improve the efficacy of cancer treatments.[7] Much attention has been focused on creating drug delivery systems that can temporarily passivate platinum complexes such as cisplatin and enable transport to the tumor site. Candidate systems include liposomes, micelles, polymers and inorganic nanoparticles. For all untargeted nanocarrier systems, however, effective deposition in tumor tissue relies primarily upon the enhanced permeability and retention effect (EPR). This effect is highly dependent upon the characteristics of the tumor, which may cause limited and/or heterogeneous extravasation of nanoparticles in solid tumors.[8,9] Consequently, more sophisticated “active” delivery strategies may need to be applied to improve tumor uptake. For example, it has been demonstrated that ultrasound can be used both to

target drug release from nanocarriers and enhance extravasation and distribution of chemotherapy agents in tumor tissue.[10]

The following sections outline the mechanisms of action and limitations of cisplatin and other platinum chemotherapy agents, and review strategies for improving the therapeutic ratio by physical delivery of nanocarriers, with a focus on polymeric encapsulation of cisplatin and ultrasound mediated delivery.

Mechanism of action of cisplatin

Cisplatin's structure and mechanism of action is shown in Figure 1. The most recognized mode of cytotoxic activity is the creation of unrepairable platinum-DNA adducts on purine bases, ultimately resulting in sufficient DNA damage to trigger apoptosis in the cell. Accumulation of cisplatin molecules within the cell is directly linked to their toxicity. It has been shown that the greater the number of DNA adducts of cisplatin, the greater the cytotoxic effects seen within the cell. Cisplatin initially enters the cell *via* both passive diffusion and active uptake, primarily through the copper membrane transporter CTR1.[11] In the bloodstream, cisplatin is relatively stable and maintains its neutral state, due to the high concentration of chloride ions (~100 mM). Once inside the cell, however, the relatively low chloride ion concentration (~4-12 mM) causes cisplatin to undergo aquation, whereby a chloride is displaced by a water molecule.[12] As shown in Figure 1 this is a key step as the aqua-cisplatin complexes do not readily diffuse from the cell, and importantly the mono-chloride form is a potent electrophile that will rapidly react with nucleophiles such as DNA. In DNA, this results in binding to the nitrogen in the N^7 position on purine bases with loss of the water molecule.[13] The remaining chloride is then subsequently aquated allowing the cisplatin to crosslink to another purine. Crosslinking between adjacent

guanine residues is considered to be crucial to the cytotoxicity of cisplatin.[14] The adjuncts interfere with DNA replication and transcription causing cell cycle arrest and potentially activation of pro-apoptotic signals. Cell cycle arrest leads to activation of DNA repair pathways, particularly nucleotide excision repair (NER). The NER complex is capable of repairing DNA adducts of cisplatin by excising the damaged region and could allow for cell survival. However, should the DNA damage be too extensive to repair, apoptosis will be the likely outcome.

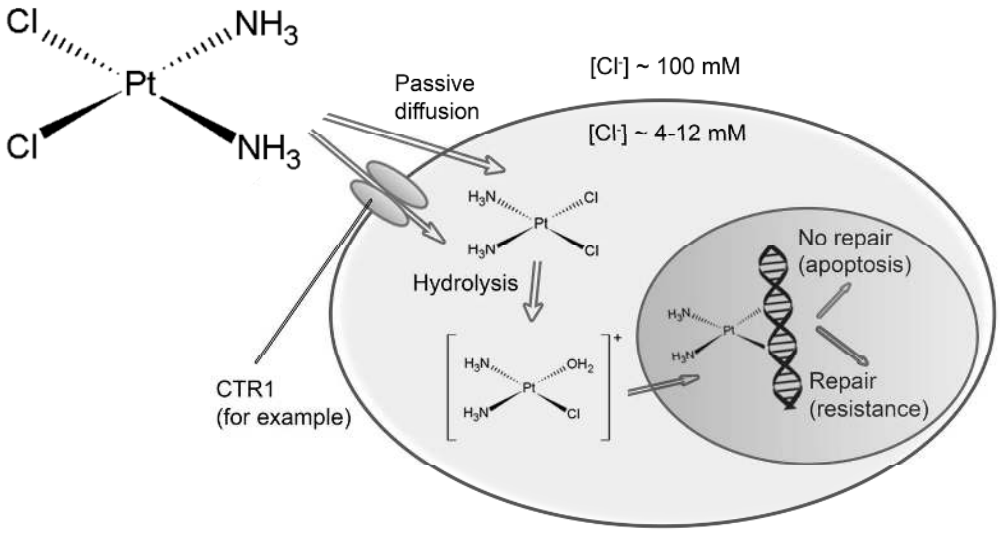


Figure 1. Cisplatin structure and mechanism of action.

DNA damage is not the only mechanism by which cisplatin may trigger apoptosis. Cisplatin's interaction and reaction with other proteins has been linked to cellular damage. In particular, the induction of oxidative stress during cisplatin treatment can lead to mitochondria damage and dysfunction,[15] glutathione depletion, lipid peroxidation, apoptotic pathway activation, and other deleterious effects. This combination of apoptotic effects results in a potent therapy against malignant solid tumors.

Limitations of cisplatin in chemotherapy

The highly toxic nature of cisplatin is also its main drawback as a chemotherapy agent. Systemic administration of cisplatin produces severe side effects, ranging from hearing loss to hemolysis. The most significant dose-limiting side effect is nephrotoxicity, as cisplatin accumulates in the kidneys, which can cause unacceptable levels of renal failure at dosages over 120 mg/m^2 body surface area.[16] This process manifests itself in the destruction of nephron tubules, exacerbated by a loss of renal vasculature and the stimulation of a robust inflammatory response.[17] Other common side effects in normal tissue include neurotoxicity and ototoxicity. Research has demonstrated that a combination treatment including antioxidants such as glutathione can reduce this damage without hampering therapy, however, the occurrence of these side effects requires a reduction of dosage and consequently a lowering of therapeutic effect. Other platinum containing drugs have also been developed that offer reduced side effects. For example, carboplatin has eliminated nephrotoxic effects but the reduced toxicity means a fourfold dose increase is required to match cisplatin's efficacy. The relative ease of cisplatin modification has led to much focus on altering the structure to reduce the toxicity, with a particular focus on the platinum (IV) (Pt(IV)) prodrug. These inactive prodrugs can be reduced inside the cell by glutathione to active platinum (II), *i.e.* cisplatin. The additional binding sites formed on the platinum ion by this modification also provides a covalent attachment point for nanocarrier loading, construction of platinum cage forms[18] or to other prodrugs, so called "dual threat" agents, such as histone deacetylase inhibitors.[19-21] The research

into Pt(IV) prodrugs has been recently reviewed by Johnstone *et al.* and Kenny *et al.* [22,23]

The other major concern associated with cisplatin is the relatively rapid development of resistance. There are multiple pathways by which a cell becomes resistant to cisplatin, but the key one appears to be a reduction in uptake. Whilst cisplatin is small enough to diffuse through cell membranes, its short half-life, both in terms of activity and elimination from the body, would not allow sufficient dose to enter cells. Instead, as previously mentioned, cisplatin is also taken up by active transport, primarily through CTR1. When stressed with cisplatin, cancer cells have been shown to reduce the expression of this transporter, necessitating an increasing dose of cisplatin for therapeutic effect.[24] Additionally, cells may increase production of glutathione, which sequesters cisplatin,[25] or increase DNA repair.[26] Furthermore, in a clinical situation, it is often difficult to achieve a therapeutic concentration of drug throughout a solid tumor as a result of the tumor microenvironment.[27] Cells which are far from a feeding vessel may receive a sub-lethal dose and become progressively more resistant with repeat dosing. To mitigate these factors, cisplatin is almost always given as a combination treatment, but cisplatin resistance remains a significant challenge.

Cisplatin delivery using nanocarriers

In order to address the aforementioned drawbacks of platinum containing drugs, much attention has been given to drug delivery strategies. One area of great interest in this field is encapsulation within nanoscale particles or "nanocarriers". The complementary aims of this approach are first to reduce systemic toxicity by

temporarily passivating the drug during its transport through the blood stream and second to increase tumor uptake through targeting of the nanocarriers, thereby improving the therapeutic ratio (recently reviewed in depth in Johnstone *et al.*[22]). An ideal nanocarrier should thus encapsulate the drug with high efficiency, prevent premature degradation of the drug or interaction with healthy tissue and deliver its payload in a targeted and controlled manner. The simplest form of (passive) targeting exploits the differences between cancerous and healthy tissue to promote drug uptake in the tumor. Tumors typically feature "leaky" blood vessels and poor lymphatic drainage.[28-30] Thus, whilst typical low molecular weight free chemotherapy agents will diffuse non-specifically through the walls of both healthy and tumor tissue, drugs loaded into nanocarriers can only extravasate in the highly permeable tumor capillary beds. The nanoscale dimensions of the carriers not only prevent their extravasation in normal tissues but also removal by renal clearance, making the size of delivery vectors very important. The cut-off size for extravasation into tumors has been reported as ~400 nm during experiments with liposomes of different mean size,[31] however the consensus from different studies is that particles with diameters <200 nm are more effective.[32]

Cisplatin and other platinum agents have been loaded into a variety of polymeric, lipid and inorganic nanocarriers, including liposomes, nanoparticles, and nanotubes. The most prominent attempts at reducing side effects have focused on liposomal encapsulation, which has been successfully utilized for encapsulation of another chemotherapy drug, doxorubicin. Doxorubicin is toxic to heart muscle, which can limit its usage for certain patients with pre-existing cardiomyopathies or in certain drug regimes, such as concurrent usage with Herceptin for breast cancer metastases. The two available liposomal encapsulated forms, Doxil (Johnson & Johnson, New

Brunswick, NJ, USA) or Myocet (Teva Pharmaceutical Industries, Petah Tikva, Israel), reduce the cardiotoxicity whilst maintaining therapeutic effect.

However, utilizing the same liposome formulation for cisplatin, known as SPI-77 or Stealth® cisplatin, showed poor clinical results. Whilst accumulation of liposomes was demonstrated within tumors, the rate of cisplatin release was insufficient to produce a significant cytotoxic effect and clinical trials were halted.[33,34] Recently, a fusogenic liposome formulation, Lipoplatin (Regulon Inc., Mountain View, CF, USA), has completed a number of phase II and phase III clinical trials on non-small cell lung carcinoma and pancreatic cancer. Like SPI-77, 10-50 times accumulation in tumors *versus* adjacent normal tissue was seen, but with a therapeutic effect similar to or greater than cisplatin only, typically when used in combination with paclitaxel.[35] Notably, Lipoplatin caused negligible toxicity.[36] Several liposomal formulations of cisplatin or analogues have undergone clinical investigation, reviewed recently in Liu *et al.*[37]

Other incorporation techniques that have been used with platinum based drugs utilize different types of solid nanoparticles made of polymers (*e.g.*, poly(lactic-co-glycolic acid) (PLGA)), proteins (*e.g.*, human serum albumin and right handed coiled coil[38,39]) or inorganics (*e.g.*, silica NPs, gold NPs, iron oxide NPs, metal oxide frameworks, and carbon nanomaterials). Such nanoparticles utilize different strategies to load drugs. For example, PLGA particles consist of a permeable polymer mesh that provides sustained release of the encapsulated drugs. On the other hand, silica NPs have a high mesoporosity, with pores sizes from a few to tens of nanometers, and easily tunable surfaces which allows for a high loading capacity and slow release of drugs. Albumin based NPs have the advantage of albumin's natural binding affinity to cisplatin, which reduces renal excretion and, despite the irreversible binding, appears

to retain cisplatin's activity.[40] There are several well-established techniques for producing loaded nanoparticles. These enable the properties of the nanoparticles, such as their size, shape, charge and permeability to be carefully tailored to the specific requirements of the application and the drug in question.

Whilst promising, and potentially capable of numerous chemical modifications for targeting or release purposes, only two particle-based cisplatin agents have undergone clinical trials to date. Whilst not strictly a nanoparticle, BP-C1 (Meabco A/S., Copenhagen, Denmark) a benzene-poly-carboxylic acid complexed with cisplatin, recently completed a phase I and II trial for stage IV metastatic breast cancer *versus* a placebo. It was found that BP-C1 controlled tumor growth, had low toxicity and mild side effects, and improved quality of life.[41] A 100 nm PEGylated, micellar nanoparticle, NC-6004 or Nanoplatin™ (Nanocarrier Co. Ltd., Kashiwa, Chiba, Japan), consisting of cisplatin bound to hydrophobic polymers is currently under clinical trial investigation for pancreatic (phase III), head and neck (phase I) and other solid tumors (phase II). Dose escalation studies have shown good tolerance of the NC-6004 with mild adverse events and some evidence of disease stabilization[42] with reduced kidney damage in comparison to cisplatin treatments from a different study.[43]

These cisplatin nanocarriers are important in demonstrating reduced toxicity and adverse events, concurrent with accumulation in tumors. However, whilst the reduction in toxicity is of enormous benefit to a patient's quality of life, the comparable efficacy to free cisplatin indicates that further strategies are required to increase uptake and release from these nanocarriers to improve the clinical outcome.

Solid tumor barriers to passive delivery

Passive delivery of untargeted nanocarrier systemic therapeutics to a therapy-resistant solid tumor, is complicated by the pathophysiology of its microenvironment. Effective delivery *via* the EPR effect is complicated by a poorly organized and tortuous blood supply within a tumor. Whilst the leaky, ill-formed endothelial layer allows the extravasation of nanocarrier drugs, the abnormal flow conditions hinder their delivery to the tumor site.[28-30] Additionally, the interstitial pressures of tumors is high, due to the rapid proliferation of cells in a tight area, vascular leakiness, and lack of development of lymphatic drainage, which further disrupts blood flow by squeezing vessels and preventing the pressure gradient-driven diffusion of large molecules out of the circulation.[27,44] The rapid proliferation of cells and poor vasculature lead to regions of cells far removed from the circulation, increasing the diffusion distance required for therapeutics and inducing a treatment resistant hypoxic nature.[45] Tumors can also exhibit a poorly organized extracellular matrix (ECM) high in collagen and charged glycosaminoglycans which obstructs tumor interstitial flow and prevent the penetration of large molecules deep into the tumor.[46,47] These barriers to nanoparticle delivery have been previously reviewed in detail elsewhere.[48,49]

With these barriers to delivery and the heterogeneity of tumors, any evidence for EPR effect requires careful consideration.[50] In some cases, it has been estimated that EPR may only increase uptake in tumors two-fold in comparison to other organs and will depend highly on the tumor type, location and vascularity of the tumor.[51] As such, nanoparticle delivery to target sites can be hindered by a lack of extravasation and/or retention ability in the most commonly used, unmodified vectors.[52] Additionally, the highly disorganized nature of tumor tissue and blood vessels can lead to non-uniform distributions of nanoparticles. Alternative strategies are therefore

required to improve drug uptake and drug release in a tumor. The following sections will detail the different methods that have been explored to improve delivery of cisplatin.

Methods of Delivery

Nanoparticle design

The simplest approach to increasing uptake in tumors is to vary the physical parameters of the nanoparticle (recently reviewed by Blanco *et al.*[53] and Durymanov *et al.*[54]). As mentioned earlier, size, shape and charge[55] can all play an important role in the extravasation of nanoparticles. These parameters also affect the clearance route and lifetime of the nanoparticle in circulation. For example, nanoparticles below 5 nm have excellent penetration and distribution within tumors but are rapidly cleared *via* the kidneys. Additionally, lowering the size of nanoparticles may compromise loading efficiency.[56] For spherical particles, a twofold reduction in nanoparticle radius lowers the maximum loading volume eightfold, but also increases the specific surface area, which can affect release rate and interactions. As such, the most appropriate nanoparticle design will depend upon its specific application.

Active targeting

One method is to provide active targeting to tumor tissues by identifying distinct biomarkers. Tumor cells and surrounding healthy cells typically display an abnormal set of membrane bound receptors and proteins. Antibodies raised against these targets can be attached to nanocarriers to assist accumulation at the tumor site.[57] Examples of such receptors include vascular endothelial growth factor receptor, VEGFR, which is expressed by the endothelial cells of growing blood vessels, as typically found in

nutrient starved solid tumors. Other receptors, such as folate receptor, biotin receptor, HER2, EGFR and interleukin-4, can all act as targets for antibody, peptide or small molecule targeting.[58-60]

This form of targeting is relatively simple to achieve with surface modification of the nanoparticle (reviewed in[61] and has formed part of a number of targeted cisplatin nanoparticles strategies.[57, 62-64] However, there are some important considerations: First, for this type of targeting to be effective, the nanoparticles must come into sufficiently close proximity to the relevant cells. As previously mentioned, the EPR effect may only improve nanoparticle extravasation in a tumor site by twofold compared to normal organs, meaning that the majority of nanoparticles will rarely come into close contact with tumor cells. Thus, whilst those nanoparticles that enter the intracellular space may be better retained in the tumor, active targeting may not significantly improve uptake in large solid tumors with poor vascularization. Second, some targeting markers, particularly endothelial markers and others such as folate, can lead to rapid clearance[65] and third, these markers may also be strongly expressed off-target.[66]

Direct injection

Several physical methods have also been proposed to increase local delivery and retention. The simplest method is to directly insert the drugs into the tumor tissue. Intraoperative approaches for debulking or eliminating residual tumor tissue include the insertion of chemotherapy drug pellets or wafers directly at the target site.[67,68] An internal radiotherapy, or brachytherapy, works by a similar method and is typically performed in surgically challenging locations. For nanoparticles, intratumoral injection has been investigated as a way to ensure complete drug delivery in the target

site without dilution or loss in the circulation.[69-71] Direct injection can also improve the distribution of the drug within the tumor.[56, 71] However, intratumoral injections are not commonly used in clinical practice because of the invasiveness of the technique for deep tumor sites and the established nature of standard surgical or radiotherapy techniques for accessible tumor sites. Historically, investigations into direct injection of free drugs demonstrated rapid clearance, poor drug distribution and toxicity to surrounding tissue.[72,73]

Tissue hyperthermia

Tissue hyperthermia is a simple technique that can have a range of effects on a tumor's microenvironment. Fluid flow around the tumor is improved, resulting in a reduction in interstitial pressure and improved chemotherapy drug uptake and effect,[74] along with a notable synergistic effect for cisplatin due to cellular changes.[75,76] Heating of cell membranes also increases lipid fluidity and permeability to drugs.[77] Finally, heating increases the diffusion rate of drugs, and can reduce hypoxia, a major barrier to effective drug delivery.[78,79] There are many methods to apply heating to a target region, both invasively and non-invasively, and hyperthermia has been attempted with several different nanoparticles formulations.[80,81] Indeed, the effect of hyperthermia in tumors can have further useful effects for the delivery of nanoparticles. Li *et al.* demonstrated that local, sub-lethal hyperthermia in a windowed, subcutaneous tumor model could induce gaps in the endothelial layer of up to 10 μm, with the vasculature still permeable up to 8 hours.[82] This led to an increase in the accumulation and retention of 85 nm, fluorescently labelled liposomes, as shown in Figure 2.

However, as hyperthermia is a relatively non-specific delivery technique, heating must be localized to the target area to ensure effective target site delivery and reduce the effect on surrounding tissue. Heat transfer is subject to tissue and tumor heterogeneity, as well as cooling from blood flow. For instance, heating near bone can be particularly problematic due to the relatively low thermal conductivity of ossified tissue in comparison to soft tissue, which can lead to unintentional thermal necrosis or off-site delivery.[83,84] The difficulty in assessing heat transfer impacts the treatment planning. Temperature monitoring can be performed, but this requires either implanting temperature probes, an invasive procedure which provides only single point information, or thermometry by magnetic resonance imaging (MRI), a costly procedure which limits the materials that can be used.[85,86]

Whilst tissue hyperthermia does increase nanoparticle delivery, it is typically applied in combination with a nanoparticle modification aimed at triggering drug release under hyperthermic conditions as discussed later in the section on thermal release.

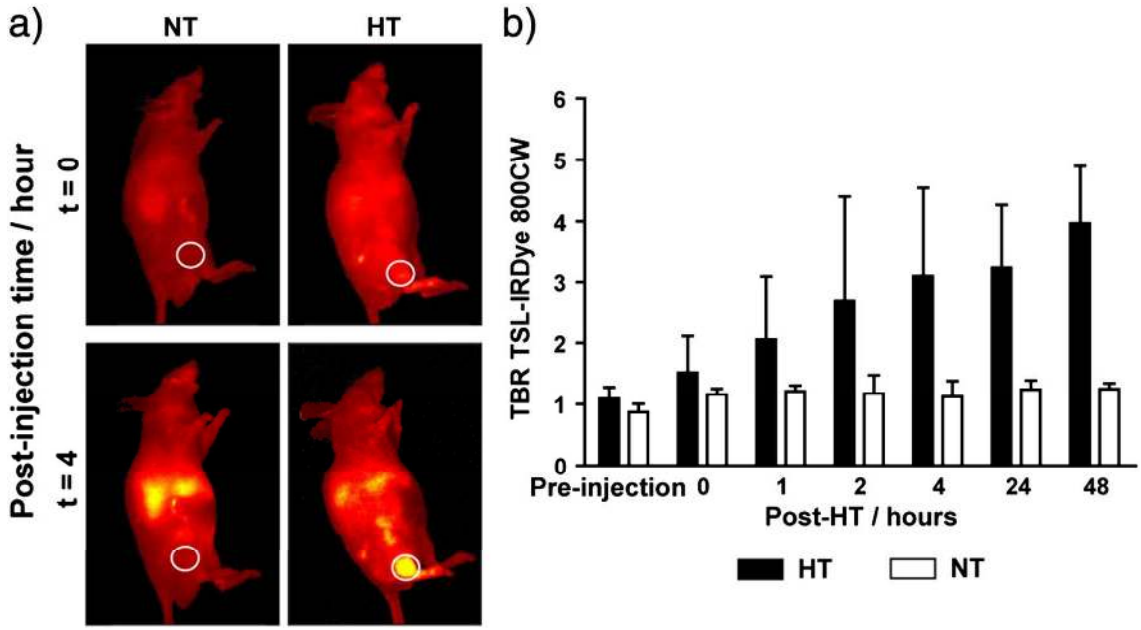


Figure 2. Accumulation of fluorescently labelled liposomes (TSL-IRDye 800CW) in a hind-limb subcutaneous tumor mouse model. a) Whole body imaging shows significant fluorescent signal from tumors four hours after liposome injection, when preceded by one hour of sub-lethal hyperthermia (HT) in the tumor bearing limb in comparison to normothermia (NT). Absolute tumor fluorescence peaked at 4 hours for hyperthermia treated mice but b) the tumor-to-background ratio (TBR) continued to increase as liposomes were cleared from blood circulation but retained in the tumor. Reprinted from Reference 82. Copyright (2013) with permission from Elsevier.

Magnetic targeting

Magnetic targeting has also become an attractive approach for cisplatin based drug delivery with the increasing availability of biocompatible superparamagnetic nanoparticles. Their ability to enhance MRI contrast to allow imaging,[87-90] to localize in specific regions under external magnetic fields,[91-94] and to cause local hyperthermia under oscillatory magnetic fields (discussed in the section on thermal release),[95-97], makes them popular agents to include in drug formulations. Superparamagnetic iron oxide nanoparticles (SPION), are commonly used to add a magnetic response to larger nanoparticles or other vector particles, but require stabilization to prevent aggregation, oxidation and loss of magnetic properties.

Cisplatin has been loaded extensively into solid and lipid based magnetic nanoparticles.[94, 98-101] In one such study, Wagstaff *et al.* prepared 60 nm to 120 nm cisplatin loaded gold-coated iron-oxide nanoparticles for use against cisplatin sensitive and resistant cell lines.[102] The conjugation of chemotherapy drugs on to gold nanoparticles has been shown to enhance uptake and cytotoxic effect, particular for

cisplatin and other platinum based chemotherapy drugs.[103-106] The gold nanoparticle also stabilizes the iron oxide, preserving magnetic response. Gold was coated onto an iron oxide core and hydrated cisplatin conjugated to the gold *via* polyethylene glycol linkers (See Figure 3). The combination of the gold and cisplatin resulted in nanoparticles with over 100-fold improvement in the half maximal inhibitory concentration (IC_{50}) values in cisplatin-sensitive cell lines. Inhibition of proliferation was also seen in specific regions when combined with a magnet. However, the unloaded gold-iron oxide nanoparticle itself displayed potent cytotoxicity and cisplatin resistance in a resistant cell line was not overcome with the loaded particle. Additionally, cisplatin release from the nanoparticle was not directly demonstrated and the strong coordinate bonds used to tether cisplatin to the nanoparticle to prevent systemic release, may prevent target site release and likely interfere with its mode of action.

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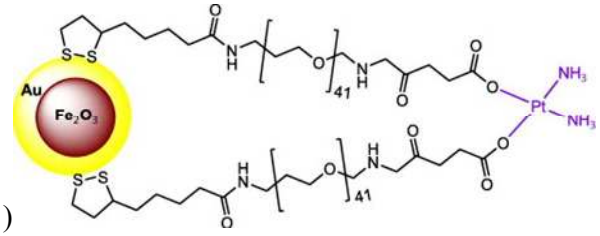

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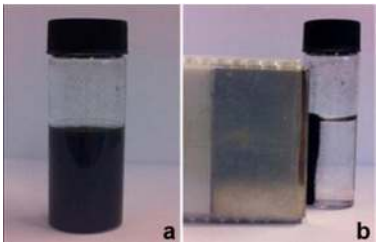

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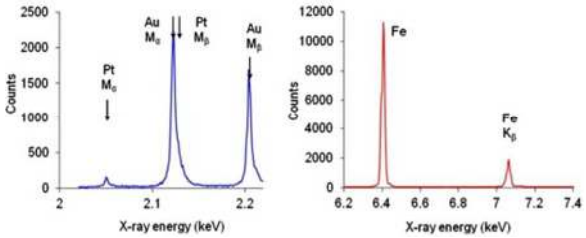

Figure 3. A potential nanoparticle design combining the improved cytotoxicity of cisplatin and gold nanoparticles, with an ability to magnetically target to a location. (1) Schematic showing the final cisplatin bound, PEGylated gold-coated iron oxide nanoparticle. The nanoparticle was (2) magnetically active and (3) loaded with cisplatin. Reprinted from Reference 102. Copyright (2012) with permission from Elsevier.

One of the great challenges with this approach is the practical generation of sufficient magnetic field gradients in confined locations in deep tissue. Additionally, overlaying tissue is unavoidably subjected to magnetic retention and the technique may be limited to tumors close to an accessible surface, *e.g.* skin, muscle, nasal, *etc.* or during surgery. However, some of these challenges are being addressed with optimized magnet designs, with a recent publication reporting the design of a Halbach array magnet for brain drug delivery applications with a useable depth of up to 50 mm.[107] A further consideration is the potential of cytotoxicity. SPIONs that are clinically approved for use have low or no toxicity at low levels, however at high exposure levels, or in their uncoated forms, cytotoxicity is seen.[108] It will be vital to ensure the biological safety in their increasingly complex use. The safety of SPION agents has been reviewed previously in the literature, albeit not recently.[109]

Electroporation and Electro-motive force

Electroporation is the use of short electrical pulses to increase the permeability of cell membranes, by the formation of pores. Sufficiently high voltages cause unrecoverable pores to form in the cell, a process known as irreversible electroporation, which is typically fatal for the cell. Whilst this is currently under

investigation in clinical trials as a potential method of tumor ablation, reversible electroporation, where lower voltages cause only temporary poration, increase the cellular permeability to typically membrane impermeable drugs.[110-114] The combination with chemotherapy, clinically termed electrochemotherapy (ECT), has been extensively used clinically to treat cutaneous or sub-cutaneous tumors, usually with bleomycin or cisplatin.[115-118] ECT is a promising technique with a short treatment time, low side effects, and tumor response rates generally greater than 80% against a range of tumor types, but the technique is still limited to superficial tumors, is typically used for palliative management and requires the placement of two electrodes either side of the target site, which can be complicated depending upon the pathology. The clinical focus is now on targeting internal tumors,[119,120] however as side effects include muscle contraction and pain, some areas will likely remain untreatable. Additionally some research is looking at the potential combination with nanoparticle formulations to improve targeting and guidance to a tumor before electroporation,[121,122] although this has not been extended to the use of cisplatin yet.

Alternatively, the application of a constant electric direct current causes iontophoresis; the movement of ions or charged molecules under an electric field. When electrodes are positioned on either side of a target tissue site, charged drugs will be forced into tissues and cells. Clinically, this is termed electro-motive drug administration (EMDA), and has been used in patients for dermal and intravesical, *i.e. via* the bladder, delivery of anti-cancer drugs.[123-127] Iontophoresis is less disruptive than electroporation, although conversely treatment times are longer. Like electroporation, it is also capable of transporting nanoparticles into tissues, although again, the use has been primarily focused on dermal delivery, which benefits from non-invasive placement of electrodes. To the best of the authors' knowledge, the use

of cisplatin loaded nanoparticles coupled with electroporation has not been reported in the literature.

Ultrasound mediated delivery

Ultrasound, a high frequency pressure wave well known for its clinical diagnostic use, has a number of therapeutic applications. For delivery purposes, the mechanical agitation and thermal effects of pressure waves upon tissue have been shown to increase both the uptake and extravasation of drugs in target tissues. Ultrasound-mediated delivery (UMD) is an attractive option for cancer therapy due to its non-invasiveness, site and depth specificity, low cost, short lived bioeffects and good *in vivo* safety profile. Several potential methods are responsible for the increase in nanoparticle uptake in a target area and are described in greater detail below.

The propagating pressure wave of ultrasound generates a pressure gradient in the tissue due to the absorption of energy. This primary acoustic radiation force (ARF) is in the direction of ultrasound propagation and can be sufficient to cause a net displacement of tissue and particles in the focal region. ARF can cause loosening of endothelial junctions and tissues,[128-132] reducing tumoral interstitial pressure, as well as increased permeability in deep tissue by heterogeneous motion of tissue.[133-135] ARF can also cause movement of therapeutics directly into the target sites, a sonophoresis effect.[131,136] These effects can lead to improved uptake and effect of free chemotherapeutics[137-139] and nanoparticles in tumors,[131, 140] but has not been used on cisplatin loaded nanoparticles. The transfer of momentum from the propagating wave to the surrounding fluid can also set up fluid flow within the tissue, known as acoustic streaming,[141] which may also increase drug uptake.[142]

Just as SPION nanoparticles can act as theranostic agents for magnetic targeting applications, there are similar agents available capable of responding to externally applied ultrasound for both imaging and therapeutic purposes. These agents, described here as cavitation nuclei but divided broadly into microbubbles, nanodroplets and gas entraining particles, have significant vector capabilities and much research has gone into modifying these to improve drug and gene delivery.[143-145] The exact mechanism of action varies depending upon the agent, but broadly speaking, in the presence of an acoustic field, these agents undergo cavitation; the generation, oscillation and collapse of a gas/vapor bubble in a pressure field. The fluid motion and acoustic emissions produced by these oscillating and collapsing bubbles can increase local permeability by blood vessel rupture,[146-149] disruption of cellular junctions and temporary poration of cell membranes.[150,151] It has been demonstrated that microbubbles are susceptible to radiation forces and can be manipulated *in vivo* to ensure close proximity to the endothelial wall[152,153] for improved endothelial rupture.[154,155] This disruption increases permeability to co-delivered drugs and has been demonstrated to improve uptake and cytotoxicity to free cisplatin in target tumors *in vivo.*[156-162]

A further attractive feature of cavitation nuclei is their potential for surface functionalisation. As permeability changes are temporary, it is essential that the drug and cavitation event are proximate. Cavitation nuclei typically consist of a gas bubble or phase change liquid encapsulated in a biocompatible shell, which can be surface functionalized to allow loading of drugs and/or nanoparticle drug carriers,[163-165] as reviewed in several publications.[166-168] For instance, microbubbles, an agent used both diagnostically and in therapeutic research, range in size from 1-10 μm, allowing considerable nanoparticle loading. Burke *et al.* demonstrated improved skeletal

muscle delivery in mice using fluorescent PLGA-based nanoparticles covalently attached to microbubbles compared to unbound co-injections of nanoparticle and microbubble,[169] highlighting the importance of localizing drug and cavitation. Subsequently, this "composite-agent" loaded with fluorouracil was used to target gliomas in mice (See Figure 4).[164] However, typical microbubbles have a short half-life in circulation and are particularly lost during pulmonary passage. Some microbubbles are also particularly susceptible to Kupffer cell phagocytosis in the liver.[170] The potential effect of this on the loaded drug clearance and off-site effects is not well understood.

It should also be noted that although the components and concepts in nanoparticle loaded cavitation nuclei have been previously licensed for clinical purposes, the combination, and in particular the therapeutic use of cavitation nuclei, would almost certainly need to be demonstrated to be safe and significantly more effective than current approaches in extensive clinical trials. The consequence of this has already been seen in the choice of clinical trials that have been performed on the UMD concept. For instance, Dimcevski *et al.* examined the safety, toxicity and potential of improving gemcitabine delivery by UMD in 10 patients with inoperable pancreatic cancer.[171] For this application, a clinical ultrasound machine and the diagnostic cavitation agent SonoVue® (Bracco Imaging Scandinavia AB, Oslo, Norway) were used. Although neither is designed for therapeutic purposes, these materials have been used safely and extensively for diagnostic imaging for decades. The positive outcome of the trial with an increase in median survival from 8.9 months with gemcitabine alone (from a historical study of 63 patients) to 17.6 months with the combination treatment, with no additional toxicity, does highlight the future potential of UMD. However, the therapeutically focused formulations of loaded cavitation nuclei

typically used in pre-clinical research will likely face substantial hurdles before clinical approval.

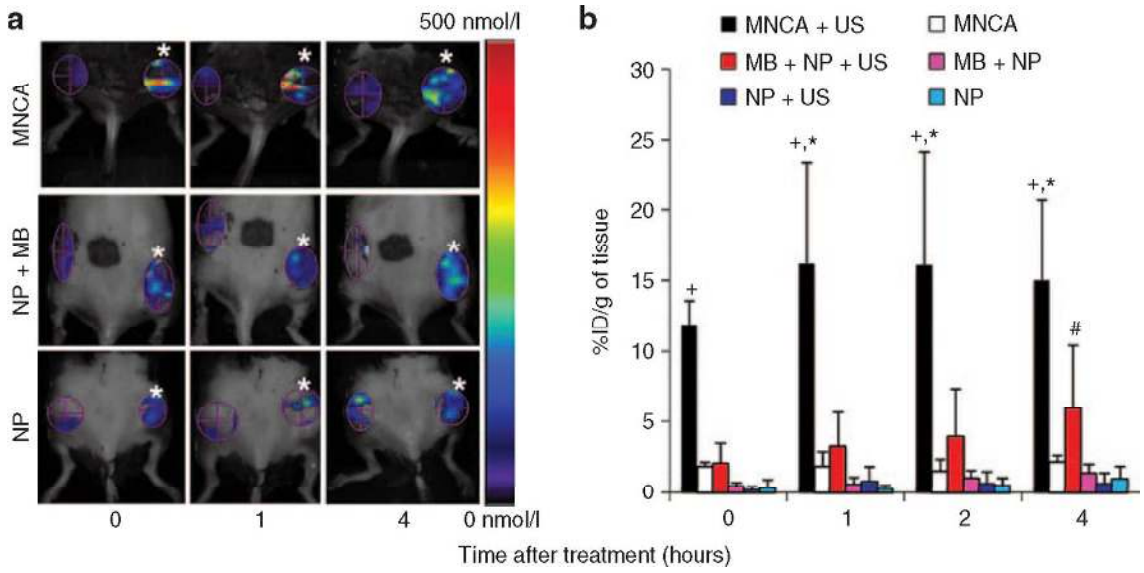

Figure 4. Increased uptake of nanoparticles in gliomas treated with ultrasound (US) and a microbubble-nanoparticle composite agent (MNCA). a) Fluorescence-molecular tomography scans and b) fluorochrome analysis of *ex vivo* tissue demonstrate a significant uptake of the PLGA based nanoparticle in comparison to a co-injection of nanoparticles and microbubbles (MB + NP) or nanoparticle only (NP) controls. Reprinted from Reference 164. Copyright (2014) with permission from Elsevier.

Only one conference proceeding regarding the combined use of cavitation nuclei and encapsulated cisplatin could be found in the literature. Yang *et al.* presented work demonstrating a focused ultrasound treatment combined with microbubbles and a targeted liposome encapsulated cisplatin (Lipoplatin) could reduce tumor progression compared to untreated controls in glioblastoma rat brain model, with intact skull.[172] Whilst promising, it is difficult to determine the advantage of the treatment or the targeting due to a lack of appropriate controls and the effectiveness of the untargeted

Lipoplatin-only treatment. However, the authors' previously published literature with doxorubicin loaded liposomes does suggest the ultrasound treatment is an effective addition.[173]

Finally, high intensity, focused ultrasound (HIFU) is capable of producing significant temperature rises. As mentioned, acoustic energy is absorbed by tissue as the pressure wave propagates. Besides kinetic motion, energy is lost as heating of the tissue. When the acoustic wave is focused by a curved array or multiple elements, HIFU can lead to significant hyperthermia in a discrete region.[174] Used primarily for clinical ablation, the highly localized nature of HIFU has seen a significant amount of research and trial use as a targeting and drug release technique, and will be covered in more detail in the section on thermal release.

Ultrasound mediated delivery appears to be a potentially effective, non-invasive drug delivery technique capable of deep tissue targeting. However, there is still uncertainty regarding the mechanism by which acoustic energy or cavitation nuclei can improve delivery, and as such, the most appropriate choice regarding therapy. Additionally, although permeability has been reported up to 8 hours after ultrasound treatment,[175] the typically short recovery times of tissue permeabilisation[176,177] may indicate a need to focus on short-lived pharmaceuticals with poor target site uptake.

Current work is also looking at overcoming the short lifespan of most cavitation agents *in vivo,*[178,179] and potentially using submicron scale cavitation nuclei to extravasate into leaky tissues before activation. Finally, UMD cannot easily be applied in areas of overlying bone or gas. Bone is a strong absorber and scatterer of ultrasound, affecting both focusing and potentially causing unintended heating.[83] In gas rich regions, ultrasound can be strongly reflected and may cause cavitation or mechanical damage to tissues at their tissue-gas interface.[180]

Lithotripsy

Lithotripsy is a short-impulse pressure wave generated by extra-corporeal shock wave devices and is typically used for breakup of stones in kidneys and the gall bladder. The high energy shockwaves (HESW) generated are typically very short in duration (10 ns), have a low pulse repetition frequency and very high positive pressures. Lithotripsy devices are not commonly used for drug delivery in tumors, although some early attempts were made with free cisplatin,[181,182] as the low frequencies and high pressures insonify large regions. Fine targeting of tumors is difficult[183] and the uncontrolled nature can, in some cases, cause additional animal death[184] and potential metastasis.[185]

More recently, some work has looked at the potential combination of HESW and polymethyl methacrylate (PMMA) nanoparticles loaded with meso-tetrakis (4-sulfonatophenyl) porphyrin (TPPS),[186] a photosensitizer drug with high tumor affinity which generates reactive oxygen species when excited with light or ultrasound. Loading TPPS onto nanoparticles before HESW treatment resulted in a significant decrease in neuroblastoma cell proliferation *in vitro*. TPPS and HESW treatment without nanoparticles had no effect on cell proliferation. The rough surface of the nanoparticle was thought to act as a cavitation nuclei source for activating the drug and was also shown to improve the uptake of the drug into cells over 12 hours, although the mechanism for this was not described. Follow up work using radiotracer-labelled drug in tumor bearing mice demonstrated increased uptake in spleen and liver *versus* free drug. HESW treatment also increased tumor uptake of the loaded drug, with associated growth reduction.[187] Lithotripsy continues to find some application for sonodynamic therapy research,[188,189] where ultrasound is required primarily for

drug activation rather than delivery, but is not a commonly used ultrasound-mediated delivery technique for chemotherapy, and no references could be found for the combination of HESW, cisplatin and nanoparticles.

Targeted release

Thermal release

Whilst successfully targeting nanoparticles to tumors is in itself a challenge, it is compounded by the need to release the drug efficiently at the target site. Slow release of the drugs from nanoparticles is useful to avoid premature leakage, but can be a barrier to achieving effective release at the target site. As such, further methods have been tried to either use external methods or aspects of the intracellular tumor environment to improve release.

As mentioned earlier, hyperthermia has been used to increase drug uptake in target tissues.[190] Additionally, nanoparticles have been modified to improve their release kinetics under heating. Although not the topic for this review, thermosensitive liposomes (TSLs) loaded with cisplatin have been used to investigate potential delivery.[191,192] TSLs are designed such that the lipids in the bilayer undergo phase transitions at sub-lethal temperatures (39-43^{o}C) resulting in release of their payload. In their thesis, Landon describes the production of cisplatin loaded lipid TSLs for use in targeting xenograft or orthotopic rodent cancer models, with thermal energy provided by a water bath or specialized heating element, with a resulting increase in anti-tumor effect and reduced side effects *versus* free drug.[193] TSLs have been recently reviewed in depth by Grüll & Langereis.[194]

Submersion of targeted areas in heated water is a simple method to cause hyperthermia, however if accumulation in the target tumor is not guaranteed, this can

lead to off-site release. Instead, targeted techniques of heating have also been applied, much as has been done for hyperthermic delivery. Ultrasound is a modality capable of generating heat at target sites deep within tissue. By focusing the acoustic pressure wave generated by either a single curved transducer element, or multiple smaller elements, high energy absorption can be caused at the focal site, resulting in heating. Clinically, HIFU has been used for the targeted ablation of fibroids and is under investigation for non-invasive, thermal ablation of tumor tissue[174, 195] combined with common chemotherapeutics;[138, 176, 196-200] including cisplatin.[201,202]

For nanocarriers, HIFU has been used to increase both delivery and release in a target tissue. Increased tumor uptake and drug distribution has been demonstrated with many TSLs,[203-206] with one such agent, ThermoDox®, currently under investigation in a clinical trial (NCT02181075, https://clinicaltrials.gov/ct2/show/study/NCT02181075). Delivery of nanocarriers by HIFU hyperthermia is typically done using lower ultrasound intensities or reduced pulse durations, to maintain a mild hyperthermia rather than cause ablation, and has great translation potential as MRI guided HIFU machines are already clinically available and allow real-time, non-invasive thermometry and treatment.

Besides TSL and standard liposomes, thermal HIFU has also been used in conjunction with nanoparticles. Oh *et al.* found increased delivery of docetaxel loaded pluronic nanoparticles in tumors using 0.8 MHz, 20 W/cm^2 HIFU treatment at 10% duty cycle.[80] This also correlated with increased apoptotic regions in tumors compared to an untreated control, however a hyperthermia only control was not performed. No temperature monitoring was performed *in vivo*, although the authors do state previous work at the chosen intensities lead to a 4-5^{o}C temperature rise, and the higher intensities tested lead to thermal ablation. The authors, however, do state

that a mechanical ARF effect may also be responsible, as discussed previously for ultrasound based delivery strategies.

Although HIFU is capable of non-invasive heating of an area deep within the body, the small focal area requires multiple transits of the ultrasound beam to achieve homogenous heating across a large target area. Additionally, the heating is not applied specifically to the nanocarrier, but to the tissue. An alternative approach is to modify the nanocarrier to respond to an external force directly. It has been demonstrated that magnetic nanoparticles can undergo significant heating in an alternating magnetic field (AMF). This can be used for tissue hypothermia to increase cisplatin uptake,[207,208] or combined with drug loaded liposomes or solid nanoparticles to trigger drug release. This approach has been combined with cisplatin in a number of different nanocarrier formulations.[209-212]

Other thermal approaches have included phototherapy and radiotherapy. Gold nanoparticles comprise an essential part of photothermal and chemotherapy approaches when combined with anticancer drugs, including cisplatin. For example, gold nanorods with a covalent cisplatin-polypeptide wrapping and folic acid conjugation were recently developed for the targeted photothermal and chemotherapy of highly aggressive triple negative breast cancer.[213] The hybrid nanoparticles delivered systemically could significantly inhibit the growth of the tumor when combined with a near infrared laser illumination (See Figure 5).

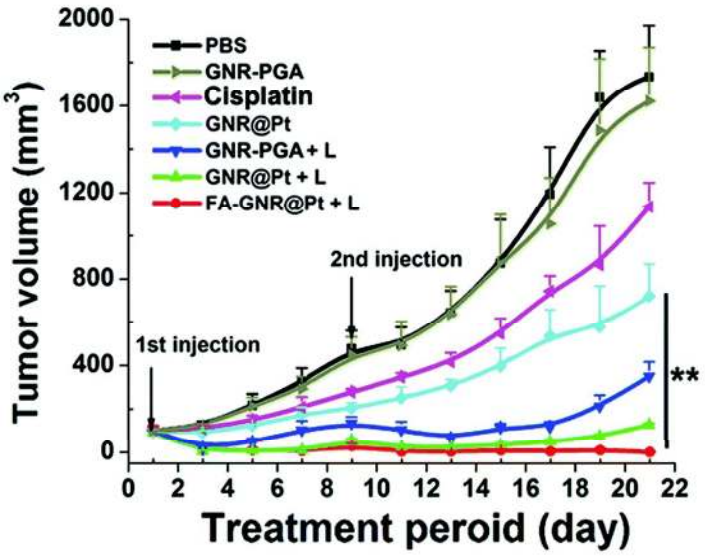

Figure 5. Tumor growth after treatment in a triple negative breast cancer mouse model. Folate acid (FA) targeted gold nanorods (GNR) wrapped in biocompatible polypeptide poly(L-glutamic acid) (PGA), were loaded with cisplatin (Pt) and intravenously administered to animals. Laser irradiation (+ L) was applied to the tumor sites and tumors monitored over 22 days. Treated animals showed significant prevention in tumor growth *versus* controls to the point of complete elimination of tumor cells in the target region and no lung metastasis when examined by histology. Reproduced in part from Reference 213 with permission of The Royal Society of Chemistry.

Carbon based nanostructures are also particularly effective at absorbing laser irradiation. DeWitt *et al.* report on the use of 100 nm single-walled carbon nanohorns conjugated to cisplatin, although the change in cellular uptake mechanisms for nanohorns at mild hyperthermia unfortunately resulted in a decrease of toxicity.[214] An alternative photothermal approach using micelles loaded with a near-infrared cyanine dye and a Pt(IV)-prodrug resulted in complete ablation of both cisplatin-sensitive and

–resistant lung carcinomas in a mouse model.[215] The penetration depth of laser light through tissue is always an issue for non-topical applications of phototherapy, however the technique can be easily paired with standard invasive procedures, such as endoscopies, catheters, *etc.* Intraoperative photodynamic therapy, where photosensitizers are administered and the relevant laser stimulation applied during surgery, is already in clinical trials for several tumor types that are difficult to fully resect.[216,217] Additionally, photothermal near-infrared (NIR) absorbing nanoparticle formulations encapsulating cisplatin have been created, to overcome the limitation of poor tissue penetration of visible light.[218,219] However, hyperthermia induced release of photosensitive drug loaded nanoparticles is still at the pre-clinical stage.

Environmental sensitive release

The tumor can present a unique environment in the body which can be exploited for triggered drug release and is the subject of a number of detailed reviews.[220-222] As the focus of this review is primarily physical methods of delivery and release, these will only be briefly covered in this section.

Due to the high glycolysis rate in cancer cells and poor waste removal in tumors, there is often a build-up of lactic acid in the tumor resulting in acidification of the environment. Additionally, the intracellular environment of tumor cells can be highly reductive, due to the increased presence of glutathione caused by high levels of glycolysis in the rapidly dividing cell.[223] Constructing nanoparticles using redox sensitive, acid labile bonds, or pH sensitive materials can result in both better delivery of and release from nanoparticles in target sites.[103, 224,225] In particular, Lin *et al.* have prepared redox sensitive Pt(IV) prodrugs as part of the structure of in silica coated metal-organic framework nanoparticles.[226,227]

Li *et al.* developed an interesting, multi-stage, polymeric, pH and redox sensitive cluster nanoparticle, dubbed an "iCluster", to overcome certain barriers for cisplatin delivery.[228] A reductive sensitive Pt(IV)-prodrug, an approach used in several cisplatin nanoparticle formulations,[62, 229,230] was conjugated to ~5 nm nanoparticles, which in turn, self-assembled into ~100 nm nanoclusters. Li *et al.* demonstrated that at pH 6.8, the release of the 5 nm drug-loaded nanoparticles was significantly increased compared to the physiological pH 7.4. Additionally, the prodrug itself was only significantly released as cisplatin in a reductive environment, as would be found intracellularly, irrespective of pH. The "iCluster" loaded with Pt(IV)-prodrug showed significantly increased circulation time, penetration into tumors and cisplatin content in *in vivo* tumor models of pancreatic cancer, cisplatin-resistant lung cancer and highly invasive breast cancer, resulting in significantly improved tumor growth prevention and survival (See Figure 6).

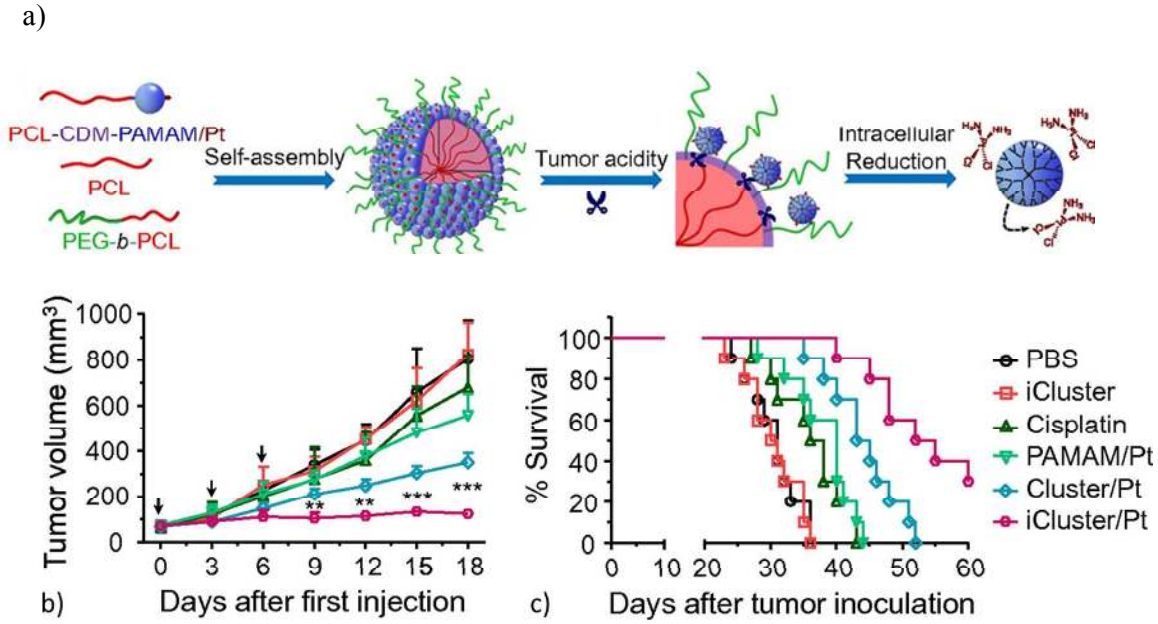

Figure 6. a) Concept and mechanism of the "iCluster" nanoparticle. b) The construct effectively inhibited tumor growth in a drug-resistant human lung cancer mouse model. c) Survival was also improved in a metastatic triple negative breast

cancer mouse model. Adapted from Reference 228. Copyright (2016) with permission from PNAS.

A further strategy is to use enzymatically degraded bonds. The inside of a cell contains many bioactive molecules which can degrade nanoparticles, to potentially allow the release of encapsulated drugs. This is an important consideration for nanoparticles taken up into lysosomal compartments within the cell. An interesting multi-drug construct based on polysaccharides was recently demonstrated by Deshpande and Jayakannan.[231] Amphiphilic dextran molecules were synthesized to self-assemble into vesicles ranging from 160-210 nm in diameter with a hydrophilic core and hydrophobic shell. Succinic molecules attached to the dextran allowed conjugation of cisplatin to form its pro-drug. The amphiphilic nature of the dextran-polymer vesicle also allowed loading of either water-soluble doxorubicin or water-insoluble camptothecin or both. Dual and triple loaded polymeric vesicles showed a significant increase in release in the presence of esterases, as would be found in lysozymes, and also protected cisplatin from inactivation from glutathione. Ultimately, when compared to free drug, the single-, dual- and triple-loaded drugs showed significant *in vitro* cytotoxicity in a cisplatin resistant cell line, at lower drug concentrations, and in addition to strong additive or synergistic interactions between the drugs further reducing the required dose. One remaining concern is that these polysaccharide-based particles may not be cell type specific, and that further modification or techniques would be required to improve specificity to the target cancer.

Ultrasound triggered release

Just as ultrasound can disrupt cellular membranes, it can also be used to release encapsulated drugs from loaded nanoparticles. Work by Schroeder *et al.*, examined the release issues with SPI-77, an early liposomal formulation of cisplatin capable of long circulation and passive tumor uptake that ultimately failed in clinical trials due to the excellent stability of the liposome, resulting in negligible therapeutic benefit. Schroeder *et al.* demonstrated an increase in cisplatin release from liposomes in murine tumors treated by 20 kHz ultrasound, sometimes termed low frequency ultrasound (LFUS), from <3% in the untreated tumors, to almost 70% in treated tumors and an almost 3 fold rise in cisplatin present.[232] This increase in local cisplatin concentration in a C26 footpad murine model, resulted in negligible growth of the tumor over 29 days in comparison to untreated controls. However, free cisplatin and the free cisplatin plus LFUS control also demonstrated a strong anti-proliferative effect, indicating the C26 cell line or applied dosage may not have been appropriate. The potential improvement in side effects was also not commented upon in the study. In their study, and follow-up modelling work on release rates,[233] Enden and Schroeder determined the mechanism of release was primarily an increase in diffusion rather than liposome disintegration, rather than improved uptake into the tumor. On the basis of previous work, the authors suggest the mechanism of LFUS on liposomal release is transient pore-like defects due to the mechanical or cavitation effects at the surface of the liposome.[234]

Similar effects were seen with TSLs and temperature insensitive liposomes (TILs) at higher ultrasound frequencies. Oerlmans *et al.* used 1 MHz, continuous wave HIFU (CW-HIFU) or direct heating on TSLs and TILs loaded with encapsulated fluorescein.[235] As expected, TSLs were sensitive to direct heating and CW-HIFU,

releasing 80% of their encapsulated fluorescein. Interestingly, TILs did not respond to the direct heating but significant release did occur with CW-HIFU. Oerlmans *et al.* further investigated using pulsed wave HIFU (PW-HIFU), a treatment regime that applies the same energy but over a longer period of time, and mostly eliminates hyperthermia. The TSLs and TILs underwent gradual increasing release of fluorescein, indicating a non-thermal method of release. Further experiments determined that cavitation was also not a factor in release, indicating a third method of ultrasound-triggered release. As no significant changes in liposome size was seen during HIFU, only a temporary disruption of the liposome membrane occurred. The authors contend that collision of liposomes with the sample chamber walls, due to acoustic streaming, and the resulting shear forces, caused the reversible destabilization. Most intriguingly, this release was also demonstrated with a lipophilic dye in the liposome lipid membrane, which could not be released from the TSLs by direct heating, indicating a potential method of releasing lipophilic drugs from nanoparticles. However, the authors note that effective release during a non-thermal PW-HIFU regime, would require a much longer treatment time than is typically used for pre-clinical work, up to 30 minutes. Additionally, motion of liposomes and nanoparticles may be restricted in solid tumors.

Besides liposomes, acoustically responsive nanoparticles have been trialed for targeted release of loaded therapeutics. Similar to the previous study on mechanical release from liposomes, Deckers *et al.* found that mPEG-b-p(HPMAm-Lac_n) micelles would also undergo temporary destabilization under ultrasound exposure, an effect that was reduced with increased crosslinking between polymers and that was unrelated to any chemical changes of the polymer, thermal effects or cavitation. Instead, the effect was likely due to shears stress induced by micelle convection under

the acoustic radiation force within the sample chamber.[236] Alternatively, Husseini *et al.*, investigating acoustic release of doxorubicin from stabilized and unstabilized Pluronic P105 micelles, detected harmonic acoustic emissions during release, which can indicate the presence of cavitation. They ascribed the release phenomenon to the generation and collapse of bubbles in the solution, causing shear stress disruption of the micelles.[237] The study was performed at low ultrasound frequencies (70 kHz), which is more capable of generating cavitation than the higher frequencies (1.5 MHz) used in the Deckers *et al.* study. Such low frequencies have excellent tissue penetration, but it may be more difficult to focus the cavitation effect to a specific area due to the wavelength resolution.

Finally, solid mesoporous silica nanoparticles (MSNs) have also been shown to be capable of ultrasound based release after modifications.[238,239] MSNs form as a series of open tubes, which allows convenient and efficient drug loading, but requires further modifications to trap the drug molecule within. Specialized polymers conjugated to the MSNs, called "gate keepers", fulfil this role, by blocking the end of the tube and typically containing a labile bond (*e.g.* heat, acid, *etc.*) to allow triggered release. In a recent case, Paris *et al.* used an ultrasound-labile polymer to effectively cap the silica nanoparticle. In its native form, the polymer is hydrophobic, but after cleavage at the labile bond, become hydrophilic, effectively opening the MSN and allowing drug release.[240] Paris *et al.* were able to demonstrate significant increase in the release of different fluorescent model drugs and doxorubicin from loaded MSNs when exposed to ultrasound (See Figure 7). Although it was demonstrated that the ultrasound caused a change in the chemical structure of the labile polymer that was essential for drug release, the ultrasound mechanism at work was not fully explored, which may be an issue if transferred to an *in vivo* situation.

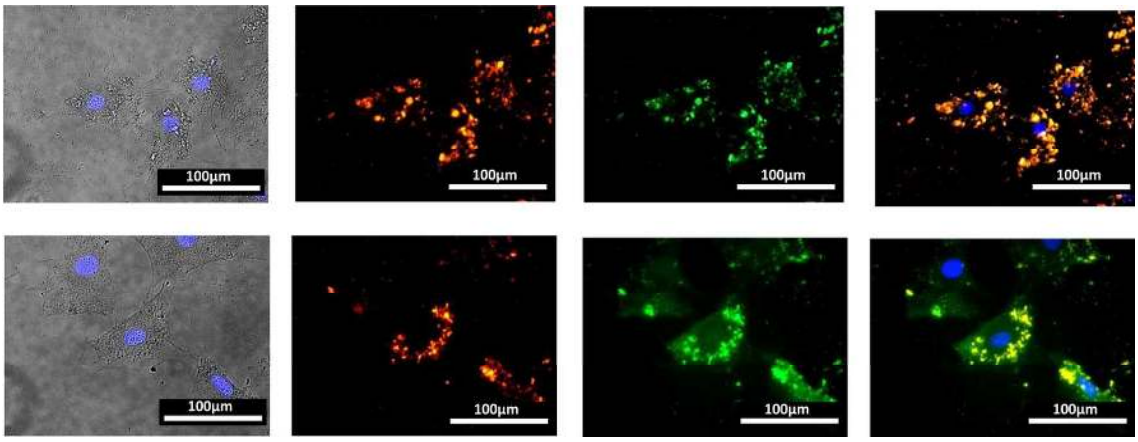

Figure 7. LNCaP cells were incubated for 2 hours with rhodamine B labelled MSNs, loaded with fluorescein and capped with an ultrasound labile polymer, and either immediately fixed (top panel) or treated to 5 minutes ultrasound exposure before fixing (bottom panel). From left to right, cells were imaged under bright field with their nuclei stained with DAPI, for red fluorescence from the MSN, for green fluorescence from the fluorescein, and fluorescence channels were overlaid for the final image. In comparison to the untreated cells, ultrasound exposure has resulted in the release of fluorescein; as indicated by the green fluorescence throughout the cell cytoplasm and drop in co-localization between the MSNs and fluorescein. Reproduced from Reference 240. Copyright (2015) with permission of The American Chemical Society.

Photorelease

In addition to hyperthermia, novel strategies have been employed using photon absorption to trigger release of cisplatin. Li *et al*. manufactured a block polymer based nanoparticle encapsulating cisplatin and the photosensitive indocyanine green (ICG) dye.[241] The block polymer was modified to contain a tellurium, which can bind to the platinum in cisplatin, but is rapidly oxidized by reactive oxygen species (ROS). Upon

stimulation with an 808 nm NIR laser, the ICG dye generates singlet oxygen which oxidizes the tellurium, causing release of the cisplatin. The initial nanocarrier complex is also highly stable, with less than 20% leakage of the cisplatin or ICG over 120 hours, but releasing over 60% of the loaded cisplatin within 8 minutes of laser irradiation. When used *in vivo* on a xenograft breast cancer mouse model, significantly improved tumor regression was seen in comparison to free cisplatin and controls. In two of the five animals, no tumors were present after 26 days. Additionally, although tellurium is a mildly toxic metal, 5 days after treatment, negligible differences in biochemical organ function test and organ histology were seen between saline only control and the treated group. This was in stark comparison to the significant toxicity seen in the free cisplatin group. This approach highlights an interesting method to reduce cisplatin leakage from nanocarriers and specific release at potentially deep target sites due to NIR good tissue penetration. It should be noted though, that the animals treated with the loaded nanoparticle but without the laser irradiation, also demonstrated tumor growth control comparable to free cisplatin. The cause of this was not commented upon by the authors and may need further investigation in future. Additionally, 7 doses were supplied over the 26 days of treatment, followed 24 hours later by laser irradiation at the tumor site. This treatment regime may prove difficult to implement in the clinic, although this would likely be a minor concern. Finally, tellurium is one of the rarest metals on the planet, which could make this approach costly upon scaling up.

A similar technique focusing on NIR as the release source, is to use rare earth metal lattices to form nanoparticles capable of “upconversion”. In simple terms, these lattices are capable of absorbing multiple photons of lower energy, *i.e.* NIR, and emit photons at higher energy, *i.e.* visible or ultraviolet light. This ability to create visible

or ultraviolet light deep within tissue, has allowed the nanoparticles combining photodynamic therapy and cisplatin to target deep tissue sites.[242] In addition, the UV radiation emitted by these nanoparticles has been utilized to both release Pt(IV) prodrugs from UV-liable polymers[243,244] and linked to the increased conversion of Pt(IV) prodrugs to active cisplatin in a polymer nanoparticle.[245]

Concluding Remarks

Platinum based drugs such as cisplatin offer a highly potent treatment for solid tumors, but to fully realize their potential several challenges still need to be addressed. Multiple nanoparticle formulations have been proposed and tested for cisplatin delivery. The combination of nanoparticle delivery with physical methods offers opportunities but also further challenges that may need to be reflected in the choice of formulation. For instance, should the agent be designed for rapid or sustained release? This in turn will affect the choice of delivery method, whether it relies upon thermal effects – *e.g.* the inclusion of thermosensitive linkages or polymers; magnetic targeting – *e.g.* the inclusion of magnetic material; cavitation nuclei – *e.g.* potential methods of attachment and issues of clearance with nuclei, or, acoustic radiation force – *e.g.* particle size for transit through the ECM.

A topic not discussed in detail in this review is that of clinical approval. This review has focused on methods to improve the delivery and release of cisplatin loaded nanoparticles, however it should be noted that no nanoparticle or liposomal formulation of cisplatin has been approved for use at this time. Some of the challenges of nanoparticle design and approval are detailed in Anselmo and Mitragotri.[246] In particular, cisplatin nanoparticles have typically demonstrated

lowered side effects and toxicity in clinical trials, but have rarely demonstrated a clear advantage over cisplatin alone. Additionally, the advent of other platinum based antineoplastic drugs, *e.g.* Carboplatin, Oxaliplatin, *etc.* has addressed some of the toxicity issues of cisplatin without the additional regulatory hurdles of nanoparticle agents. Many of the approaches detailed above may help the development of more effective cisplatin nanoparticles, but the lack of an approved formulation in clinical use may inhibit uptake by the pharmaceutical industry.

Aspects of the tumor environment, such as the vascularity, the state of the supporting ECM, the presence of multiple cell types and heterogeneous cancer cell population, and the emerging role of immunological processes, all affect the deposition, delivery and effectiveness of a chosen therapeutic. In future, it is likely this choice will be driven by a more detailed characterization of a patient's tumor, so called personalized medicine, and delivery mechanisms will undoubtedly form another factor in these important decisions.

Acknowledgment

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