Published in final edited form as:

Int J Hyperthermia. 2016 ; 32(1): 76–88. doi:10.3109/02656736.2015.1120889.

Hyperthermia Using Nanoparticles – Promises and Pitfalls

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Abstract

An ever-increasing body of literature affirms the physical and biological basis for sensitization of tumors to conventional therapies such as chemotherapy and radiation therapy by mild temperature hyperthermia. This knowledge has fueled the efforts to attain, maintain, measure and monitor temperature via technological advances. A relatively new entrant in the field of hyperthermia is nanotechnology which capitalizes on locally injected or systemically administered nanoparticles that are activated by extrinsic energy sources to generate heat. This review describes the kinds of nanoparticles available for hyperthermia generation, their activation sources, their characteristics, and the unique opportunities and challenges with nanoparticle-mediated hyperthermia.

Keywords

cancer; hyperthermia; nanoparticles

1. Introduction

The notion that heat can be used for therapeutic purposes has been described in the medical literature for centuries. Commonly, heat has been used as a means of ablating cancer cells similar to surgical removal of tumors. We define this as *thermoablation*, a modality that utilizes lethal levels of heat to irreparably coagulate proteins and other biological molecules and induce cell death. A milder rise in temperature which does not cause cell death by itself may limit some of the collateral damage to normal tissues beyond the ablation zone and could still have therapeutic benefits. We define this as *hyperthermia* (HT) and nominally

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Conflict of Interest: The authors report no conflicts of interest.

assign it a temperature range of 41-50°C, with a temperature above 50°C being considered thermoablation. It is noteworthy that the consequences of a rise in temperature are dependent on duration of HT, homogeneity of temperature in tissue, tissue type, and context of treatment. The induction of cell death via necrosis and/or apoptosis of cancer cells can occur even with temperature regimes as low as 42°C maintained for more than one hour (1). HT causes a sustained improvement in blood flow preferentially within tumors (and not normal tissues) resulting in increased perfusion and oxygenation of (and delivery of chemotherapy to) the poorly vascularized hypoxic core of the tumor which is generally resistant to ionizing radiation. Cells residing in this hypoxic core of tumors also tend to be more acidotic, a feature that makes them more sensitive to thermal damage but less sensitive to radiation therapy (2). Collectively, these phenomena lead to an altered extracellular microenvironment that is more sensitive to heat-induced cellular damage. HT-induced cellular damage is cell-cycle-independent, in contrast to radiation therapy and many chemotherapy regimens that are more cytotoxic when cells are in specific phases of the cell cycle. Complementary cell cycle sensitivity of HT and radiation therapy allows synergy between these two treatment modalities. HT also activates immunological responses to prime tumors for eradication by the host immune response by surface display of tumor antigens for activation of tumor-specific effector T cell responses. Beyond the physiological and microenvironmental levels, at the molecular level, the targets of traditional thermal therapy are a diverse array of proteins including cytoskeletal structures, plasma membrane components, intracellular enzymes and signal transduction molecules, heat shock proteins that orchestrate the refolding of damaged proteins and proteins associated with DNA repair, apoptosis and necrosis (3). Taken together, the intent of HT is to minimally raise the temperature of the target tissue to alter its physiology and biology and in practice, prime the tissue for a greater response to other therapeutic modalities, like chemotherapy or radiation therapy (4).

Beginning with the early observations by Dr. William Cooley in the 1890s that infections in cancer patients are associated with tumor regression and that injection of cocktails of attenuated bacterial cultures induces a fever and a significant anti-tumor effect, there has been a revival of interest in HT for cancer therapy (5). As a new wave of thermal therapy began to find its place in modern medicine, HT saw itself as an adjunct to standard cancer therapies rather than a stand-alone therapeutic modality. Numerous clinical trials have documented that mild temperature HT can be safely administered to patients and that clinical benefits may be realized in terms of improved local control, palliation of symptoms and even overall survival (6). Despite knowledge of the therapeutic benefits of HT in cancer treatment, HT is underutilized in the clinic for a variety of reasons including the invasive methods of generating HT, the difficulty in maintaining temperatures at desired levels, the lack of monitoring and modeling of HT, and the logistical hurdles with delivering and reimbursing this treatment. Development of alternative methods of HT has been galvanized by the recognition that the *effectiveness* of HT is not really the problem but that the practical challenge of *effecting* HT drives its poor acceptance and adoption in the clinic.

2. Conventional HT

A variety of techniques have been employed to achieve HT in the clinic in modern practice including electromagnetic radiation (like laser, microwave and radiofrequency) or high-

intensity focused ultrasound. Depending on the extent of the body's exposure to heat, there are three types of HT conventionally employed in clinical practice – whole body, regional and local HT. Whole body HT is achieved using hot water blankets and thermal chambers whereas regional HT includes the perfusion of a tumor-bearing limb with part of the patient's blood which is taken out and warmed *ex-vivo* and the perfusion of the peritoneum with a heated solution of anticancer agents in cases of peritoneal malignancies like mesothelioma (7). These methods are not tumor-specific and are technically challenging to perform repeatedly with reproducible heating levels. The third type is local HT, which is tumor-specific. Luminal and interstitial HT techniques employ customized probes and applicators placed close to the tumor individually or as a grid to achieve relatively uniform heating of the tumor. Placement of external heating sources within tumors or metal antennas that are activated by an external energy source are generally invasive, challenging for deep-seated tumors, and often result in heat distributions across the tumor that are non-uniform. External heating techniques such as ultrasound and electromagnetic phased-arrays are not invasive like luminal and interstitial implants but come with their own challenges with attaining and maintaining reproducible and uniform heating levels. While HT is a treatment modality that holds a lot of promise for cancer therapy, the methods of attaining, maintaining, monitoring and modeling it suffer from many inadequacies. Therefore, there remains a continuing need for newer methods of generating HT. Ideally, these would be tumor-focused, minimally invasive, and more uniform in generating HT.

3. Nanoparticles for HT

Nanoparticles are traditionally defined as materials with their longest dimension less than 100 nm although particles up to 1 micron in size are also often lumped within this definition. This review focuses primarily on metallic nanoparticles. In the case of such particles, when compared to their bulk counterparts, metals at a nanoscale often demonstrate unique properties which can be exploited for therapeutic gain. This is often due to the much larger surface-area to volume ratio of nanoparticles that allows elemental metal atoms at the surface to have greatest potential for interaction with surrounding molecules. Given that most components of the cellular machinery operate at this scale to maintain structure and function, there is the possibility that scaling down to this level affords an opportunity to interact, modulate and control such cellular and/or subcellular structure and function. Advances in fabrication and engineering of nanoparticles now allow an unprecedented level of precision in the design of particles with finely tuned surface properties that can be further modified for interaction with biological systems. It is within this context that nanoparticles are envisioned as conduits for generating HT (8). Nanoscale transducers can be engineered as specific absorbers of tuned electromagnetic radiation for efficient conversion of this energy to heat, which in turn is coupled and transmitted to the tissues the nanoparticles reside within (9). The most common energy sources used for generating heat using nanoparticles are light and alternating magnetic fields; these are outlined under each section on individual types of nanoparticles. This is not meant to be an exhaustive review of all studies to date with formulations of nanoparticles capable of generating heat. Rather, this is more of a bird's eye view of the landscape of nanoparticle-mediated HT and a perspective on the promise and the pitfalls of this technology.

Before describing the different nanoparticles that generate heat for biological applications, there are some unique properties of nanoparticles that warrant being highlighted. First, metallic nanoparticles, being excellent conductors of heat, efficiently transmit heat generated within them to adjacent tissues. Second, when administered intravenously, they can accumulate preferentially in tumors via the enhanced permeability and retention (EPR) effect wherein particles smaller in size than a couple of hundred nanometers passively extravasate from leaky, chaotic and immature tumor blood vessels via large fenestrations (60-400nm) in their vascular lining and are inefficiently cleared by an underdeveloped lymphatic drainage system (10, 11). Counterbalancing this fortuitous synergy between nanoparticle size and pore size of tumor vasculature but not normal vasculature is the barrier to tumor accumulation posed by innate scavengers of foreign objects in circulation. Circulating macrophages and resident reticuloendothelial cells in the spleen and liver recognize these particles as foreign, engulf them and clear them from circulation. Reducing the size of the particles to below 5.5nm has been shown to aid renal clearance and avoid reticuloendothelial capture (12). An alternative method to evade capture is to maintain a near-neutral charge on the surface of particles so plasma proteins do not adsorb to their surface, activate the complement cascade (opsonization) and trigger macrophage clearance. Another method employed is that of decorating the surface of particles with substances that render them some stealth properties. Commonly, the surface of nanoparticles is functionalized with hydrophilic and biocompatible materials which are protease-resistant, non-immunogenic and non-antigenic such as polyethylene glycol (PEG), dextran, or chitosan. The resulting increase in circulation time allows greater EPR-mediated passive accumulation in tumors. Third, over and above passive accumulation in tumors, nanoparticles can be decorated with targeting molecules such as tumor-homing peptides and antibodies that allow docking to cancer-specific antigens ("active targeting") to achieve even greater tumor accumulation and specificity, thereby reducing collateral thermal damage to adjacent normal tissues when activated via an external trigger (13). From a mechanistic standpoint, targeted delivery of nanoparticles to tumors reduces the side effects of treatment (14). Untargeted nanoparticles enter the cells through innate cellular mechanisms of endocytosis (15) where the plasma membrane engulfs and encloses the nanoparticle; this pocket detaches from the membrane to form an endocytotic vesicle as it continues on its path from an early endosome to a late endosome and eventually an endolysosome (16). Internalized nanoparticles can sterically hinder cellular processes and cause cell stress, which manifests as lower proliferation rate (14), induction of oxidative stress (17), cytoskeleton disruption (18), hindered differentiation (19) and DNA damage (20). They can also cause autophagy and lysosomal dysfunctions and other effects, which can eventually trigger apoptosis or induce necrosis (21).

We will now highlight some of the types of nanoparticles typically used for hyperthermic treatment of cancers. The typical modes of nanoparticle delivery and activation are illustrated in Figure 1.

3.1 Magnetic Nanoparticles

When ferromagnetic material is magnetized to saturation (alignment of all magnetic domains) in one direction, it takes a magnetic field in the opposite direction to return it to its

original state. Alternating magnetic fields (AMFs) magnetize and demagnetize these materials in opposite directions in a loop called a hysteresis loop. When these materials are scaled down to small nanoparticles, their magnetization can fluctuate back and forth in direction randomly and rapidly with changes in temperature and this results in measurement of magnetization as zero (superparamagnetism) in the absence of an external magnetic field. However, in the presence of a magnetic field, these superparamagnetic nanoparticles behave like a paramagnet with a single magnetic domain (the sum of all the electron spins of all atoms in the nanoparticle aligned into a single giant magnetic moment) but with much greater magnetic susceptibility. When these superparamagnetic nanoparticles are then placed in a tuned AMF they can be resonantly excited to generate heat. The mechanism of conversion of AMF energy to heat by superparamagnetic nanoparticles can be explained by a number of theories. Néel relaxation causes energy to be released when the magnetic dipole of a particle flips between two stable orientations within a magnetic field that are separated by an energy barrier. Brownian relaxation invokes random collision with other particles and physical rotation of particles within an AMF. Hysteresis losses can occur in a magnetic material placed in an AMF. Anisotropic magnetic particles can generate frictional heating during physical rotation. While AMF can generate heat in nanoparticles in tissues, non-magnetic material in tissues can also get heated unintentionally due to dielectric losses in a low electrical conductivity material and eddy current losses in a high electrical conductivity material. Careful calibration of size, shape and coercivity of nanoparticles and tuning of the applied AMF frequency and amplitude can result in resonant excitation of just the nanoparticle and minimal off-target effects in normal tissues. The efficiency of conversion of absorbed AMF energy by a nanoparticle to heat is described as its specific absorption rate (SAR). In biological systems, the intracellular degradation and aggregation of nanoparticles can influence the preservation of SAR values. In general, superparamagnetic nanoparticles tend to be less than 20nm and they resonate within magnetic fields ranging from 10kHz - 10MHz, which can easily penetrate soft tissues and bones.

A notable attribute of magnetic HT is that AMF fields are typically directed towards the entire body rather than focused specifically on the tumor alone. This means that untargeted particles residing in normal organs can also be heated while the tumor is heated within the AMF. This makes treatment of tumors close to the liver or spleen challenging when nanoparticles are administered intravenously. An alternative approach is to inject the particles directly into the tumor to overcome this limitation of collateral heating of off-target tissue that has accumulated nanoparticles. This approach is associated with an inhomogeneous distribution of nanoparticles through the tissue for uniform heating. Nonetheless, direct injection affords the opportunity to administer sufficient quantities of nanoparticle into the tumor to generate HT. Other challenges with implementation of magnetic particle HT include the inability to accurately perform thermal dosimetry and the dependence of heating efficiency on thermal dose rate. Larger dose rates are achieved by applying large field amplitudes in a short time frame and result in a more sustained rise in temperature before heat is dissipated by thermal washout (blood coursing through vessels at body temperature and creating a heat sink) (22). Nonetheless, iron oxide particles that are nontoxic, nonimmunogenic, biocompatible, stable, and possibly biodegradable can be generated by careful design.

3.1.1 Iron oxide nanoparticles—Iron oxide nanoparticles are visualized on magnetic resonance imaging by their shortening of T1 (longitudinal relaxation – spin-lattice) relaxation time, which leads to a brighter signal on T1-weighted images and T2 (transversal relaxation – spin-spin) relaxation time, which leads to a darker signal on T2-weighted images than in the surrounding tissue. As noted previously, when placed in an AMF, they can generate heat. For clinical translation, their additional favorable attributes include their biocompatibility and the versatility of fabrication of particles with a wide range of diameters (23). They can be modified with appropriate coatings including organic molecules like dextran, dendrimers, chitosan, or aminosilanes or inorganic molecules like gold to prevent aggregation and increase their circulatory half-life (23). A formulation of 12nm Fe₃O₄ nanoparticles coated with aminosilane has been tested clinically by MagForce AG wherein ~5ml of the formulation is injected directly into recurrent glioblastomas in patients who are then subjected to twice weekly treatment in a 100kHz AMF while receiving 30Gy of radiation therapy at 2 Gy/fraction. This magnetic fluid hyperthermia (MFH) holds great promise for thermotherapy of deep-seated tumors and has demonstrated a median overall survival of 13.4 months in a cohort of 59 glioblastoma patients (24). This is considerably longer than the typical median survival of about 6 months for this subset of patients. Importantly, this was achieved with minimal toxicity – reported toxicities included sweating, fever, tachycardia and convulsions (24). In the post mortem analysis of glioblastoma patients on this phase II study who had received treatment with aminosilane-coated iron oxide nanoparticles, nanoparticles were localized to macrophages in areas of geographic tumor necrosis rather than the cancer cells themselves (25). The lack of dispersal beyond the areas of instillation argues for the need for multiple routes of instillation to cover the entire tumor and visualization (pre-treatment imaging) of adequate coverage of the tumor when nanoparticles are directly injected into the tumor. In contrast, when particles are injected intravenously, their biodistribution is highly dependent on the formulation used, the organ evaluated and the time point of assessment. For instance, in one study, the maximum accumulation of Fe₃O₄ nanoparticles was in the lung and kidneys at 6 hrs, liver, brain, stomach and small intestine on day 1 post-administration and the heart and spleen on day 3 post-administration. The highest concentrations of nanoparticles were observed in the liver (one-day post administration) and spleen (three days post administration) (26). A potential strategy to overcome the need for direct injection of magnetic nanoparticles into tumor is to administer them intravenously and home them specifically to tumor cells or subcellular compartments of tumor cells to enhance treatment efficacy. One such proof-of-principle concept study is an in vitro assessment of iron oxide nanoparticles coated with crosslinked dextran and decorated with tumor-homing peptide CREKA that binds to fibrinogen in the extracellular matrix. AMF exposure of cells treated with these particles (generating HT) and cisplatin resulted in greater cytotoxicity than treatment with HT alone or cisplatin alone (27). It remains to be seen whether this strategy will achieve HT in vivo with intravenous administration of the nanoparticle.

3.1.2 Super-paramagnetic iron oxide nanoparticles (SPION)—As an extension of iron oxide nanoparticles, they can be synthesized as SPIONs. SPIONs randomly flip their magnetic dipole orientation in the absence of a magnetic field but align themselves along the magnetic field direction when placed in an external magnetic field. The resultant magnetic

susceptibility is considerably higher than that of paramagnetic materials. AMF causes the particles to rapidly flip their magnetic polarity. However, there is some hysteretic loss involved in the flipping, which manifests as heat. Loading a tissue with SPIONs and then subjecting it to an AMF, generates heat. Several factors can influence the extent of this heating – the magnitude of the AMF, the size and characteristic of the SPIONs, the concentration of SPIONs in the tumor and the depth of the tumor within the body. One approach to embed large quantities of SPIONs into tumors via direct injection is to use hydrogels and organogels which entrap SPIONs within them and prevent distant migration. An exploratory study with thermosensitive chitosan and poloxamer polymers as hydrogels, alginate solution as an ionic crosslinker, and organogels derived from single-solvent or co-solvent exchange used as carriers of SPIONs noted that organogel formulations possessed the most favorable properties, i.e., high loading of SPIONs, efficient generation of heat, localization within the center of the tumor, and low toxicity (28).

Similar to the recent studies with actively targeted iron oxide nanoparticles, SPIONs functionalized with anti-human epidermal growth factor receptor-2 aptamers have shown HT induction at ninety-fold lower concentrations than untargeted SPIONs (27). In another study, functionalization of ultrasmall paramagnetic iron oxide particle with cyclic arginine-glycine-aspartic peptides to target integrin $\alpha_v\beta_3$ and $\alpha_v\beta_5$ resulted in preferential uptake by breast cancer cells compared to unconjugated SPIONs as assessed by MR imaging, confocal microscopy and Prussian blue staining (29).

3.1.3 Doped iron oxide—The presence of dopants in iron oxide nanoparticles has been shown to increase their specific absorption rate (SAR) and enhance efficiency of heat generation in an AMF (30). This can be further tuned by varying the size of the nanoparticle and the anisotropy of the crystal. For instance, manganese, cobalt, platinum, and nickel have been shown to significantly enhance the specific power loss (SPL) of SPIONs. However, they may also increase the oxidative instability and free radical induced toxicity of such doped nanoparticles in biological systems. In a recent study, iron oxide doped with cobalt and mineralized within a ferritin protein cage decorated with PEG and α -melanocyte-stimulating hormone peptide has demonstrated greater magnetic anisotropy and thermal transduction efficiency than undoped samples, resulting in a strong reduction in melanoma cells in vitro (31).

Even greater levels of efficiency of converting AMF energy to heat are attainable with core-shell nanoparticles with exchange-coupling of elements in a magnetically hard core with elements in a magnetically soft shell (32). However, even with a ten-fold increase in SPL with this formulation, generation of HT required direct injection of the nanoparticles into the tumor. Though not classical for doping of iron oxide with another element, intratumoral injection of Yttrium aluminosilicate microspheres doped with iron oxide nanocrystals dispersed throughout the glass matrix resulted in localized therapeutic HT (43°C) in rats with liver tumors subjected to AMF treatment (33).

3.2 Gold Nanoparticles

In contrast to magnetic nanoparticles that are activated in an alternating magnetic field to generate heat, gold nanoparticles are photothermally activatable. Resonance of surface plasmons of gold atoms on the surface of nanoparticles upon illumination with light at the resonance wavelength evinces unique properties of matter at a nanoscale, one of which is the efficient conversion of light energy to heat. The wavelength of greatest interest in clinical applications is the optical window where light penetrates deepest in biological tissues (with least absorption by water, melanin, oxy-hemoglobin, and deoxy-hemoglobin), i.e., the near infrared (NIR) end of the spectrum between 700 and 850nm. Altering the size, shape and composition of gold nanoparticles allows for tuning of the resonance wavelength to the incident light (34). Accordingly, photothermally activatable gold nanoparticles have been fabricated with a surface layer of gold and an inner core that is hollow, made of dielectric material like silica, or gold itself. Gold is considered biologically compatible because it is an inert noble metal that is thermally and chemically stable. It is also easy to conjugate biomolecules to on its surface (for PEGylation or decoration with tumor-homing peptides and antibodies). Lastly, gold has a long history of use in clinical practice (for treatment of rheumatoid arthritis).

3.2.1 Silica-gold nanoshells—Core-shell gold nanoparticles have a layer of gold surrounding a core made of dielectric material, most commonly silica due its biocompatibility. This metal-dielectric structure causes a red shift of the characteristic plasmon absorption spectrum of gold to the NIR region of the electromagnetic spectrum. The plasmon resonance frequency is tunable by altering the core to shell ratio. Silica-gold nanoshells (GNSs) are usually 50-150nm in diameter, stable in solution at low temperatures, and biologically non-reactive particularly if coated with a layer of PEG. GNSs measuring about 150nm in diameter (120nm silica core and 15nm gold shell) are plasmon resonant at NIR wavelengths and highly efficient photothermal activators. Their size allows for EPR-mediated passive accumulation in tumors, albeit not deep penetration into tumors. Decorating their surface with covalent attachment of PEG that binds rigidly to the surface gold atoms renders them additional biocompatibility by minimizing opsonization, immune activation and sequestration in the liver and spleen, thereby increasing their blood circulation time (35). In preclinical biocompatibility, toxicity and biodistribution studies performed with these GNSs prior to clinical use, there was no indication of toxicity for durations of up to 404 days (36).

Initial studies used these GNSs for thermoablation and demonstrated that tumor-bearing mice treated with intravenously administered GNSs prior to NIR laser illumination had substantially longer survival durations than those treated with GNSs and sham irradiation (35). Studies show that the increased temperatures generated by the GNSs following NIR light absorption are sufficient to produce irreversible photothermal damage in subcutaneous tumors. Actively targeted gold nanoparticles to tumors in mice have demonstrated improvements in survival when compared to passively targeted nanoparticles (37). However, thermoablative monotherapy has the potential for collateral damage of adjacent normal tissues beyond the ablated tumor. In view of this concern and by extension of the observations on photothermal activation of GNSs for cancer applications, a subsequent study

noted that mild temperature HT with laser illumination of GNSs passively accumulated in tumors results in sensitization of tumors to radiation therapy. Murine tumors pre-treated with GNSs when subjected to mild temperature HT (about 41°C for 20 minutes) followed immediately by a single dose of radiation therapy (10 Gy with 125 kV X-rays) showed a tumor volume doubling time that was nearly twice that with radiation therapy alone (38). This was attributed to an initial increase in perfusion of tumors (thereby delivering more oxygen to the radioresistant hypoxic core of tumors) and a subsequent disruption of vasculature (due to the perivascular sequestration of the GNSs) (38). Furthermore, a preferential sensitization of radioresistant cancer stem-like cells was noted in follow-up studies (39, 40). The first of these studies noted that breast cancer xenografts decreased in volume after radiation and even more so after radiation and GNS-mediated HT but the relative proportion of stem-like cells increased in the radiated group while they did not in the group treated with radiation and HT. A lower frequency of tumor formation was observed after transplantation of the cancer cells in limited dilutions from residual tumors following combined treatment as compared to analogous transplantation of cells from residual tumors following radiation therapy alone. This is presumably due to the localization of stem-like cells in perivascular niches and the heat generated by GNS-mediated HT being most intense in this region where the GNSs reside after passive extravasation from tumor vasculature.

Tumor photothermal ablation has progressed significantly in the past few years with multiple on-going clinical trials based on a FDA IDE (investigation device exemption) granted for their use as AuroShell® particles (Nanospectra Biosciences, Inc., Houston, TX). Pilot studies have been performed in prostate cancer patients to demonstrate passive accumulation in tumors upon intravenous administration (41). Understandably, the limited penetration of NIR light in biological tissues restricts applications of GNSs to superficial tissues only, unless interstitial laser fibers are used for illumination of deep-seated tumors. A clinical trial of interstitial laser-based illumination for photothermal ablation of recurrent or refractory head and neck cancers is also on-going ([clinicaltrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00848042) NCT00848042)

3.2.2 Gold nanorods—Gold nanorods (GNRs) are cylindrical solid gold nanoparticles that are also photothermally activatable. Altering the aspect ratio (ratio of GNR length to GNR diameter) changes plasmon resonance wavelength of the GNRs. Tuning the longitudinal resonance to the NIR region by changing the aspect ratio facilitates the use of GNRs for photothermal applications. Polarization of spheres into cylindrical structures requires a surfactant during fabrication. Commonly, cetyl trimethylammonium bromide (CTAB) is used as the surfactant but potential toxicity concerns with CTAB motivate attempts to remove it physically by centrifugation, filtration and dialysis or replace it with PEG (42). GNRs are smaller (less than 50nm typically) than GNSs, have longer circulation times and are more efficient as photothermal activation agents (43). The strong extinction coefficient of GNRs at the longitudinal surface plasmon resonance wavelength accounts for their potent photothermal activation properties and this results in six-fold faster heating per gram of gold with GNRs than with GNSs (43, 44). Photothermal ablation of tumors in nude mice after intravenous administration of PEGylated GNRs resulted in the formation of a minor scar without tumor regrowth whereas tumors rapidly regrew after treatment with saline with or without laser illumination and PEGylated GNRs without laser illumination

(43). Following intravenous administration and GNR accumulation in the tumor, HT is localized to the tumor tissues with high spatial confinement as long as particles accumulate preferentially in tumor compared to adjacent normal tissues (45). As noted in another study, the tumor accumulation and anti-tumor efficacy of photothermal therapy following intravenous administration of GNRs are comparable to that following intratumoral administration of GNRs (46). This suggests that intravenous administration is capable of achieving sufficient intratumoral accumulation to effect anti-tumor effects following photothermal activation without the need for direct intratumoral injection.

3.2.3 Hollow gold nanoshells—Hollow gold nanoshells (HGNSs) comprising a hollow core with a thin outer gold shell are smaller in size (< 100 nm) than silica-gold nanoshells while still being plasmon resonant at NIR wavelengths. Their synthesis involves beginning with a core-shell nanoparticle with a cobalt or silver core and a gold shell and then oxidizing and sacrificing the core to leave behind a hollow center. In turn, this hollow cavity can be used as a vehicle for drug delivery (47). The smaller size of these shells than GNSs potentially allows greater extravasation from vasculature with smaller fenestrations. As with other nanostructures, functionalization of their surface allows tumor-specific homing and delivery. Examples of this strategy include HGNSs functionalized with epidermal growth factor receptor (EGFR) antibodies for targeting and ablating cells overexpressing EGFR (48) and HGNSs functionalized with α -melanocyte-stimulating hormone analogs for ablation of subcutaneous murine melanoma xenografts in vivo (49). One concern raised with these shells is that of in vivo instability of the hollow structure and that the residual silver or cobalt in the hollow cavity could result in standalone cytotoxicity since it does not stay contained within the HGNS (50). One potential advantage of residual cobalt present in the metallic shell is that it broadens the plasmon resonance peak of cobalt-HGNSs, allowing tuning of the plasmon peak without changing nanoparticle diameter (51).

3.2.4 Gold nanomategyoshkas—Nanomategyoshka are gold nanoparticles that maintain the photothermal activation properties of GNSs but are smaller in size. They comprise a gold core coupled to a thin silica shell with a gold epilayer around them; the resultant multilayered particle relies on plasmon hybridization which involves strong coupling between the plasmons of the gold core and the shell to make them intensely photothermally activatable. As with GNSs, the plasmon resonance is tunable to NIR wavelengths by altering the relative thicknesses of the core, the shell and the epilayer. Synthesized in this fashion, nanomategyoshkas smaller than 100nm have a low-energy plasmon subradiant mode at 783 nm and a high-energy superradiant plasmon mode at 560 nm, thereby allowing greater clinical applicability (52). Initial results with these gold nanomategyoshkas suggest that they accumulate passively in orthotopic xenografts five times more efficiently than GNSs and are more effective for photothermal therapy than GNSs (52). These promising results argue for a role for nanomategyoshkas alongside GNSs in clinical translational applications.

3.2.5 Hybrid nanoshells—Hybrid gold nanoshells preserve the desirable optical properties of NIR localized surface plasmon resonance of GNSs and can be synthesized in size regimes between 10 and 100 nm. These particles have varied, functional (nonplasmonic) core components ranging from “hard” semiconductor quantum dots to superparamagnetic

nanoparticles to “soft” liposomes made using poly-L-histidine as a template to direct Au deposition (53). These particles are generally engineered with the intent of lending additional functionality to GNSs or nanomatryoshkas either for imaging (with magnetic resonance imaging compatible agents or with fluorescent molecules) or therapy (with SPIONs for AMF activation).

3.3 Carbon nanotubes

Carbon nanotubes (CNTs) are one dimensional nanomaterials composed of sheets of honeycomb-like lattice of carbon atoms (graphene) rolled in the shape of a tube hundreds of nanometers to microns long but a few nanometers in diameter. Single walled carbon nanotubes (SWCNTs) have a single roll of the graphene sheet whereas multiwalled carbon nanotubes (MWCNTs) are comprised of such tubes concentrically stacked within each other. CNTs absorb incident energy over a broad frequency spectrum including visible light, NIR light, and even radiofrequency waves; the extinction coefficient of such absorption is considerably higher than that of natural chromophores such as melanin, hemoglobin, and water. Upon activation by electromagnetic waves, electronic transitions within the nanoparticle are activated; the relaxation that follows results in amplified vibrational modes within carbon lattice. Tunable properties include the arrangement of carbon atoms in the wall of the cylinder for potent thermal conductivity, alterations in length and diameter, number of concentric tubes and dopants within or outside the cylinder. The tunable electrical, thermal and spectroscopic properties of these CNTs make them highly versatile conduits for multiple biological applications including the generation of HT.

Direct injection of SWCNTs into head and neck squamous cell carcinoma tumor xenografts in mice followed by NIR laser illumination for 3 minutes resulted in durable complete eradication of tumors albeit with an eschar formed on the skin due to thermal injury (54). The nanotubes were cleared from the tumors over the course of two months largely through renal and biliary excretion. MWCNTs, with enhanced absorption cross-sections when compared to SWCNTs, have shown potent anti-tumor activity when injected intratumorally in renal cancer xenografts in mice prior to exposure of these tumors to short pulses (30-sec treatment) of low-power (3 W/cm^2) laser illumination (55). The extent of tumor regression was dose-dependent and durable long term tumor control was observed with the higher doses of MWCNT. Alternatively, these MWCNTs can be activated by tuned radiofrequency waves, potentially at the risk of some off-target toxicity. A persisting concern with carbon nanotubes is the possibility of developing granulomas reminiscent of mesotheliomas arising from asbestos exposure when the mesothelial and pleural linings of mice are exposed (56).

4. Potential Advantages of Nanoparticle-Mediated HT

The previous sections have highlighted some of the methods of generating HT with nanoparticles and outlined how systemically administered or locally injected nanoparticles with a high absorption cross section for transduction of an extrinsic energy source to heat can deliver heat to the tumor while preventing thermal injury to surrounding healthy tissues. In this section, we underscore several potential advantages of this form of HT over both global and focal HT achieved without nanoparticles.

4.1 Tumor Targeting

A unique attribute of nanoparticle-mediated HT is the potential for confinement of HT conformally to the tumor via active targeting of the nanoparticle to the tumor as a means of enhancing the tumor-to-normal-tissue ratio of nanoparticle accumulation. This is typically achieved by decoration of nanoparticles with peptides and antibodies that allow tumor-specific homing and accumulation. The benefit of active targeting extends beyond an increase in tumor accumulation to a change in the distribution of the nanoparticle within the tumor at the level of the cell and/or tissue, i.e. cellular internalization or confinement to specific cells of the tumor microenvironment such as fibroblasts, endothelial cells and immune cells (57). In a classical approach, glycosylated antibodies can be directionally conjugated to gold nanoparticles using a heterobifunctional linker like using hydrazide-PEG-dithiol to link the Fc region of the antibody to the gold surface (58).

4.2 'Inside-Out' HT

Mathematical and experimental studies and first principles of thermodynamics speak to the dissipation of heat from a source following a temperature gradient away from the source with a sharp fall-off of the temperature with increasing distance from the heat source. For all external heat sources, the tumor-vascular interface is a heat sink where blood traveling through the tumor vasculature at body temperature quickly dissipates the heat generated by HT within the parenchyma of the tumor. In contrast, nanoparticles that extravasate from tumor vasculature after intravenous administration tend to reside in the perivascular space if they are larger (like GNSs) or have a gradient away from the vessel with the highest concentration being in the juxtavascular area. Therefore, HT-range temperatures within tumor parenchyma are generated by a heat source sitting at the vascular interface and radiating heat from within the tumor. The vascular interface is no longer a 'cold spot' in the heat (isotherm) map of the tumor but a 'hot spot'. This has two important consequences. First, the lack of adequate heating of the vascular interface (the heat sink effect) and the resulting poor efficacy of treatment due to inadequate heating of this region is no longer a therapeutic challenge. Second, vascular endothelial damage or disruption may occur and synergize with tumor cell killing to increase anti-tumor efficacy. This reversal of heat loss compared to other forms of HT is a distinctive feature of nanoparticle-mediated HT that we refer to as "inside-out" HT. On the flip side, it is possible that heat generated by perivascularly confined nanoparticles may not be conducted efficiently to cell populations farther from the vasculature that tend to be hypoxic and most likely to benefit from HT. While experimental data on direct measurement of heat generated with hypoxic and normoxic areas of tumors are hard to come by, at least one study suggests that contrast perfusion into (and by inference, oxygenation of) the hypoxic cores of tumors is improved immediately following GNS-mediated HT (38).

4.3 Cancer Stem Cell Sensitization

In many cancers, cancer stem cells are seen as a source of tumor initiation, therapeutic resistance, and tumor metastases. Elimination of cancer stem cells has proven particularly challenging with traditional therapy. Some studies suggest that stem-like cells are sensitized to radiation therapy by GNS-mediated HT. In the first such study, treatment of animal

models of breast cancer with radiation and HT resulted in a greater decrease in tumor size than treatment with radiation alone but more interestingly, treatment with radiation increased the relative proportion of stem-like cells in the tumors whereas treatment with radiation and HT did not (59). Furthermore, when treated tumors were digested and transplanted into syngeneic mice in limiting dilutions, more cells were needed to regrow tumors from mice treated with radiation and HT than mice treated with radiation alone. This lends credence to the notion that stem-like cells were probably sensitized to radiation by HT. One hypothesis is that stem-like cells reside in the perivascular niche of tumors and nanoparticle-mediated HT creates a large rise in temperature within this region due to the 'hot spot' formed here, as noted above. An alternative explanation is provided by an in vitro study that noted that GNR-mediated HT selectively eliminated cancer stem cells in culture and attributed this to greater and faster uptake of GNRs by these cells (40). In another study comparing MWCNT-mediated HT to waterbath HT in triple negative breast cancer, cancer stem cells and non-stem cells were equally sensitized to HT with MWCNT-mediated HT whereas cancer stem cells were resistant to waterbath HT (60). This study posited that the high surface temperature of MWCNT-mediated HT irreversibly permeabilizes cell membranes and causes necrotic cell death whereas waterbath HT causes apoptotic cell death. Tumors derived from a breast cancer stem cell line were eliminated after treatment with laser-induced MWCNT-mediated HT.

4.4 Theranostics

The versatility of synthesis of nanoparticles of different compositions and functionalities allows fabrication of custom nanoparticles that can be visualized by contemporaneous imaging modalities and/or newly developed imaging modalities. This would allow detecting, sensing, imaging, targeting and treatment of tumors with the same nanoparticle. This dual capability of both diagnosis and therapy is referred to as theranostics.

An important consideration when using nanoparticles for cancer therapy is quantification and/or visualization of particles within tumors. For instance, magnetic nanoparticles can be visualized on magnetic resonance imaging as areas of signal void when high enough concentrations of iron oxide nanoparticles are present and this can be used for visualizing particles as well as for treatment planning and thermal dosimetry (61). With direct injection of magnetic nanoparticles, imaging and dosimetry are readily doable. Gold nanoparticles are detectable by computed tomography when large quantities of gold are present but not readily visualizable with concentrations achieved from intravenous administration. Alternative techniques to detect gold nanoparticles in tumors include diffuse optical spectroscopy where changes in the optical absorption and scattering properties of tissue in the presence of plasmonic gold nanoparticles can provide accurate quantification of gold content. One such study validated these measurements in phantoms and tumor xenografts in mice by neutron activation analysis (62). However, diffuse optical spectroscopy does not provide spatial information on the distribution of gold nanoparticles within tumors. Narrowband imaging capitalizes on the strong absorption of gold nanoparticles at their plasmon resonant wavelength and distinguishes between blood and NIR-resonant nanoparticles by imaging in narrow wavelength bands in the visible and NIR, respectively (63). This provides accurate spatial and geographical information on the heterogeneous distribution of gold nanoparticles

within tumors but is restricted to shallow depths of penetration of light. Another technique to discriminate gold nanoparticles and carbon nanotubes within tissues is photoacoustic imaging, where incident optical energy from a laser source is absorbed by plasmon resonant gold nanoparticles leading to acoustic emissions that are detected by an array of ultrasound transducers (64). In addition to visualizing native HT-generating nanoparticles by these techniques, nanoparticles can be readily functionalized to aid visualization by magnetic resonance imaging (by doping them with SPIONs or gadolinium), fluorescence imaging (by doping them with fluorescent dyes) and positron emission tomography (by radiolabeling them with radioactive elements). Visualization of spatial distribution of nanoparticles within tumors opens up the possibility of modeling HT generated by these particles and inverse treatment planning. For instance, gold nanoshell-mediated HT can be computationally modeled using the diffusion approximation of light transport theory for light distribution of lasers in tissue and a modified bioheat equation to quantify temperature rise from photothermal activation (65). In principle, this approach can handle heterogeneity of nanoparticle distributions within tumors and provides information about the contribution of each heat source to the global increase in temperature. Alternatively, using the Green's function method to modify the heat equation, the change in optical properties of tissue when laden with gold nanoparticles can be used to quantify heat generated when illuminated with a laser (66). Similarly, knowledge of the location of magnetic nanoparticles within a tumor (from CT or MRI scans) can be used to predict heat generated using the bioheat equation and to generate a treatment plan (67). As with all models, the heat transfer in biological systems is an index of conduction loss (a physical parameter) and perfusion (a parameter defined more by physiology). Heterogeneity of heating within tumors and the effect of heat itself on perfusion (the physiological parameter, where the body regulates local temperature by increasing blood flow to dissipate heat) make modeling and treatment planning challenging. Nonetheless, visualization of nanoparticles allows prediction of heat generated by them, at least to a first approximation, and non-invasive measurement with MR thermometry would provide validation and confirmation of the modeling.

4.5 Drug Delivery

Nanoparticle formulations that generate HT can often be custom synthesized to deliver drug payloads. Such formulations allow externally-triggered thermally-mediated release of payloads such as peptides, antibodies, toxins, and oligonucleotides within the confines of the tumor alone. This circumvents the undesirable biodistribution of drug within healthy organs of the body when administered intravenously and avoids off-target accumulation and dose-limiting toxicity. For instance, HGNSs have been loaded with doxorubicin such that the spatial and temporal coupling of thermal injury and drug-induced toxicity will increase the efficacy of nanoparticle-mediated thermal therapy (68). In another approach, when a 33-nm polyethylene glycol-coated colloidal gold nanoparticle with an incorporated TNF-alpha payload was used to treat mice bearing SCK mammary carcinomas, dramatic increases in tumor growth delay were observed when the formulation was combined with HT (69). Conceivably, using a nanoparticle that generates HT instead of the gold colloid would combine drug delivery and nanoparticle-mediated HT into a single construct. Similar to delivery of drugs, oligonucleotide delivery for replacement therapy or gene silencing can be achieved using plasmon resonant nanoparticles decorated on their surface with the

oligonucleotide where light triggers HT generation and release of the oligonucleotide (70). An elegant example of siRNA delivery into cells in vitro is the recent demonstration that a TAT-peptide coated plasmonic HGNS with dense siRNA loading can efficiently enter an undifferentiated human embryonic stem cell and deliver siRNA to the cytosol upon NIR illumination (71). Lastly, the HT-generating nanoparticle can be encapsulated within a traditional drug-delivery nanoparticle platform such as a liposome or a polymer like chitosan, dextran, polylactide, or poly(DL-lactide-co-glycolide).

4.6 Immunomodulation

Normal body temperature is regulated by neurovascular homeostatic mechanisms, which assist in heat dissipation during exposure to warm environments and heat generating exercises. Fever is a “cardinal sign” of infection and inflammation in both endotherms and ectotherms. Owing to the prominence of fever in infectious or inflammatory environments, the immune consequences of fever have been studied extensively. Reduced IL-2 production from co-stimulation of CD4⁺ T cells via CD28 ligation has been reported during fever (72). Macrophages, the body’s scavengers of cellular debris, rapidly respond to various “alarm” signals generated from inflammatory sites by releasing pro-inflammatory mediators (73). Raising the core temperature to 39.5°C with mild systemic heat treatment significantly enhances the serum TNF- α and IL-6 concentration, and modulates the macrophage function in BALB/c mice challenged with lipopolysaccharide (74). This thermally-enhanced production of pro-inflammatory cytokine can produce synergistic effects with conventional cancer therapy, be beneficial in eliminating pathogens, and hasten the resolution of inflammation (75). Furthermore, integrated actions of these immune phagocytes aids in eliminating pathogens, repairing damaged tissues, initiating an adaptive immune response, and most importantly, restoring tissue homeostasis (76). As demonstrated by the use of IL-12 in combination with fever-range whole body HT in BALB/c mice, additional immunomodulation beyond fever-range HT can also exert potent anti-tumor effects (77).

As with differences noted between traditional HT and nanoparticle-mediated HT in terms of location of hot spots of temperature within tumors, sensitization of cancer stem cells and induction of necrosis vs. apoptosis, it remains to be determined whether nanoparticle-mediated HT might generate unique signatures of immunomodulation that can be exploited for therapeutic benefit. While tumor-associated antigen presentation to activate effector T cells is a hallmark of traditional HT, nanoparticle-mediated ‘inside-out’ HT could not only expose additional endothelial antigens from angiogenic tumor vasculature but also increase permeability of vasculature (or vascular disruption) to allow greater penetration of effector T cells into the tumor microenvironment. Similarly, nanoparticles internalized into cells may expose a different array of tumor neoantigens after HT generation than when HT is generated extrinsically. Lastly, enormous interest has recently been focused on mobilizing the immune system against cancer and designing agents which stimulate complementary cancer killing mechanisms (78). Nanoparticle-based drug delivery systems hold great promise in the evolving arena of cancer immunotherapy (79). A new delivery system for cisplatin describing a platinum prodrug-modified PEGylated phospholipid micelle encapsulating biocompatible iron oxide nanoparticles has recently been reported. This nanoconstruct was functionalized with polyinosinic-polycytidylic acid (a double stranded

RNA analog widely used as an adjuvant in clinical trials of cancer immunotherapy) and demonstrated significant enhancement of cytotoxicity in tumor cells and concomitantly activated dendritic cells in vitro (80). On the flip side, nanoparticles may generate immune effects that are deleterious as well. Given the concerns with carbon nanotube toxicity, a recent study noted that functionalized CNTs do not adversely affect the viability of the immunocompetent cells (B cells and T cells) and macrophages in vitro and stimulated macrophages to secrete pro-inflammatory cytokines (81). Similarly, plasma proteins that adsorb onto gold nanoparticles and form a protein corona around them can influence opsonization, complement activation, immune cell uptake and clearance and thereby alter biodistribution. A recent study noted that the molecular weight of the PEG coating on such particles dictates the amount of protein binding within the corona but only slightly alters the composition of the protein corona (82). Furthermore, the presence of specific proteins within the corona *per se* does not imply that the protein is activated or altered in function; only specific functional assays such as coagulation and complement activation assays can determine this.

5. Persisting Challenges with Nanoparticle-Mediated HT

5.1 Biocompatibility and scale-up

Although the development of nanoparticle-based HT systems has been heralded as an important biomedical breakthrough, concerns about their biocompatibility need to be addressed individually for each formulation and each application. As described before, concerns have been raised about the toxicity of CNTs when organ surfaces like peritoneum or pleura are exposed to them (56). Also, the CTAB used during synthesis of GNRs can be toxic if present in large quantities or if dissociated from the GNR in vivo. A recent report on the biocompatibility of GNSs highlights the typical battery of tests conducted to confirm biocompatibility and safety even though the bulk metal (gold) is considered safe (36). These include cytotoxicity, pyrogenicity, genotoxicity, in vitro hemolysis, intracutaneous reactivity, acute systemic toxicity in the mouse after single and multiple injections, and long-term toxicity in rodents and dogs monitored for over a year. Maintaining consistency in physicochemical characteristics between batches of formulations and upon scale-up from small batches to large batches are also pre-requisites for clinical translation. In addition to the standalone toxicity of the nanoparticle, the toxicity of excipients, toxicity of functionalization moieties, and preservation of association between the core particle and its epilayers or functionalization moieties also needs to be ascertained. The Nanotechnology Characterization Laboratory at the National Cancer Institute assists investigators from academic institutions and industry with some of the biocompatibility testing of promising nanoparticles being considered for clinical translation. Cataloging their experience with characterizing over 250 different nanomaterials from over 75 different investigators, this group outlined some common mistakes and oversights in nanomaterial formulation in a monograph that serves as a valuable reference material for all investigators pursuing nanoparticle formulations for eventual clinical use (83). Another issue with scale-up, not in terms of manufacturing of the particles but with extending findings from rodents to humans, is that of thermoregulation in rodents being different from that of large animals and humans. Humans can readily dissipate heat by evaporation and cutaneous vasomotor alterations;

rodents compensate for greater heat loss due to a higher surface-area to volume ratio of their bodies by maintaining a high metabolic rate to keep body temperature constant. Consequently, the physiological responses to hyperthermia are more robust and vigorous in rodents and extrapolation to humans can be problematic. Similarly, appropriate animal models for HT studies may be orthotopic, syngeneic or genetically engineered mouse models rather than subcutaneous xenograft models. These models are less sensitive to cutaneous thermoregulatory and homeostatic responses to HT, have greater fidelity to true tumors in humans, and in the case of syngeneic and genetically engineered mouse models, have a full complement of immune functions similar to that encountered in humans.

5.2 Heterogeneity of biodistribution

When injected into the systemic circulation, nanoparticles are distributed heterogeneously within the tumor and within the body in organs and tissues. This is dictated by shape, surface charge, hydrodynamic diameter, extent, the length and branching of the polyethylene glycol surface coating often used to confer “stealth” properties for immune evasion from the reticuloendothelial system, and surface functionality of the nanoparticle (84). As noted previously, spherical nanoparticles with a zwitterionic surface charge and hydrodynamic diameter of approximately 5.5nm are cleared by the kidneys and not entrapped within the reticuloendothelial system whereas larger nanoparticles of approximately 20nm are captured by the reticuloendothelial macrophages (38, 85). Irrespective of size of particles, when administered intravenously, there is a gradient away from the vasculature, and even tumor-specific targeting does not overcome this. Much larger particles remain in the perivascular space and do not penetrate deep into tumor parenchyma (38, 85). This potentially provides a therapeutic advantage due to focal vascular disruption following laser treatment and HT as noted in the radiosensitization study with gold nanoshells described earlier (38).

5.3 Efficiency of Extrinsic Energy Transduction

Large local concentrations of magnetic nanoparticles are required in a tumor for generation of heat when placed within an AMF field. This is typically not achievable with intravenous administration. Consequently, all magnetic nanoparticle-mediated HT applications to date have relied on intratumoral injection which is both invasive and non-uniform. Nevertheless, the extent of coverage of the tumor is readily visualized by MRI and deep-seated tumors can be accessed for intratumoral injection and AMF activation. On the other hand, photothermal activation of plasmon-resonant gold nanoparticles is achievable with lower concentrations of nanoparticles in tumor readily attainable via intravenous administration of the nanoparticle. In addition, light can be focused directly on the tumor and surrounding tissues with lower (off-target) accumulation of the nanoparticle can be shielded by collimation of the incident laser beam. Such focusing of AMF is not possible currently. Despite the high efficiency of transduction of extrinsic energy to heat using plasmon resonant nanoparticles, the greatest limitation of this approach is the restricted depth of penetration of incident light. Consequently, this would be a viable therapeutic strategy only for superficial tumors, the operative bed, endoscopically accessible tumors and/or tumors that can be infiltrated with light-emitting catheters.

6. Conclusions

HT has a long history of clinical use as a potent sensitizer of cancer to traditional therapies such as radiation therapy and chemotherapy. Nonetheless, adoption of HT in clinical practice in the modern era has been hindered by the challenges associated with achieving, maintaining, and monitoring HT. Recent advances in our understanding of fundamental properties of nanoparticles provide a framework for the use of nanoparticles as conduits for generating HT. This review outlines some of the types of nanoparticles that can be used for HT generation, their unique attributes that can be exploited for distinct gain in nanoparticle-mediated HT applications, and challenges faced with incorporation of nanoparticles into clinical workflow routines. Critical prerequisites for advancement of such nanoparticle-based HT strategies are the identification and validation of optimal nanoconstructs, methodologies for thermal activation using extrinsic energy sources, tumor-specificity, image-guidance, and thermal dosimetry. One such formulation that has made inroads into clinical practice is the use of iron oxide nanoparticles directly injected into tumors under image-guidance prior to exposure to an AMF source. While its therapeutic efficacy is being validated in larger randomized trials, similar approaches with other nanoparticles warrant being evaluated in coming years. Different techniques of nanoparticle-mediated HT will have different clinical scenarios where they will find optimal utility and these will need to be defined as clinical translational efforts are advanced. Conceivably, with successful demonstration of clinical efficacy of nanoparticle-mediated HT combined with standard-of-care cancer therapies, this technology may provide a boost to on-going efforts to integrate HT into the workflow in routine clinical practice.

Acknowledgements

This work was funded in part by institutional support from Morehouse School of Medicine and U54 CA118638 (to P.K.), University of Dammam (to A.A.) and National Institutes of Health (1R01CA155446) and the John E. and Dorothy J. Harris Endowed Professorship (to S.K.).

Abbreviations

AMF	alternating magnetic field
CNT	carbon nanotube
CTAB	cetyl trimethylammonium bromide
EPR	enhanced permeability and retention
GNRs	gold nanorods
GNSs	silica-gold nanoshells
HT	hyperthermia
HGNS	hollow gold nanoshells
MFH	magnetic fluid hyperthermia
MWCNT	multi-walled carbon nanotubes

NIR	near-infrared
PEG	polyethylene glycol
PTT	photothermal therapy
SAR	specific absorption rate
SPIONs	super-paramagnetic iron oxide nanoparticles
SPL	specific power loss
SWCNT	single walled carbon nanotube

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Fig. 1.
Schematic illustration of modes of nanoparticle delivery and activation.