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Multi-functional polymeric nanoparticles for tumour-targeted drug delivery

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The use of nanoparticles as drug delivery vehicles for anticancer therapeutics has great potential to revolutionise the future of cancer therapy. As tumour architecture causes nanoparticles to preferentially accumulate at the tumour site, their use as drug delivery vectors results in the localisation of a greater amount of the drug load at the tumour site; thus improving cancer therapy and reducing the harmful nonspecific side effects of chemotherapeutics. In addition, formulation of these nanoparticles with imaging contrast agents provides a very efficient system for cancer diagnostics. Given the exhaustive possibilities available to polymeric nanoparticle chemistry, research has quickly been directed at multi-functional nanoparticles, combining tumour targeting, tumour therapy and tumour imaging in an all-in-one system, providing a useful multi-modal approach in the battle against cancer. This review will discuss the properties of nanoparticles that allow for such multiple functionality, as well as recent scientific advances in the area of multi-functional nanoparticles for cancer therapeutics.

Keywords: drug delivery, molecular imaging, multi-functional, polymeric nanoparticles

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1. Introduction

In the search for successful cancer treatment is the quest for the ultimate cancer therapeutic. Although conventional treatment options such as chemotherapy and radiation have experienced many advances over the past decades, cancer therapy is still far from optimal. Effectiveness of cancer therapy depends on a fine ratio that is determined by the ability of the therapeutic to eradicate the tumour while affecting as few healthy cells as possible. In this case, systemically administering bolus doses of powerful chemotherapeutics often results in intense side effects due to the action of the drugs on sites other than the intended target. With such nonspecific drug action, the concentration of drug rendered available at the tumour site itself is potentially beneath the minimal effective concentration, entering the patient into a vicious predicament between choosing a near-toxic effective dose and a comfortable ineffective dose. To alleviate this difficulty, decades of research have focused on developing cancer-specific drugs or delivery systems that can preferentially localise existing agents to the tumour site. Recent advances in nanotechnology promises further developments in target-specific drug delivery systems.

Stemming from the nanotechnology revolution, nanoparticles came onto the scene as a type of drug delivery vector. Nanoparticles are colloidal systems of submicron (< 1 μM) size that can be constructed from a large variety of materials in a large variety of compositions. Commonly defined nanoparticle vectors include: liposomes, micelles, dendrimers, solid lipid nanoparticles, metallic nanoparticles, semiconductor nanoparticles and polymeric nanoparticles, although the scope of nanoparticle formulations that have been applied to cancer therapy is far more elaborate. Despite the

large variety of formulations available, this review will focus primarily on natural and synthetic polymer-based solid core nanoparticles, including metal and nanocrystal formulations, due to their role in the multiple functionality of the vector.

Depending on the chemical composition of the nanoparticles, these can carry a wide variety of compounds, making them efficient drug delivery vehicles. In addition, there also exists the ability to introduce to the particle a metallic core or shell, giving the particle optical, magnetic, or hyperthermic properties; or to covalently bind antibodies or lectins, whereby enhancing targeting efficiency of the particle. Such variant properties allow for the trend towards multiple functionality of nanoparticles that will be discussed in this review. Examples of biocompatible and biodegradable polymers that have been used to prepare nanoparticles for tumour-targeted delivery include poly(D,L-lactide-co-glycolide), poly(ε-caprolactone), and poly(β-amino esters) [1-4].

Nanoparticles are excellent tumour-targeting vehicles because of a unique inherent property of solid tumours. Due to the rapid growth of solid tumours, many tumours present with fenestrated vasculature and poor lymphatic drainage, resulting in an enhanced permeability and retention (EPR) effect [5], which allows nanoparticles to accumulate specifically at the tumour site (Figure 1). Although nanoparticles protect the drug from rapid metabolism and clearance, as well as nonspecific recognition and distribution, stealth-shielding nanoparticles (using PEG surface modification [6]), in addition, will help to avoid uptake by the reticuloendothelial system [7] and mononuclear phagocytes [8]. Altogether, this results in the property of nanoparticles to circulate for prolonged periods of time, allowing them to eventually reach the tumour vasculature where, guided by the EPR effect, they specifically extravasate through the fenestrated capillaries to accumulate drugs at the tumour mass. It has been shown that nanoparticle and polymer conjugate delivery can allow concentrations of the drug near the vicinity of the tumour to reach 10- to 100-fold higher than when administering free drug [6]. Beyond the passive tumour-targeting properties by the EPR effect, intratumoural localisation of nanoparticles can be further improved by active targeting through conjugation of the particle with tumour-specific recognition of small molecules such as folic acid [9], thiamine [10] and even antibodies or lectins [11]. In addition, at the tumour site, nanoparticles offer one further advantage: they can be endocytosed/phagocytosed, enhancing cell internalisation of the drug, and leading to delivery of the drug closer to the intracellular site of action [8].

It was soon discovered that tumour-specific accumulation of nanoparticles provided not the means for drug delivery to the tumour, but also an opportunity to further conjugate a metallic core or shell to exploit for optical imaging or MRI in tumour diagnostics, guided hyperthermia therapy and guided radiation therapy. After the use of first-generation nanoparticles as either drug delivery carriers, imaging agents or guided therapy agents alone, the possibility to combine

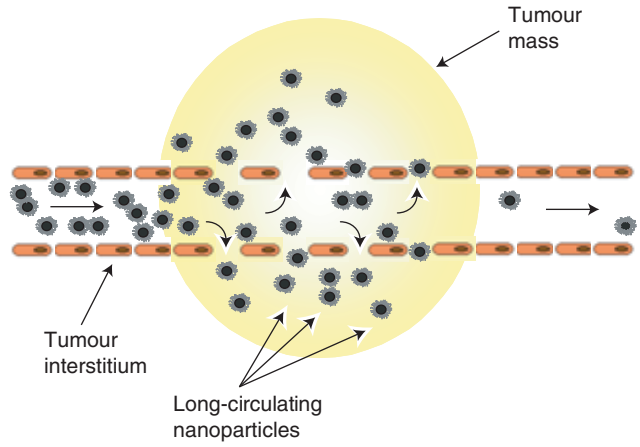

Figure 1. A schematic representation of the nanoparticle localisation in solid tumours by the enhanced permeability and retention effect. Long-circulating nanoparticles, shielded by water-soluble polymer such as poly(ethylene glycol), preferentially accumulate in the tumour mass by extravasation through the fenestrated tumour interstitium.

these differing properties soon emerged. Subsequently, by exploiting the varied chemistry of the polymeric nanoparticle, one could encapsulate multiple drugs and tag on tumour-specific targeting moieties; therefore truly multifunctionalising the vector. One could also envision sequential delivery of drugs based on their location in the nanoparticles (e.g., surface bound versus encapsulation in the matrix). By confining drug molecules to a specific location, the delivery is further optimised because a particular agent is available where it has the highest effect.

This review will focus on such recent progress surrounding the multiple functionality of nanoparticles for improved cancer therapy, advancing from simple multiple functionality of the nanoparticle by inclusion of targeting moieties and co-encapsulation of variant therapeutics, to complex multiple functionality of the nanoparticle by combining targeting, imaging and therapy together into one system.

2. Simple multiple functionality of polymeric nanoparticles

Polymer chemistry allows for many variations, whereby polymeric nanoparticles can be easily manipulated without the loss of their desired physical, chemical, and biological properties. In one manner, this principle can be used to greatly improve the function of the nanoparticle in cancer therapy through the attachment of tumour-specific targeting moieties (e.g., antibodies or receptor ligands), directed at cell surface markers unique to the cancer cell. Alternatively, this principle can be used to improve the function of the nanoparticle for simultaneous delivery of a combination of drugs to the cancer cell, creating a multivalent therapeutic strategy. Such manipulations

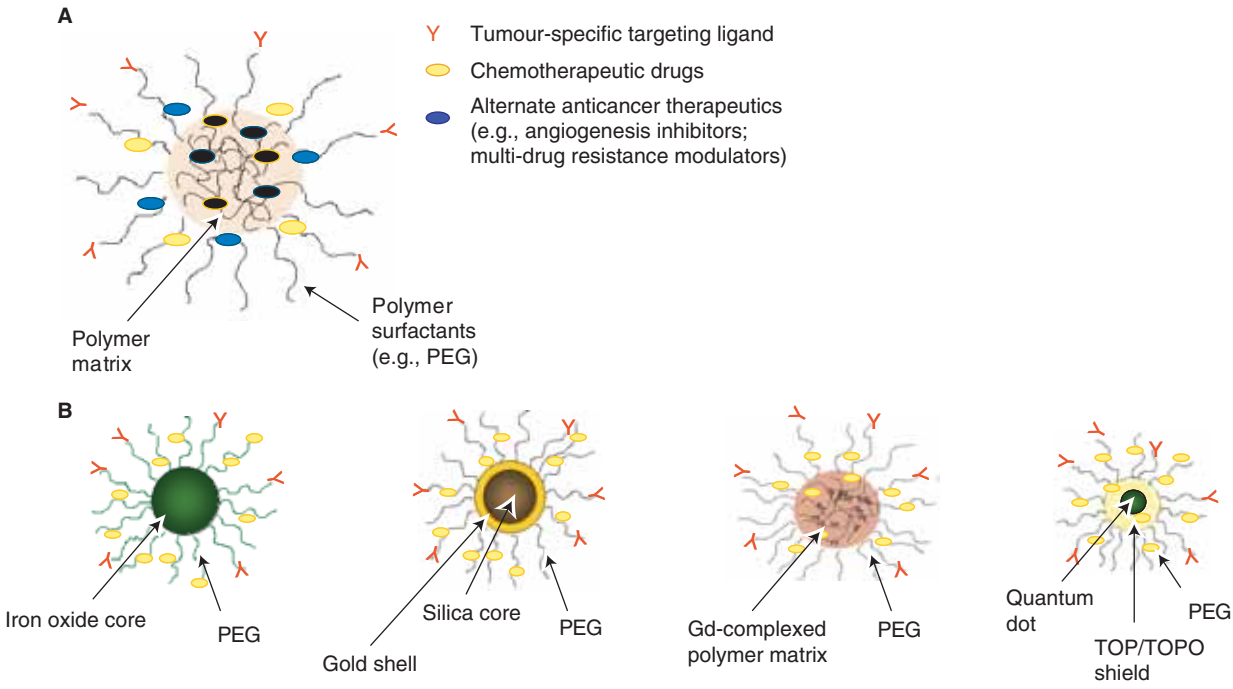

Figure 2. A schematic representation of nanoparticle formulations. A) Simple multi-functional nanoparticles are formulated from a solid polymer core in which chemotherapeutic drugs and/or alternate anticancer therapeutics (such as antiangiogenic drugs or multi-drug resistance modulating drugs) are encapsulated. The core is surrounded by PEG chains, which promote prolonged circulation and to which tumour-targeting ligands can be covalently attached. **B)** Complex multi-functional nanoparticles include (from left to right) iron oxide nanoparticles, gold nanoshells, Gd nanoparticles and quantum dots. Surface modification allows for covalent attachment of tumour-targeting ligands and encapsulation of anticancer drugs.
Gd: Gadolinium; PEG: Poly(ethylene glycol); TOP: Tri-*n*-octyl phosphine; TOPO: Tri-*n*-octyl phosphine oxide.

of the nanoparticle formulation allow for simple multiple functionality directed at enhancing cancer therapy (Figure 2A).

2.1 Targeted constructs

Despite preferential accumulation of nanoparticles in the tumour mass by the EPR effect, the functionality of these nanoparticles by an inclusion of tumour-targeting moieties enhances tumour-specific localisation of the nanoparticle and its payload. In addition, it allows for targeting of the nanoparticles to much smaller and earlier stage tumours, as well as to the cancerous cells that do not belong to a solid tumour mass, such as metastatic cells and cancerous leukocytes. Using the expression of specific recognition markers by the tumour, bioconjugation of the nanoparticles with antibodies directed against such tumour markers improves localisation of the particles specifically at the cancer cells. Two tumour markers most commonly used as targets for directed therapy are the folic acid receptor and the EGFR-2 (erbB2/HER2), as their implication in tumorigenesis results in their overexpression on the cancer cell surface of a wide variety of tumour types [12-16]. For example, folic acid-coated polymeric nanoparticles showed enhanced localisation and internalisation of nanoparticles intended for drug delivery to the breast cancer cells [12], whereas on the other spectrum, folic acid coating also improved localisation and internalisation of magnetite nanoparticles intended for tumour imaging of breast cancer cells [17]. Similarly, tagging the anti-HER2 to the nanoparticle surface greatly improved cell internalisation of gelatin/albumin [15] and gold nanoparticles [18,19], regardless of the fact that the nanoparticles differ in structure and intended function. Along these lines, delivery of the therapeutic can be enhanced by the functionality of nanoparticles with targeting moieties directed against any number of tumour-specific markers.

2.2 Co-encapsulation of multiple therapeutics

As cancer research has progressed, it has became evident that therapy with cytotoxic drugs was not the only effective option for cancer treatment. On one hand, an alternate strategy arose that has opened up a different direction of anticancer drug development, mainly therapy directed at inhibiting angiogenesis at the tumour mass. However, on the other hand, the necessity to design alternate drugs directed at mechanisms of multi-drug resistance (MDR), has emerged as multi-drug resistant cancers are unresponsive to conventional chemotherapeutics. Given such multivalent cancer therapy, nanoparticles provide a good platform to coadminister anticancer therapeutics directed at different targets, which can all converge for maximum cell-toxic effect.

2.2.1 Antiangiogenic and cytotoxic drug combination

A key regulator in the growth and survival of tumours is the influx of nutrients, which places an important role on the tumour blood supply. Without an adequate blood supply, the tumour will lose its proliferative power, thus halting tumour growth. Essentially, as tumour growth is directly dependent on the blood supply, it seemed obvious to direct cancer therapy at the tumour vasculature; specifically aimed at inhibiting angiogenesis. Luckily a plethora of angiogenesis inhibitors exists that was deemed applicable for this purpose. A small subset of this large population of angiogenesis driving factors includes fibroblast growth factor (FGF) [20], insulin-like growth factor [20], G-CSF [20], platelet-derived growth factor [20], and the most common candidate, VEGF [20]. It must be noted, however, that as with all biological processes, tumour angiogenesis is also regulated not just by genetic factors but by a combination of various factors and environmental stimuli [20]. Although angiogenesis treatments initially came into the market as alternatives for potent cytotoxic agents, it was rapidly found that anticancer therapy could be greatly enhanced when combining cytotoxic drugs with antiangiogenic drugs. Examples of such use include: the combination of VEGF receptor 2 (VEGFR2) inhibitor JNJ-17029259 with paclitaxel and doxorubicin [21]; the use of monoclonal antibodies against VEGFR2 (ZD-6474, SU-6668, IMC-1121) or anti-VEGF (bevacizumab) with cisplatin and gemcitabine [22]; and the combination of COX-2 inhibitors, which indirectly inhibit VEGF function, with temozolomide [23]. Proof of the success of such therapy came in clinical trials when the administration of bevacizumab in combination with standard chemotherapeutics (irinotecan, fluorouracil, calcium folinate and leucovorin in these studies) improved survival time for patients with metastatic colorectal cancer [24,25].

However, ongoing clinical studies involving the coadministration of angiogenesis inhibitors with chemotherapeutics revealed a problematic conundrum for this type of therapy; inhibition of angiogenesis can also limit the therapeutic efficacy of chemotherapeutic drugs to the tumour [26]. Given these results, it was hypothesised that co-encapsulation of such drugs into a nanoparticle delivery system could overcome this barrier and effectively deliver both drugs simultaneously to the target site. In a recent publication, Sengupta *et al.* provide an elegant example of this concept by developing a novel multi-functional nanoparticle formulation that, following localisation in the tumour mass, first releases the antiangiogenic drug combretastatin A4 to suppress the tumour vasculature, followed by the sustained release of the cytotoxic agent doxorubicin, which is already localised within the tumour mass [27]. Multi-functional nanoparticle formulations that simultaneously target both the vascular and interstitial compartments of the tumour mass with one system like this provide many promising opportunities to enhance anticancer therapy.

2.2.2 Chemotherapeutics and multi-drug resistance modulators

The ability of cancer cells to become cross-resistant to a variety of structurally and functionally unrelated drugs is termed MDR. This factor is a major hurdle in the fight against cancer as it renders many chemotherapeutic drugs useless. MDR is classified as either intrinsic, if the tumour cell is inherently resistant to chemotherapy, or acquired, if the tumour relapses after treatment [28]. MDR modulators are a group of drugs that can inhibit or reverse the processes that cause cancer cells to become resistant. As there are a multitude of cellular events that can lead to the development of MDR, the pool of MDR modulators is growing steadily. Acquired MDR is commonly caused when the cancer cell i) activates drug metabolising enzymes, thus prematurely inactivating chemotherapeutics [29-31], ii) activates DNA repair mechanisms, thus undoing the work of many chemotherapeutics [29,30], iii) blocks the apoptotic signalling cascade, thus inhibiting the cell-death signal [29,30,32,33], or, most importantly, iv) pumps anticancer drugs out of the cell through efflux pumps from the ATP-binding cassette (ABC)-transporter family, such as P-glycoprotein (P-gp) [29,30,34]. As it is the most common cause of MDR, many first-generation MDR modulators, such as verapamil and ciclosporin A, focused on inhibiting P-gp-induced drug efflux [35,36]. However, it was quickly found that systemic administration of these MDR modulators required near-toxic levels to give the desired effect [37]. Similar to the problems facing conventional chemotherapy, and their need for target-specific drug delivery systems to lower nonspecific drug action, encapsulation of these MDR modulating drugs into nanoparticles could help overcome these hurdles. As with combined antiangiogenic and chemotherapy, nanoparticle encapsulation allowed the opportunity for coadministration of MDR modulators with chemotherapeutics. Not only could nanoparticle-mediated delivery of these drugs then lower their systemic toxicity, intracellular nanoparticle delivery could also evade ABC-transporter-mediated drug efflux. The free drug usually enters the cell by diffusion across the plasma membrane, rendering the drug readily available for efflux through the membrane-spanning pumps. However, nanoparticles gain entry by means of endocytosis/phagocytosis, and are hereby shuttled closer to the centre of the cell where the drug load is deposisted far from the vicinity of the efflux pumps [38]. By engineering the nanoparticle system such that the intracellular drug-release kinetics is greater than the drug-efflux kinetics, one can successfully overcome P-gp efflux in drug-resistant tumours. Soma *et al.* demonstrated the success of this strategy when they successfully reversed MDR in monocytic leukaemia cell line (p388) [39], by coadministration of doxorubicin and ciclosporin A in polyalkylcyanoacrylate nanoparticles. Such data verify the potential for use and the need for development of multi-functional nanoparticles to successfully combat cancer that has attained a resistance to chemotherapeutic drugs.

3. Complex multiple functionality of nanoparticles

Beyond the ordinary use of nanoparticles as a mere vector for delivery of either therapeutic drugs or imaging contrast agents, it seemed obvious to combine these roles and create all-inclusive nanoparticle formulations that can carry imaging and drug delivery capabilities; specifically targeted to the tumour site by passive and/or active targeting (Figure 2B). Furthermore, inherent properties of the core imaging agents, such as iron oxide, gold, gadolinium and quantum dots, allowed for these nanoparticles to also function in alternate anticancer therapies, such as hyperthermia, radiation and photodynamic therapy. Hereby, the possibilities emerged to develop nanoparticles that simultaneously image and treat cancer; a more complex approach to multiple functionality.

3.1 Superparamagnetic iron oxide (magnetite) nanoparticles

A commonly used metal in nanoparticle formulations for use as MRI contrast agents is iron oxide. Two types of iron oxide have mainly been investigated for their use in magnetic nanoparticle formulation: maghemite (γ-Fe_2O_3) and magnetite (Fe_3O_4) [40], where proven biocompatibility of magnetite has caused it to be a more promising candidate [40]. One of the more important advantages of this material is that it exhibits superparamagnetism, a property that allows for stability and individual dispersion of the particles after the external magnetic field has been removed [40]. The strong magnetic property of magnetite makes it well suited for use as an MRI contrast agent. Although MRI is a very useful technique for the detection of solid tumours (by providing clear anatomical detail and soft tissue contrast), in the past MRI has been quite insensitive for smaller events in cancer imaging, such as the detection of lymph node metastasis and therapeutic efficacy of cancer treatment. Harisinghani *et al.* showed that even unmodified iron oxide nanoparticles allowed for 90.5% detection of lymph node metastasis in patients with prostate cancer, as opposed to 35.4% detection using conventional MRI [41].

Surface modification with active targeting ligands improves target localisation of the nanoparticles. A method for visualising therapeutic efficacy of cancer treatment proved possible when Zhao *et al.* targeted iron oxide nanoparticles to anionic phospolipids present on the surface of apoptotic cells. This was achieved by incorporating the C2-domain of synaptotagmin I onto the surface of the nanoparticles as an apoptotic targeting moiety [42]. The functionality of the nanoparticles by inclusion of active targeting moieties not only guides the particles beyond the solid tumour mass, but also aids in internalisation of the particles. The inclusion of folic acid on the surface of iron oxide nanoparticles improved targeting and intracellular uptake to BT20 breast cancer cells *in vitro* [17]. Keeping in mind that folic acid receptors are desired targets to direct targeted tumour therapy, Kohler *et al.* found a way to use this principle in their multi-functional iron oxide nanoparticle formulation [43]. Methotrexate is an analogue of folic acid that, in addition to recognising folic acid receptors, exerts a chemotherapeutic effect on many cancer types that express folic acid receptors. Kohler *et al.* showed that iron oxide nanoparticles can be multi-functionalised by binding methotrexate to the surface to produce a targeting construct, and once internalised by the cancer cell, lysosomal pH cleaved methotrexate from the surface allowing it to further serve as a chemotherapeutic for cancer eradication [43], hereby producing a multi-functional system that allows for simultaneous tumour therapy and real-time imaging of drug delivery. Multiple functionality of iron oxide nanoparticles by combining such tumour targeted imaging with drug delivery is an obvious and applicable step in the creation of an all-in-one cancer therapy. Magnetic particles have the limitation that their magnetic strength and bioavailability depends strongly on their size and surface chemistry [44,45]. So far, magnetite nanoparticles formulated with poly(D,L-lactide-co-glycolide) have been successful in combining the delivery of chemotherapeutic drugs to the tumour, while retaining enough magnetic strength for imaging contrast enhancement [46], although reports of a significant loss of magnetic strength (~ 40 – 50%) must be taken into consideration [47,48]. A more recent successful approach involved the formulation of iron oxide core nanoparticles with oleic acid, which were able to carry water-insoluble anticancer agents to the tumour site while retaining their magnetic strength [49]. Besides combining imaging with therapy, drug-loaded magnetic nanoparticles raise the potential to magnetically guide nanoparticles to deposit drugs at the intended target site, although *in vivo* data are not conclusive enough to prove clinical success. The major limitation of magnetic targeting *in vivo* is dampening of the external magnetic field with increasing depth in the biological environment.

Going one step beyond the ability of iron oxide nanoparticles to deliver drugs and image tumours in one multi-functional system, it was also found that the magnetic properties of the iron-oxide core could be further exploited for use in guided hyperthermia. The most obvious problem with hyperthermia treatment is the challenge of heating only the tumour region without damaging healthy tissue. With their ability to localise to a great extent in the tumour region, iron oxide nanoparticles are excellent candidates for such local heat conduction. By alternating the magnetic field externally, heat is generated around the magnetic particles due to hysteresis loss [50], and when the temperature is raised above 43°C, cell apoptosis/necrosis results [40,51]. Local hyperthermia also enhances the perfusion of systemically administered drugs into the core of the solid tumour mass. The usefulness of this principle on tumour shrinkage has been readily shown in many *in vivo* studies [52,53], and although there has been much progress using hyperthermia to enhance efficacy of separately administered chemotherapeutics [54] and gene/protein therapy [55-57], a unified polymeric iron oxide nanoparticle that combines

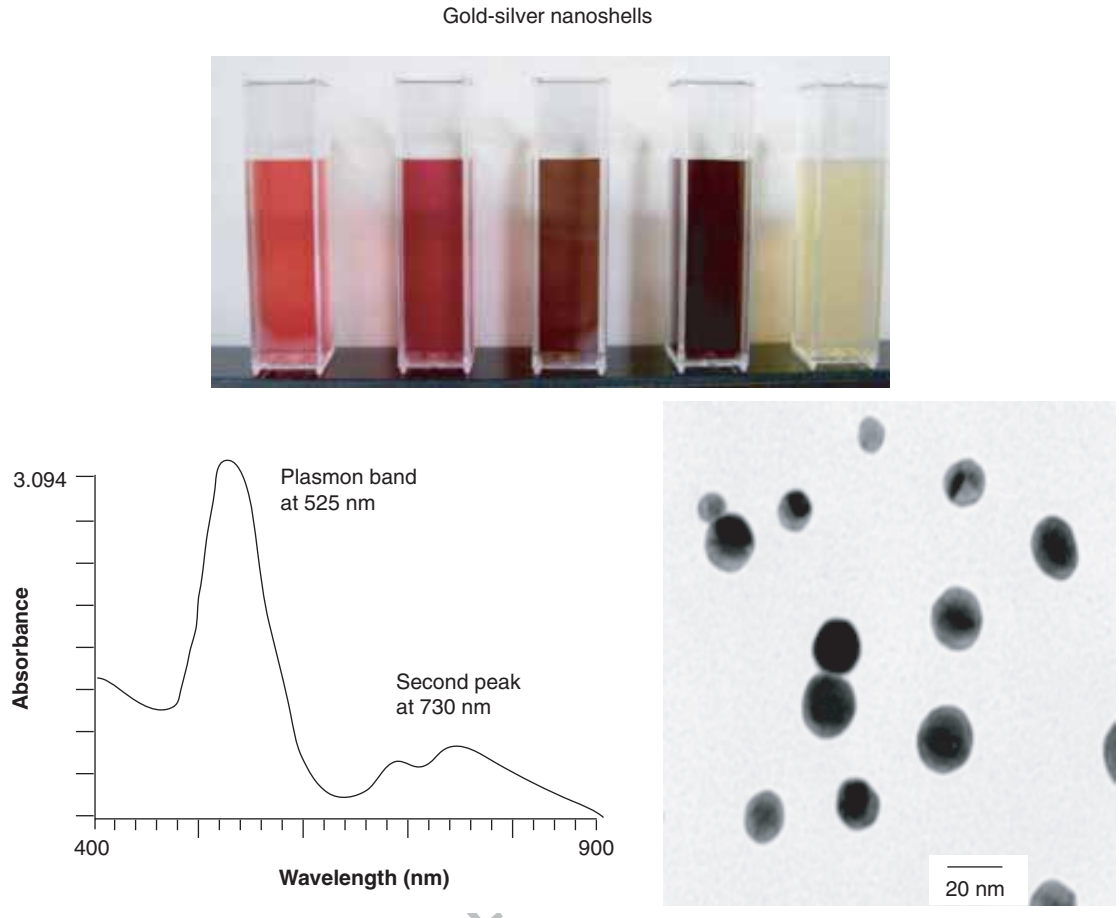

Figure 3. Resonance wavelength tuning of gold nanoshells. On account of their composition and size, gold nanoshells can be tuned to emit light at varying wavelengths, ranging from ultraviolet through visible light to near-infrared.

tumour imaging, drug delivery and hyperthermia treatment remains to be developed.

3.2 Gold nanoparticles and silica-gold nanoshells

Gold nanoparticles are another class of metal nanoparticles that have found a niche in the tumour-imaging and guided hyperthermia market. Gold nanoshells, silica core nanoparticles surrounded by a layer of gold coating, are favourable to use as contrast agents in optical coherence tomography because variations in their size and shape allows for the precise tuning of their resonance wavelength [58]. This flexibility translates into the potential to tune absorbance of the nanoshells anywhere between near-ultraviolet and mid-infrared [59] (Figure 3). For example, a gold nanoshell with a 20-nm shell on a 60-nm silica core will resonate at ~ 700 – 750 nm, whereas a nanoshell with a 5-nm shell on the same 60-nm core will resonate at ~ 1000 – 1050 nm [60]. The disperse range permissible to these nanoparticles spans the near-infrared, which immediately brings forth the second useful property of gold nanoshells; their use in thermal ablation. Light in the near-infrared region experiences optimal tissue penetration, whereas tissue absorption is minimal [61]. As opposed to conventional near-infrared dyes [62], gold nanoshells are robust, which prevents thermal denaturation. In addition, they scatter light more intensely, allowing for detection and use at femtomolar concentrations and they also do not photobleach. The latter two characteristics are important for their optical imaging properties. Combined imaging and therapeutic use of these gold nanoshells has been proven in several cancer models, both *in vitro* and *in vivo* [19,60,63]. It has been shown that thiolated PEG [19,60,63], which easily assembles onto the nanoshell surface, allows for incorporation of tumour-targeting antibodies into the nanoshell system, to functionalise the particles to target tumours actively, in addition to their passive targeting properties by the EPR effect. An antibody effectively used in conjugation is anti-HER2 [18,19,60], although it is possible to attach a wide variety of antibodies raised against tumour-specific markers. Use of a polymer linker with free carboxylic acid or amino groups expands the option for antibody/antigen incorporation, as

not all proteins have free sulfhydryl groups for covalent linkage. Recent reports investigating additional uses of gold nanoparticles helps classify these colloidal carriers as multifunctional. Paciotti *et al.* have used colloidal gold particles to successfully deliver TNF-α as an anticancer therapeutic to an MC38 colon carcinoma *in vivo* [64]. Mukherjee *et al.* reported the inhibition of angiogenesis by gold nanoparticles, through direct binding of the particles to heparin-binding growth factors (vascular permeability factor/VEGF and FGF specifically); a property that is very useful in halting tumour proliferation [65]. In addition, Hainfeld *et al.* have shown that gold nanoparticles can help to localise radiotherapy in order to prolong 1-year survival rates of mice bearing EMT6 mammary carcinomas (86% survival with gold nanoparticles versus 20% survival with X-rays alone) [66]. Although the latter three examples used colloidal gold nanoparticles rather than the aforementioned silica-gold nanoshells, which bear the combined use of imaging and thermal ablation, future research may allow for the development of a gold nanoshell or particle that bring together all these uses.

3.3 Gadolinium-containing nanoparticles

Gadolinium-157 is a stable (non-radioactive) nuclide that, following irradiation with thermal neutrons, produces cytotoxic γ-ray radiation [67,68]. This feature enables it for use in neutron capture therapy (NCT) of cancer [68]. As opposed to other radiation producing elements, such as boron-10, which are also used in NCT, gadolinium compounds are used as contrast agents in MRI diagnostics [69]. Thus, the therapeutic and imaging properties of gadolinium make it an excellent candidate for multivariant tumour therapy. Studies show that tumour-specific gadolinium localisation with gadolinium ion-containing nanoparticles significantly suppresses tumour growth and increases survival time with NCT in mice bearing a radio-resistant melanoma [68]. Delivery of gadolinium through gadopentatic acid (Gd–diethylenetriaminepentaacetate) allows for association of gadolinium into polymeric nanoparticles, a principle proven by Tokumitsu *et al.* who used this concept to associate gadolinium into chitosan nanoparticles [68,70] for NCT. Although prior use of Gd–diethylenetriaminepentaacetate as a MRI contrast agent [71] suggests the dual use of these chitosan nanoparticles in imaging and therapy, the authors have yet to investigate that particular principle. Chitosan nanoparticles have been employed in the delivery of chemotherapeutics such as paclitaxel and doxorubicin to tumours [72], suggesting a future potential to further multi-functionalise this gadolinium nanoparticle formulation for drug delivery capabilities. A currently feasible feat is the association of targeting ligands to gadolinium nanoparticles for improved site-specific localisation. Conjugation of folic acid or thiamine to the surface of gadolinium-containing nanoparticles, through distearoylphosphatidylethanolamine and a PEG spacer, greatly enhanced the cell uptake of gadolinium to cancer cells expressing receptors for folate and thiamine, respectively (*in vitro* and *in vivo*), potentially improving localisation and tumour eradiaction by NCT [73-75]. Already, gadolinium nanoparticles present multi-functional properties in their ability to image and ablate the tumour in one system. However, further multiple functionality of these vectors by conjugation with tumour-specific targeting ligands and incorporation of a drug load remains to be examined.

3.4 Quantum dots

Quantum dots are semiconductor-based nanoparticles that have emerged on the forefront as fluorescent probes for imaging purposes [76]. As for gold nanoshells, quantum dots are favourable imaging agents in that their absorption properties can be tuned from visible to infrared wavelengths, they emit highly intense signals, and they are chemically, photochemically and thermally stable [77]. Quantum dots have the unique property that with one excitation wavelength, resulting from their size and chemical composition, they can emit a signal at any wavelength between blue and infrared [78]. Therefore, a number of quantum dots, each antibody conjugated to target a different tumour marker, can be visualised simultaneously. This provides a property of quantam dots that can be taken advantage of in real-time cancer imaging, particularly in the tracking of metastatic tumours [78]. Quantum dots, miniscule in size (2 – 8 nm in diameter) [76], are easily bioconjugated with peptides, antibodies and small molecule drugs through polymer linkers, without loss of their fluorescence or tumour-localisation properties [76]. Typically, high-quality quantum dots are prepared in the organic solvent mixture tri-*n*-octyl phosphine/tri-*n*-octyl phosphine oxide at high temperatures, which caps the quantum dots with a monolayer of the nonpolar solvent [76,79]. This capping allows for surface adhesion of amphiphilic polymers (such as PEG and poly[ethylene oxide]-containing block copolymers), which not only facilitate solubility and bioavailability of the nanoparticles, but also provide a linker for bioconjuation of peptides, antibodies, oligonucleotides, or small molecule drugs [76,80], hereby multi-functionalising the quantum dot for tumour targeting, tumour imaging and potential drug delivery. A few examples of the large pool of tumour-targeting ligands that have been bioconjugated to quantum dots in this manner include: antibodies against HER2 [81], prostate-specific membrane antigen [82], heat-shock proteins [83], and P-gp [84]. The next step in quantum dot multiple functionality includes the incorporation of a drug load for simultaneous imaging and chemotherapeutic delivery to the tumour. Although the chemistry exists to load drugs into the bulk of the polymer coating, or to graft drugs onto the surface of the nanoparticle, use of such a system while retaining the imaging, biocompatibility and bioavailability properties remains to be proven. Nevertheless, through a recent discovery, it seems that quantum dots are not entirely defunct as anticancer therapeutics, thus maintaining the combined tumour imaging and therapy functions that gives these vectors their reputation as multi-functional nanoparticles. Bakalova *et al.* reported in

2004 of the potential to use quantum dots as photosensitisers in photodynamic therapy [85]. Photodynamic therapy uses light, oxygen and a photosensitiser to selectively destroy target tissue by generating reactive oxygen species, which promotes apoptosis of the target cells [85]. With quantum dots, the potential exists to specifically eradicate the cancer with chemotherapeutics and/or photodynamic therapy, following real-time visualisation of the tumour mass and metastasis. This provides a highly useful multi-functional nanoparticle tumour-therapy system in itself, regardless of the added promise to combine chemotherapeutic delivery in this system as well.

4. Conclusions and future outlook

Polymeric nanoparticles are being developed as effective delivery vehicles due to their passive tumour-targeting properties, which lead to the ability to enhance the efficacy and reduce the side effects of chemotherapeutic drugs. In addition, this unique capacity of nanoparticles to preferentially accumulate in and around the tumour mass also grants a platform for improved tumour diagnostics, hereby laying the foundation for the development of multi-functional nanoparticle systems in cancer therapy. As polymer chemistry is such a versatile and adaptable field, using polymers as the backbone for nanoparticle formulation facilitated the advancement to multiple functionality. Inclusion of tumour-targeting ligands directed against common markers, such as the folic acid receptor and the EGFR-2 (HER2), not only enhances localisation of the particles to the solid tumour mass, but also allows for targeting of the nanoparticles to small and early stage tumours, metastatic cancer cells and leukaemias. Therefore, this provides a means to greatly improve both drug delivery to and visualisation of cancerous cells. Beyond effective tumour-specific targeting of the therapeutic load comes the dilemma of delivering an effective therapy. Cytotoxic chemotherapeutics have set the standard for anticancer therapy; however, their potent toxicity, nonspecific action, and failing use in drug-resistant tumours has driven the search for alternate therapies. Polymeric nanoparticles can be manipulated with ease to allow for co-encapsulation and, thus, efficient simultaneous delivery of multiple drugs intended for differing purposes. Even though cancer therapy can be greatly enhanced by these means alone, the diverse opportunities available to polymeric nanoparticle formulation allows for true multiple functionality of the particles. Through the inclusion of metals such as iron oxide, gold, gadolinium and the various semiconductors (e.g., cadmium, selenium and zinc [80]) that consitute the body of quantum dots, nanoparticles can be formulated that combine tumour targeting, tumour imaging and tumour therapy, both by chemical and physical means, all in one system.

A future outlook for this field promises even further improvements in combining multi-modal therapy and imaging. Although iron oxide nanoparticles and gold nanoshells have been shown to combine imaging with hyperthermia treatment, or imaging with drug delivery, a cohesive formulation remains to be developed that can effectively image, deliver drugs and localise hyperthermia treatment to the tumour, whilst retaining the long circulating and tumour localisation properties of the nanoparticle. Similarly, gadolinium nanoparticles are proving to be highly useful in combining cancer imaging with cancer therapy by NCT; and although these nanoparticles bear a potential to deliver a targeted therapeutic load efficiently, successful *in vivo* proof of tumour imaging and tumour treatment, both by NCT and drug delivery, rests unproven to this day. The same is true for the quantum dots, which have been proven to carry tumour-targeting ligands and small molecule drugs without loss of their tumour-imaging strength; however, merging these properties with their use in photodynamic therapy is as yet uncharted territory. An area that also remains unexplored is the synthesis of polymeric materials with intrinsic imaging (e.g., using fluorescent monomers) and drug delivery properties (e.g., drugs as monomers).

As the development of cancer-directed polymeric nanoparticles extends to include ever more functions, concerns arise about bioavailability, biocompatibility and the loss of other properties such as imaging strength and drug release. However, so far, polymeric nanoparticle formulations have already evolved from single function through dual function to multi-functions, all the while retaining the desired properties that make nanoparticles so useful in cancer therapy. Therefore, the potential exists to successfully improve current nanoparticle formulations, gaining one step closer to the ultimate cancer therapy system.

5. Expert opinion

Due to their versatility, polymeric systems offer tremendous promise in the development of nanotechnology for cancer therapeutics. Polymeric materials that are designed by parallel synthesis, such as poly(β-amino esters) developed in Langer's laboratory at the Massachusetts Institute of Technology [86,87], allow for judicious selection for cancer targeting and delivery. In addition, many new chemical synthesis schemes are being evaluated that provide the means to develop polymers with intrinsic responsiveness to differences in solid tumour microenvironment as compared with normal tissues, such as low pH, redox, and/or hypoxia. Surface nanoengineering also provides a favourable outlook on development of polymeric nanoparticles that can simultaneously bind to multiple target sites on tumour cells, and preferentially localise the drugs and imaging agents. Multiple target access will allow for even better specificity and selectivity of the tumour mass relative to normal tissue exposure, thereby further reducing the needed drug dose, as well as the potential for harm to the cancer patient.

Second, one of the most interesting potential developments for the future of multi-functional polymeric nanoparticles is the use of biologically active polymers as building materials in nanoparticle formulations. Using biologically active polymers

(such as polymer drugs and polymers with inherent fluorescence) as the structural frame of the nanoparticle promises the development of an efficient carrier system that in and by itself is entirely a therapeutic. Ideally, with such technology, the prolonged circulation and tumour-targeting properties of the nanoparticle remain in place; however, following localisation in the tumour, the particle degrades to become the anticancer therapeutic, as opposed to merely releasing it. Polymeric drugs, such as polyanions and polysulfates, have been known to possess anticancer activity [88]; however, their success in the clinic is limited by toxic side effects [89]. Their cytotoxic anticancer effect and polymeric structure makes drugs of these class very interesting candidates for formulation as biologically active polymeric nanoparticles. PEG shielding and active tumour targeting of these nanoparticles can increase tumour-specific accumulation, thus reducing toxic side effects, and allowing the polymer to exert an antitumoural effect.

An alternate approach is in the use of environmentally responsive polymers to overcome a novel facet of tumour MDR. Research has shown that certain types of multi-drug resistant cells present with highly acidified endosomes, resulting in the entrapment of ionisable chemotherapeutics, rendering the drugs ineffective [90]. A nanoparticle composed of pH-sensitive polymers (namely basic poly[amino acids] and polyamides), can function therapeutically as an MDR modulator, by buffering the endosomal pH and thereby deprotonating the basic drug to allow for diffusion out of the endosome, back towards its intracellular/nuclear target.

Although any such biologically active polymeric nanoparticle formulation with proven function remains to be developed, the overall concept is truly elegant; the aforementioned examples are just some of many interesting possibilities. With the step towards multiple functionality of nanoparticulate carriers, comes the burden that the composition becomes increasingly dense, potentially resulting in poor biocompatibility or poor tumour availability. The use of biologically active polymers as scaffolds in the nanoparticle formulation helps to simplify the composition, providing more room in the carrier for multiple functionalities; a trend to strive for in the future development of nanotechnology for cancer therapeutics.

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