Bio/Analytik — Toxikologie/Medizin — Kolloquien 2013

Hochschule Zittau/Görlitz
University of Applied Sciences
FG Biotechnologie

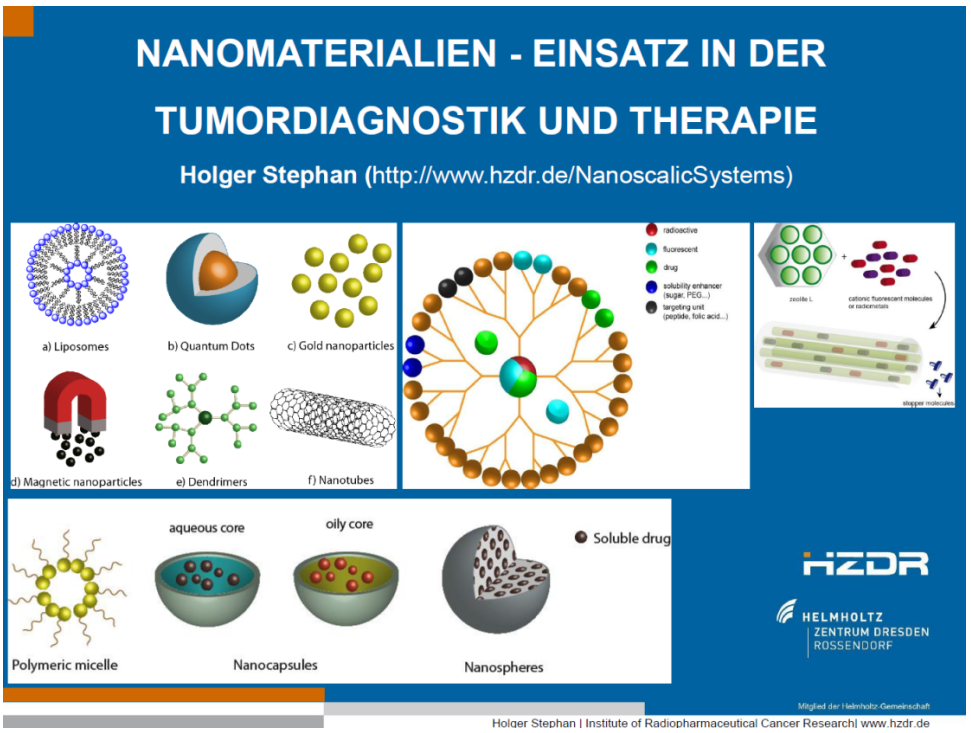
Wissenschaftliche Kolloquien 2013 - 01

Dr. rer. nat. Holger Stephan

Helmholtz-Zentrum Dresden-Rossendorf,
Institut für Radiopharmazeutische Krebsforschung

Thematik: „Nanomaterialien – Einsatz in der Tumordiagnostik und Therapie"

Termin: 08. Mai 2013, 15.00 Uhr, Marschnerstrasse 22, MB



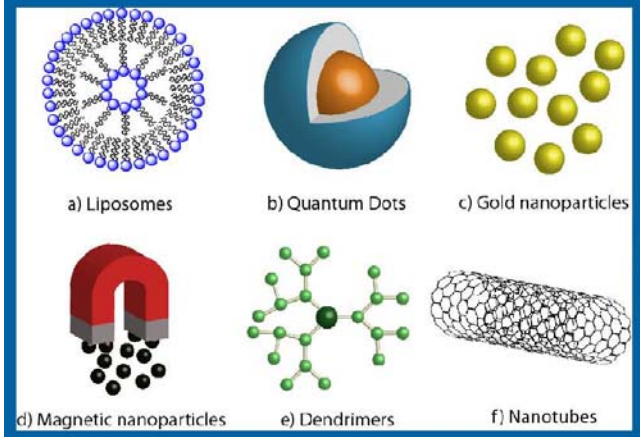
Nach den Kolloquien findet eine Nachsitzung in der Mäuseburg (MB) statt!

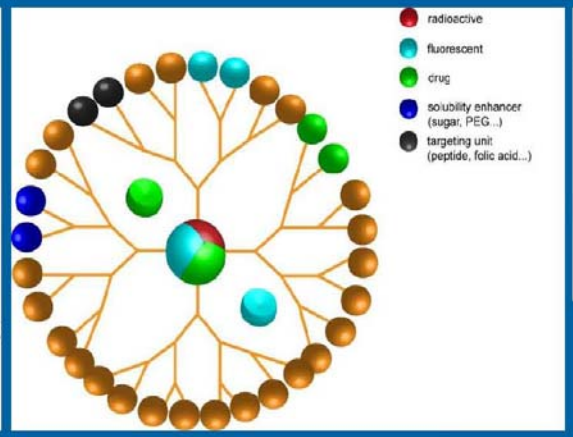
Dazu sind immer alle StudentInnen/KollegInnen sehr herzlich eingeladen!

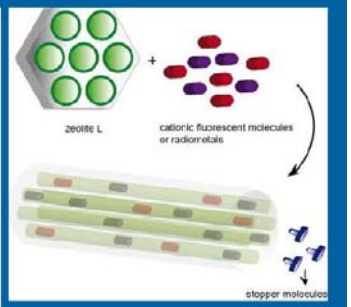
Prof. Dr. Manfred Gey

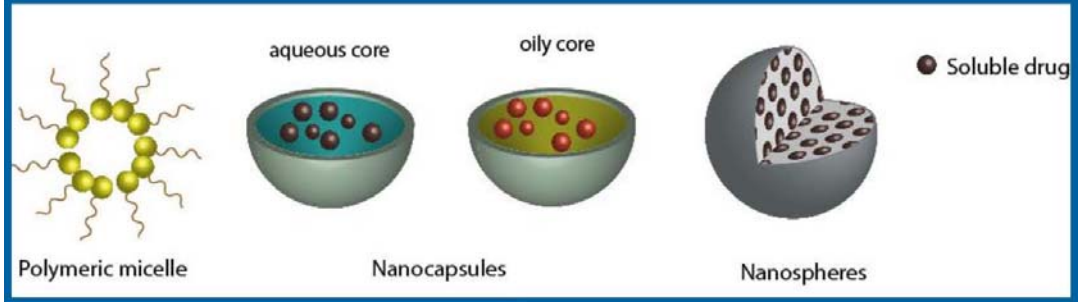
NANOMATERIALIEN FÜR DEN EINSATZ IN DER TUMORDIAGNOSTIK UND THERAPIE

Holger Stephan (http://www.hzdr.de/NanoscalicSystems)









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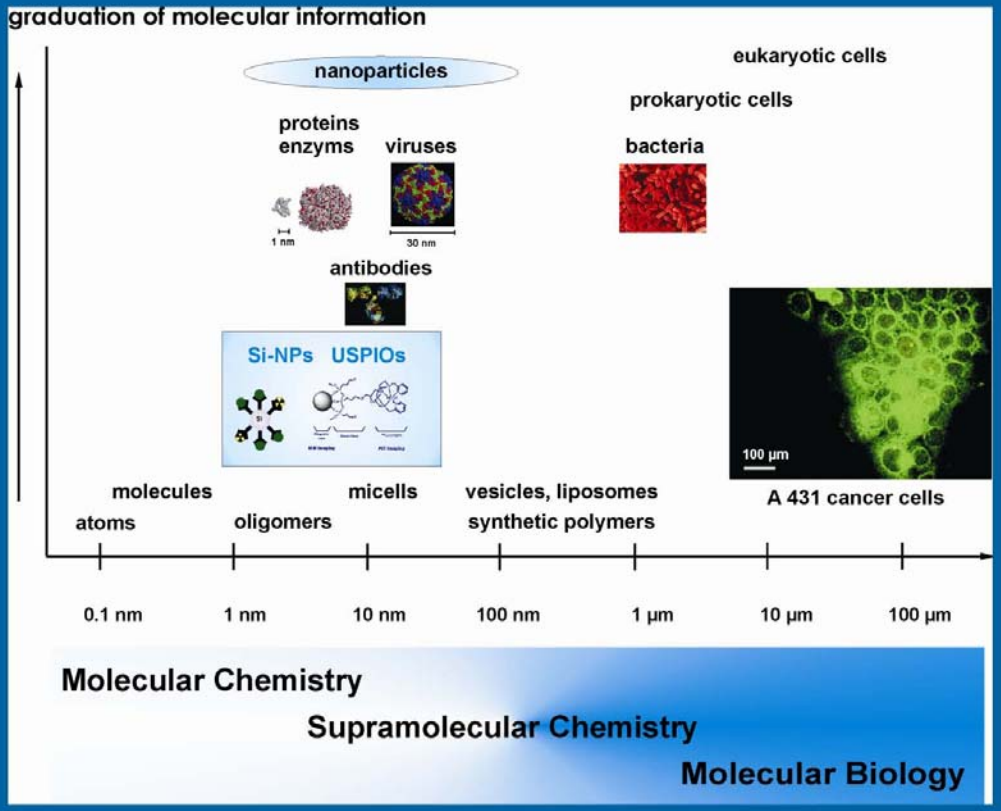
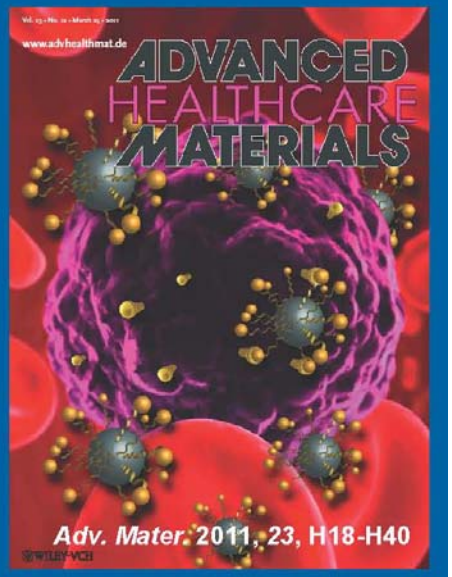
NANOMATERIAILIEN FÜR DEN EINSATZ IN DER TUMORDIAGNOSTIK UND THERAPIE





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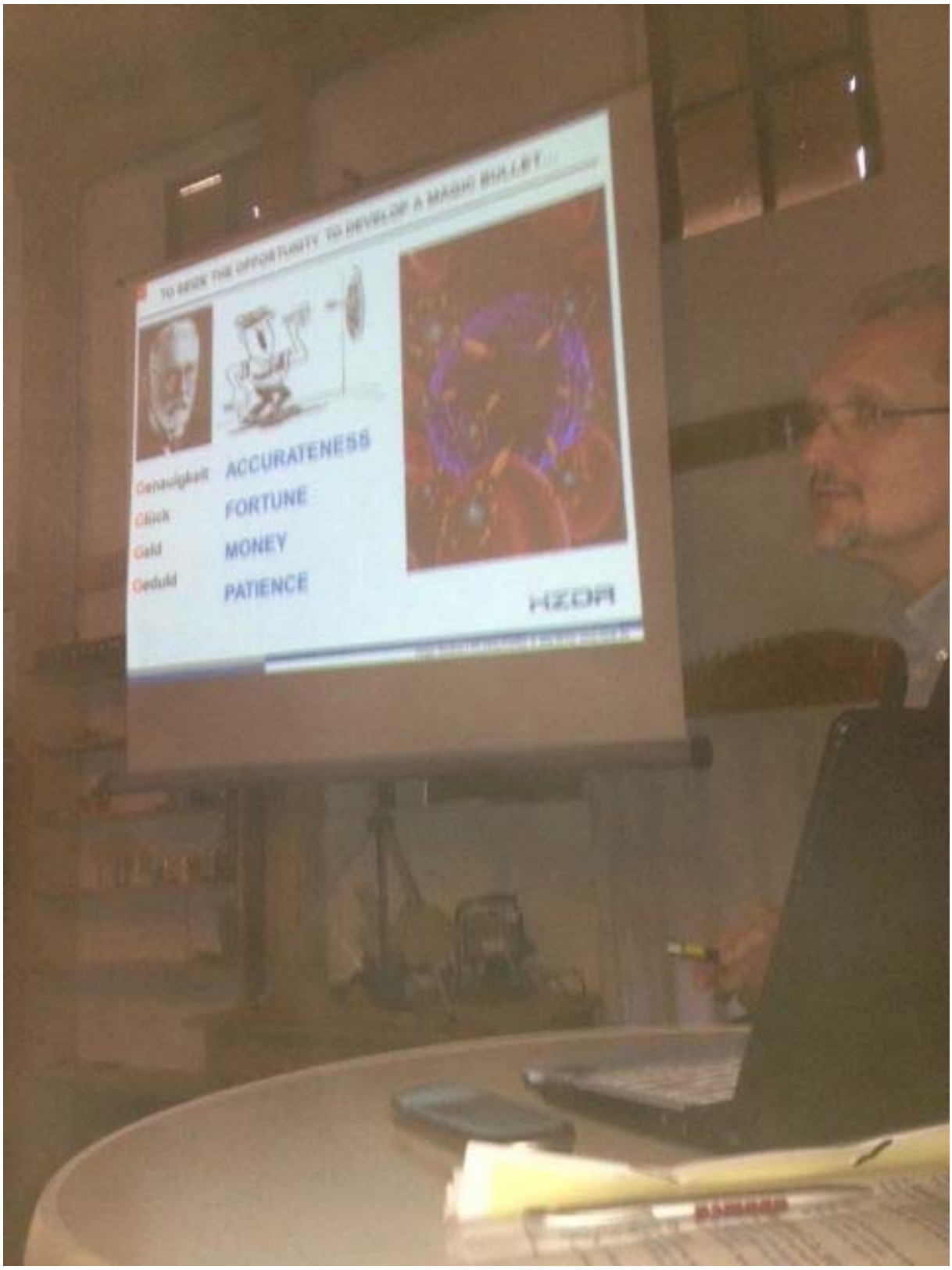
TO SEIZE THE OPPORTUNITY TO DEVELOP A MAGIC BULLET...
Genauigkeit
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Glück
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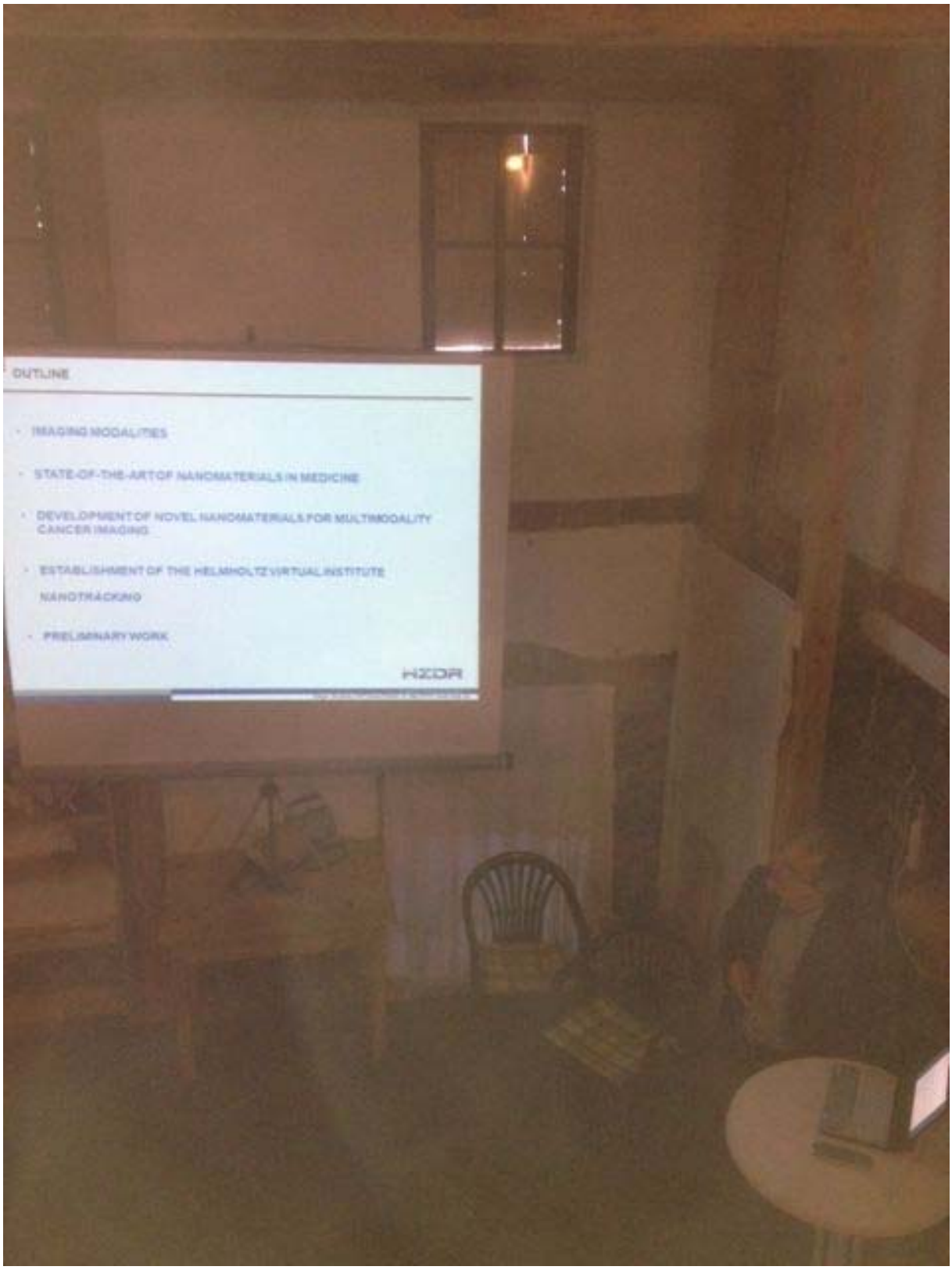
OUTLINE
IMAGING MODALITIES
STATE-OF-THE-ART OF NANOMATERIALS IN MEDICINE
DEVELOPMENT OF NOVEL NANOMATERIALS FOR MULTIMODALITY CANCER IMAGING
ESTABLISHMENT OF THE HELMHOLTZ VIRTUAL INSTITUTE
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Nanomaterials: Applications in Cancer Imaging and Therapy

José A. Barreto, William O'Malley, Manja Kubeil, Bim Graham, Holger Stephan,* and Leone Spiccia**

The application of nanomaterials (NMs) in biomedicine is increasing rapidly and offers excellent prospects for the development of new non-invasive strategies for the diagnosis and treatment of cancer. In this review, we provide a brief description of cancer pathology and the characteristics that are important for tumor-targeted NM design, followed by an overview of the different types of NMs explored to date, covering synthetic aspects and approaches explored for their application in unimodal and multimodal imaging, diagnosis and therapy. Significant synthetic advances now allow for the preparation of NMs with highly controlled geometry, surface charge, physicochemical properties, and the decoration of their surfaces with polymers and bioactive molecules in order to improve biocompatibility and to achieve active targeting. This is stimulating the development of a diverse range of nanometer-sized objects that can recognize cancer tissue, enabling visualization of tumors, delivery of anti-cancer drugs and/or the destruction of tumors by different therapeutic techniques.

1. Introduction

The development of new nanomaterials (NMs) for the diagnosis and/or treatment of different diseases has been receiving greater attention in recent years and has now become an important field in medical research. Dedicated NMs can be used to monitor the progress of a therapy or disease, to determine the blood type of patients requiring transfusion, or for tissue typing when a transplant is required.[1–6] One major advantage of NMs is their potential to be used as non-invasive diagnostic tools. Another is the capacity to combine multiple modalities on one probe, enabling higher sensitivity to be achieved and deeper insight gained into in vivo processes. NMs are also ideally suited to be applied as drug-delivery systems, making the development of a new generation of theranostics possible. The combination of molecular imaging and local treatment of lesions is unique for nano-objects and has the potential to change the current medical paradigm of "see and treat" to "detect and prevent".[1]

NMs used as diagnostic/therapeutic tools encompass a wide range of substances, both organic (e.g., liposomes, natural and synthetic polymers including dendrimers, and carbon nanotubes) and inorganic materials (e.g., quantum dots (QDs), metallic nanostructures, and metal oxides (particularly magnetic iron oxides, up-converting nanophosphors, and zeolites)). Decorated with special functionalities, NMs allow the application of different molecular imaging techniques, such as computed tomography (CT), magnetic resonance imaging (MRI), single-photon emission tomography (SPECT), positron emission tomography (PET), ultrasound imaging, and optical imaging methods.[7] Their fascinating and unique properties are also exploitable across a range of therapies, including chemotherapy, photodynamic therapy, neutron capture therapy, thermal therapy, and magneto-therapy. NMs may be engineered that permit a combination of these therapies to be used, leading to synergetic medical effectiveness.

This review focuses on recent developments of NMs for application in cancer diagnosis and treatment. A short description of tumor angiogenesis is given, and its importance for the design of new NMs is discussed. A major goal of this review is to unveil the emerging possibilities of nanovectors for therapeutic applications.

2. Cancer Pathology

Cancer is a generic group of diseases that can affect different parts of the body. According to the World Health Organization, cancer accounted for 7.9 million deaths in 2007 (13% of all deaths), and this number is expected to rise to 12 million by 2030. Cancer occurs because of mutations in genes that control cell cycles.[8] Four major characteristics of cancer cells are uncontrolled growth (uncontrolled division), invasion of adjacent tissue, metastasis (spreading to other locations in the body), and immortality (protection against programmed cell death).

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Practically all cancer cells show irregular cell-cycle control.[8] When the tumor has grown larger than ca. 1–2 mm, it develops new blood vessels (angiogenesis) by stimulation from substances released into the tumor microenvironment. Tumor vessels are abnormal, presenting spaces between endothelial cells, interruption of the basement membrane, and no smooth muscle within the capillary walls.[8] They possess a chaotic architecture, are tortuous and dilated, exhibit uneven diameter and excessive branching, and have a disorganized vasculature.[9,10] With respect to the walls, the vessels have numerous openings, such as endothelial fenestrae, vesicles and transcellular holes, interendothelial junctions, and a discontinuous basement. These factors make the capillaries more permeable than normal vessels, allowing substances to pass more easily to and from the surrounding environment.[7,8] Vascular permeability depends on the type of tumor and the organs within which it is located.

2.1. Tumor Angiogenesis

Angiogenesis refers to the growth of new blood vessels from existing ones; this process is necessary for the growth of multicellular organisms to transport oxygen and nutrients to the new cells.[9] Angiogenesis is regulated by a balance between pro- and anti-angiogenic molecules, the *angiogenic switch*. This switch is considered to be "off" when the effect between pro- and anti-angiogenic molecules is balanced, and "on" when the balance is favorable for angiogenesis.[9,11,12]

A tumor typically grows as a cluster of cells at a rate that depends on oxygen and nutrient diffusion, and can be benign or malignant (**Figure 1**). When the tumor reach a maximum size (1–2 mm),[9,11,13,14] the inner cells are unable to grow and proliferate because of a lack of nutrients and become quiescent or necrotic, leading to the formation of a three-layered structure: a core of dead cells surrounded first by a layer of quiescent cells and then by a thin rim of proliferating cells.[11] The tumor can exist in a state of dormancy where cell proliferation is balanced by cell death. Only through formation of new blood vessels (angiogenesis) can the tumor emerge from this state of dormancy. Angiogenesis provides access to an increased supply of oxygen and nutrients, thus allowing rapid growth of the tumor. The more malignant tumors have the greatest angiogenic potential.[11] Different signals have been reported to

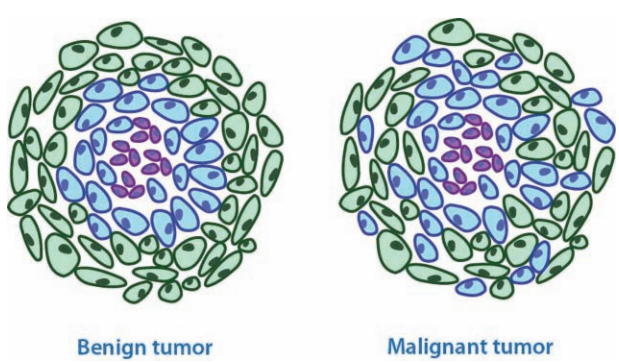

Figure 1. Types of tumors. In a *benign tumor* mutated cells remain contained within a single cluster, with a clear boundary separating them from normal cells. In a *malignant tumor*, mutated cells are mixed with normal cells, attempting to invade the surrounding tissue.

José A. Barreto, born in Bogotá, Colombia, received a Bachelor of Chemistry (Honours) from the National University of Colombia (Universidad Nacional de Colombia) in 2006. From 2006 to 2008, he worked as a researcher at the Colombian Immunology Foundation Institute (FIDIC) in the field of peptide synthesis. He is currently undertaking PhD studies in the School of Chemistry at Monash University within the group of Professor Leone Spiccia. His research focusses on the development of novel iron oxide nanoparticles as imaging agents for multimodal detection of cancer tumors by MRI and PET.

Leone Spiccia obtained his PhD degree from the University of Western Australia in Perth, Australia in 1984. He took up an academic appointment in the Department of Chemistry at Monash University in Melbourne, Australia in 1987, where he is currently Professor of Chemistry. His research interests include bioinspired water oxidation catalysis, nanomaterials for water splitting devices and dye-sensitized solar cells, metal-ion speciation aqueous environments, biosensors based on metal-complex bioconjugates, artificial nucleases and ribonucleases, therapeutic and diagnostic applications of magnetic nanoparticles, and radiolabelled bioconjugates.

Holger Stephan received his PhD degree from the Technical University Bergakademie Freiberg in 1989. He is currently leading the "Nanoscalic Systems" group at the Helmholtz-Zentrum Dresden–Rossendorf. His research interests are the development of radiometal complexes, including functionalized nanoparticles for therapeutic and diagnostic applications, dendrimer chemistry, coordination chemistry, and the themodynamics of liquid two-phase systems.

Bim Graham obtained his PhD degree from Monash University in 1999. After post-doctoral studies at the Max Planck Institute for Chemistry, Mainz, Germany, and the Commonwealth Scientific and Industrial Research Organisation, Molbourne, Australia he took up an academic post within the Monash Institute of Pharmaceutical Sciences in 2006, where he is now a senior lecturer in medicinal chemistry. His research focusses on the development of luminescent metal complex- and nanoparticle-based probes for imaging and assay applications, lanthanide tags for biomolecular NMR spectroscopy, metal complex bioconjugates for nucleic acid cleavage, and novel bioconjugation reagents and strategies.

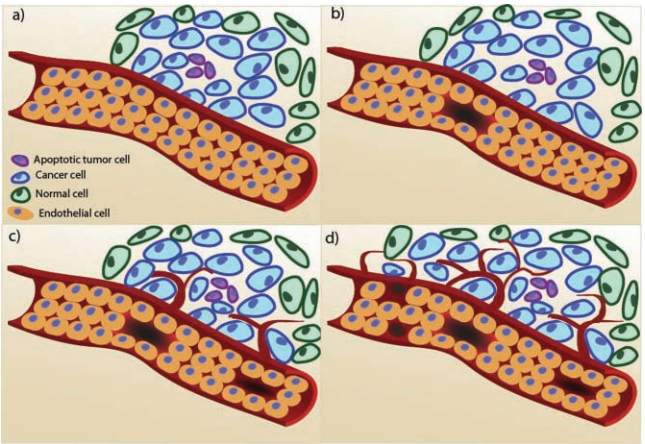

Figure 2. Key events during tumor angiogenesis: a) a tumor in a state of dormancy near a blood vessel, b) the *angiogenic switch* is turned on, causing dissolution of the basement membrane (black hole), followed by migration and proliferation of endothelial cells in order to form new blood vessels c) and d).

switch on the angiogenesis process, such as hypoxia,[9,11] low pH, hypoglycemia, mechanical stress, immune or inflammatory responses, or genetic mutations.[11]

The key events that result in the sprouting and growth of tumor vessels are similar to those involved in normal tissue angiogenesis (**Figure 2**). In quiescent endothelia, the rate of proliferation and migration is minimal (endothelial cells can, however, activate these mechanisms very quickly if required),[11] while in tumors endothelial cells are produced rapidly. The endothelial cells must be able to proliferate, degrade the extracellular matrix, change their adhesive properties, migrate to the new vascular vessels, and finally differentiate and avoid apoptosis.[15] In tumor angiogenesis, three main events must occur for new vessel formation: dissolution of the basement membrane, migration of endothelial cells, and proliferation to provide new cells for vessel growth.[16]

Once the angiogenic switch has been turned on, induced by the production of the vascular endothelial growth factor (VEGF) and other angiogenic molecules,[11,17] the endothelial cells within the vessel start to lose contact with nearby cells due to the action of proteolytic enzymes produced by these cells. Two main enzyme groups are responsible for proteolysis: the matrix metalloproteases (MMPs) and the plasminogen activator (PA)/plasmid system. The basement membrane is the first target of these proteins.[11,18,19] After the endothelial basement membrane has been disrupted, the endothelial cells are able to migrate, while other endothelial cells fill the gap left in the membrane.[11] Once the endothelial cells start moving away from the parent vessel, they begin to form small sprouts, and more endothelial cells are recruited, elongating the new vessels. These endothelial cells also start creating a central lumen that will form the initial structure of the new vessels,[11,20] and rapid proliferation of the endothelial cells begins to occur near to the sprout tips. The sprouts initially grow and expand parallel to one another, until a certain point at which they turn towards other sprouts, leading to the formation of closed loops necessary for blood circulation.[11]

In contrast to physiological angiogenesis (where the expression of angiogenic factors, migration and proliferation of endothelial cells, and proteolysis of the base membrane stops after the target tissue has been vascularized), in tumor angiogenesis the angiogenic switch remains turned on due to the continued production of angiogenic factors within the hypoxic regions of the tumor. New capillaries continue to grow through the tumor, leading to a non-mature, irregular, leaky and tortuous vasculature that lacks a stable basement membrane.[11]

An important characteristic of tumors is their pH. Whereas the intracellular pH of tumors and normal tissue is similar (ca. 7.2),[21,22] the extracellular pH for tumors can be more acidic (varying from 5.8 to 7.8, with an average of 7) compared to that of extracellular fluid/blood in normal tissue (7.4). This results from an accumulation of lactic acid and carbonic acid, produced by aerobic/anaerobic glycolysis, glutaminolysis, and ATP hydrolysis inside the cells. As the tumor has a high metabolic rate and poor blood convective and/or diffusive drainage, H^+ ions accumulate in the respective tissue.[23–25]

2.2. The Enhanced Permeability and Retention (EPR) Effect

The abnormalities present in the tumor tissue result in extensive leakage of blood plasma components and other macromolecules.[26,27] The greater permeability of tumor vessels to macromolecules compared with normal vessels, and the impaired clearance of these macromolecules from the interstitial space of the tumor (contributing to longer retention of these molecules), is called *enhanced permeability and retention (EPR) effect* (**Figure 3**).[26–30] A consequence of the EPR effect is that macromolecules can accumulate in the tumor at concentrations five to ten times higher than in normal tissue within 1–2 days.[26,28]

The EPR effect can be exploited to achieve passive targeting of tumors for imaging and/or drug release by using macromolecules and nanoparticles (NPs) instead of conventional small-molecule drugs, which, unless they are bound to tumor specific molecules, can also interact with and affect normal tissue.[10,31] In order to effectively exploit the EPR effect for both detection and treatment of cancer, a drug should have a molecular weight greater than 40 kDa.[31] Pore cut-off sizes for several tumor models range between 380 and 780 nm.[10,32] The ability of macromolecules and NPs to accumulate specifically in tumor tissue has been studied extensively since Maeda et al.[33] first described the EPR effect twenty years ago. Nowadays, most drug delivery systems for cancer are being designed based on this principle.

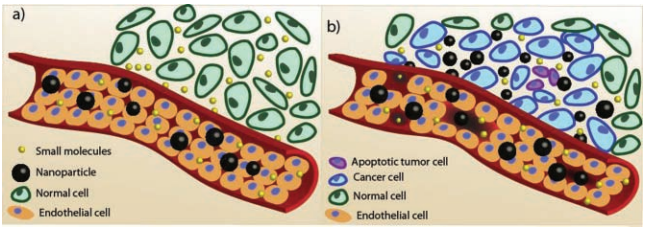

Figure 3. The enhanced permeability and retention (EPR) effect. In normal tissue, only small molecules can diffuse through blood vessel walls (a), whilst in tumors, macromolecules and NPs can diffuse inside the tissue due to the leaky vasculature (b); NPs thus reside longer inside the tumor blood vessels, allowing specific targeting of the tumors. This is the principle used in passive targeting of tumors.

3. Important Characteristics of Nanomaterials Used for Cancer Imaging and Therapy

Although the EPR effect is the major effect that is exploited for the diagnosis and treatment of cancer using NMs, other properties apart from size also influence the biodistribution and clearance of NMs from the body and are thus important considerations in NM design. These include shape, composition, hydrophilic-lipophilic balance, and surface charge of the NMs.[34–36] Most NPs are easily cleared by the reticuloendothelial system (RES) or mononuclear phagocytic system (MPS).[37]

3.1. Nanomaterial Size

The RES is part of the immune system and consists of phagocytic cells like monocytes and macrophages that are located in the spleen, lymph nodes, and Kupffer cells in the liver. The main functions of the RES are to remove senescent cells from the bloodstream and to produce phagocytic cells for immune and inflammatory responses.[38] In particular, larger NMs are easily cleared by the RES (**Figure 4**);[39–41] the smaller the nanoobjects, the longer their retention time in the bloodstream.[40] NPs smaller than 100 nm in diameter have been suggested to be ideal for cancer therapy[42,43] because of their favorable biodistribution and clearance/accumulation behavior.[44] Since the kidney is the first site where NPs can be eliminated, a lower-size threshold has been established based on the glomerular filtration of particles; particles smaller than 6 nm will be filtered and cleared from the blood, while particles bigger than 8 nm will not.[45] Also, as the normal endothelium has an average effective pore size of ca. 5 nm, particles smaller than this will rapidly extravasate across the endothelium.[45] The renal excretion cut-off size has been established to be around 5.5 nm.[41–46]

The mechanism used by the body to recognize and clear NPs involves three steps: opsonization, phagocytosis and clearance.[47] In opsonization, a foreign organism or particle is enveloped by proteins called *opsonins,* which allows recognition of the particles by phagocytic cells and subsequent clearance from the bloodstream.[47,48] Recognition and attachment of phagocytes to the surface-bound opsonins can occur in one of three different ways: i) a change of the opsonins from an inactivated state to an activated state (by conformational changes) that can be recognized by phagocytes using specific receptors on their surface; ii) non-specific association of phagocytes with the opsonins on the particle surface; iii) activation of the complement system.[47,49] After recognition, the phagocytes ingest the particle by endocytocis and degrade it by producing enzymes and other oxidative-reactive chemical factors. If not biodegradable, depending on its size and molecular weight, the particle will be removed by the renal system or stored in one of the RES organs.[47]

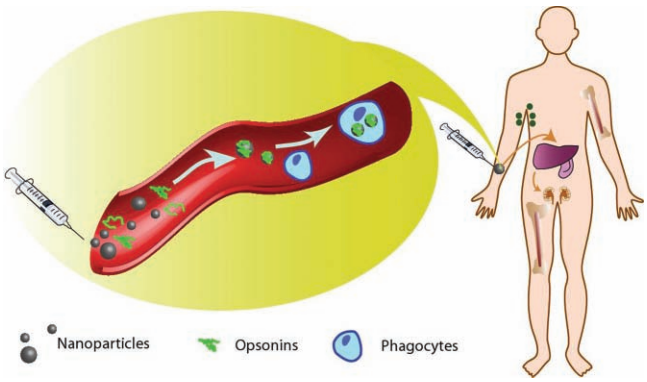

Figure 4. Clearance of NPs by the reticuloendothelial system (RES). When larger NPs are injected into the blood stream, a group of proteins called opsonins cover their surface (opsonization), enabling phagocytic cells in the blood stream to recognize the NPs and remove them by degradation, renal excretion or accumulation in one of the RES organs (liver, spleen, etc.).

3.2. Hydrophobicity/Hydrophilicity and Surface Charge

Opsonization is influenced by the hydrophilic–lipophilic balance of nanomaterials. This process is faster when the particles are more hydrophobic due to the enhanced "adsorbability" of blood serum proteins onto their surface.[34,47,50,51] The NP surface charge can also influence its uptake by the RES, uptake being favored for NPs bearing low or neutral charge.[37,40,41,47] Positively charged particles can cause nonspecific sticking to cells, while negatively charged particles can be taken up by scavenger endothelial cells in the liver.[37,44] NPs with a zeta potential higher than (+/−) 30 mV have been shown to be stable in suspension, as their surface charge prevents aggregation.[52]

3.3. PEGylation of Nanoparticles to Avoid Clearance by the RES

In order to reduce the electrostatic and hydrophobic interactions between NPs (agglomeration) and clearance by opsonization, shielding groups have been introduced onto their surface. PEGylation is one preferred method for avoiding premature clearance of NPs by the RES.[37,47,53,54] PEGylation refers to the process of covering the NPs with polyethylene glycol (PEG) or its derivatives by grafting, entrapping, adsorbing or covalently binding these molecules to the NP surface.[47,55,56] For example, Thierry et al.[57] were able to stabilize silica NPs (126 nm diameter) by covering the surface with a polyethyleneimine-polyethyleneglycol (PEI-PEG) copolymer (PEG M_w = 3400 or 5000) via electrostatic interactions with the silica surface without significantly affecting the particle size (PEI-PEG 3400, 134 nm; PEI-PEG 5000, 139 nm). The resulting NPs were resistant to nonspecific adsorption of proteins onto their surface. Prencipe et al.[58] prepared different polymers based on poly(γ-glutamic acid) (PGA) and poly(maleic anhydride-*alt*-1-octadecene) ($PMHC_{18}$), with PEG grafted for stabilization of gold NPs and gold nanorods. The NP suspensions were stable over a wide pH range, at high temperatures, and when mixed with serum. The most remarkable result from this study was the long circulation times found for the nanorods grafted with PEG polymers ($t_{1/2} \approx 22$ h for PGA-PEG-nanorods, and 19 h for $PMHC_{18}$-PEG-nanorods).

Covalent linking of PEG to the NPs avoids desorption of the PEG chains and biodegradation of the NPs.[10,47] Zhang et al.[59–61] covalently attached PEG to iron oxide NPs by conjugating PEG-dicarboxylate ($M_w \approx 600$) with triethoxypropylaminosilane (APTES). Coating of the NPs was achieved by hydrolysis (ligand exchange) of the silane-modified PEG molecules over their surface. In the initial study,[59] the NPs were coated

with PEG, followed by conjugating folic acid to the ends of the PEG chains. In a subsequent study,[60] NPs were modified with PEG-APTES conjugated to chlorotoxin, a glioma tumor targeting molecule, and the fluorescent molecule, Cy5.5. The preferential uptake of the modified NPs by glioma cells was probed by confocal fluorescence microscopy, which showed a significantly higher degree of internalization of the NPs containing both chlorotoxin and Cy5.5 compared to those with only Cy5.5. Zhang et al.[61] also demonstrated that PEGylation increases the uptake of NPs by breast cancer cells, probably due to the permeability of PEG in the cellular membrane.

Covering the surface of NPs with PEG prevents their clearance from the body as opsonins are not able to efficiently cover their surface, thereby hindering phagocytosis.[47] One explanation for this observation is based on the conformation of the PEG molecules on the NP surface. In solution, PEG chains adopt extended conformations due to their flexibility and hydrophilicity. They become compressed when an opsonin is attracted to the surface by Van der Waals forces (or other forces). This increases the energy state of PEG molecules, creating a force that repels the opsonin away from the surface.[47] PEGylation may also reduce RES clearance by shielding the surface charge of NPs and increasing their hydrophilicity (**Figure 5**).[53]

The optimal chain length of surface-bound PEG molecules necessary to avoid RES clearance has been proposed to correspond to PEG with $M_w \geq 2000$.[47] Recently, Choi et al.[62] studied the biodistribution of near-infrared (NIR) fluorescent InAs (ZnS) QDs (3.2 nm) as a function of the length of the PEG used for coating. QDs were coated with dihydrolipoic acid (DHLA) conjugated to PEG chains of different lengths ($n = 2, 3, 4, 8, 14, 22$). The PEGylated QDs were stable in rat serum, while QDs coated only with DHLA were enveloped by serum proteins and increased in hydrodynamic diameter. For $n = 2$, the NPs accumulated in the liver, whereas NPs with $n = 3$ accumulated in the kidneys and bladder. The NPs with $n = 8$ (and to a lesser extent $n = 14$) tended to accumulate in the pancreas, while those with $n = 22$ remained in the bloodstream for at least four hours post-injection. Kaminskas et al.[63] synthesized poly L-lysine dendrimers (generation 3 and 4) surrounded by PEG molecules with different lengths (M_w = 200, 570 and 2000, with n = 3, 10, or 42 ethylene glycol units, respectively), and their biodistribution was studied after labeling with tritium (^{3}H). PEGylation significantly reduced plasma clearance of the dendrimers and the plasma half-life was found to increase in all the cases. Rapid elimination of the smallest dendrimers (PEG_{200}) into the urine was found, but low uptake by the RES was also found, whereas the biggest dendrimers ($Lys_{16}(PEG_{2000})_{32}$) showed no uptake by the RES and very little renal clearance. The effect of PEG length on the biodistribution of coated QDs was also assessed by fluorescence spectroscopy in a study by Daou et al.[64] Notably, the QDs coated with PEGs of lower molecular weight (2750 and 4000 Da) accumulated in the liver faster than those coated with higher M_W PEGs (12000 to 22000 Da), which circulated for longer periods in the bloodstream.

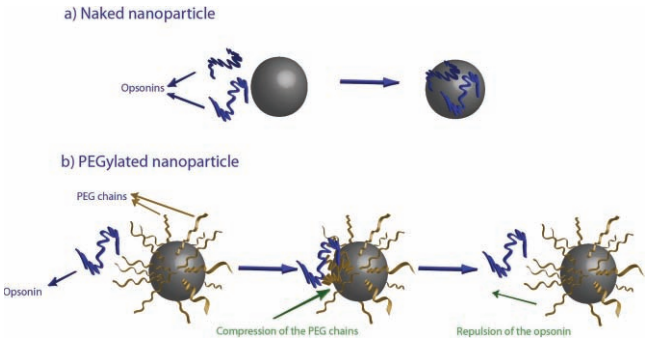

Figure 5. Representation of how PEGylation avoids clearance of NPs by the RES.

3.4. Passive and Active Targeting of Tumors

NPs and other delivery systems can be classified as first, second (passive) or third (active) generation, depending on their ability to locate and deliver a drug to the site/target of interest in the body.[39,65] First generation systems deliver substances when applied near the site of action.[65] Second generation NPs can locate the site of interest and/or deliver the drug by virtue of specific (intrinsic) properties of the carrier and/or those of the associated drug.[40,49,65] As previously noted, passive targeting can be achieved by taking advantage of the EPR effect, which allows NPs to access tumors by way of their leaky vasculature.[43,66,67] Another passive targeting method exploits the tumor microenvironment (pH and redox potential);[66] here, pH- or redox-responsive pro-drugs are converted into an active drug when they reach the acidic, hypoxic microenvironment of the tumor target. Direct local application into the tumor can avoid widespread circulation of the pro-drug in the body.[66] Third generation NPs used for active targeting are capable of specifically recognizing their target. The delivery system is equipped with a "homing device" capable of guiding the carrier to the intended target. These NMs exploit specific properties of the tumor cells, such as the presence of unique or overexpressed receptors on their surface. Small molecules, peptides, proteins, aptamers, and antibodies that interact with receptors are commonly attached to NPs for active tumor targeting.[68] NP internalization can then occur by receptor-mediated endocytosis, after which they may remain intact or be degraded (**Figure 6**). In this way, anti-cancer drugs and other substances can be delivered into cells via incorporation into NPs. The main targets for active tumor targeting with NPs are listed in **Table 1**.[69–72]

4. Types of Nanomaterials Currently Applied in Cancer Imaging and/or Drug Delivery

A wide range of NPs has been designed to reach tumors, including liposomes, QDs, polymers/dendrimers, metals and metal oxides (**Figure 7**).[42,67,73–76] (For a recent comprehensive review on inorganic NPs as materials for cancer therapy, the reader is referred to Minelli et al.)[77] Although all of these types of NPs have been studied for cancer diagnosis and tumor drug delivery and release, each type of NP can exhibit different and sometimes unique properties, which make them useful for different applications.

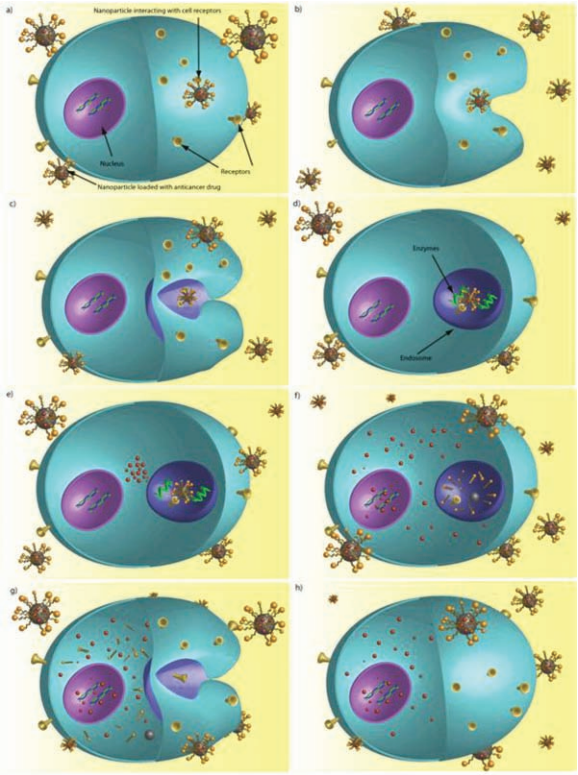

Figure 6. Schematic sequence showing the receptor-mediated endocytosis mechanism for uptake of targeted NPs by cancer cells. a) NPs flowing around the cell interact with cell receptors; b), c), d) the particle is internalized by formation of an endosome; e) the drug is released, triggered by enzymes or the lower pH inside the endosome; f) the NP is degraded while the drug distributes in the cell; g) and h) the cell receptor is recycled.

Table 1. Common targets in tumors for active targeting with NPs.[69–72]

Angiogenesis-associated targets	Cell proliferation markers
Vascular endothelial growth factor (VEGF) receptors[69,71]	Human epidermal growth receptors (HER):[69,72] TGF-α, EGF-R
$\alpha_v\beta_3$-Integrin cell adhesion receptors[69–70,71]	Transferrin receptors[69,72]
Vascular cell adhesion molecule 1 (VCAM-1)[69,71]	Folate receptors[69,72]
Matrix metalloproteinases[69]	Lectin receptors[72]

4.1. Polymeric Nanoparticles

4.1.1. General Characteristics and Synthetic Aspects

Natural polymers such as chitosan, albumin, heparin, dextran, gelatin, alginate, and collagen as well as synthetic polymers, such as PEG, polyglutamic acid (PGA), polylactic acid (PLA), polycarprolactone (PCL), poly-d,l-lactide-co-glycolide (PLGA) and *N*-(2-hydroxypropyl)-methacrylamide copolymer (HPMA) have been widely used to prepare NPs and encapsulate drugs for cancer therapy.[67,78–80] In many cases the polymeric NPs are comprised of a hydrophobic core containing the anticancer agent and a hydrophilic surface layer for the stabilization of the NPs in aqueous environment.[79]

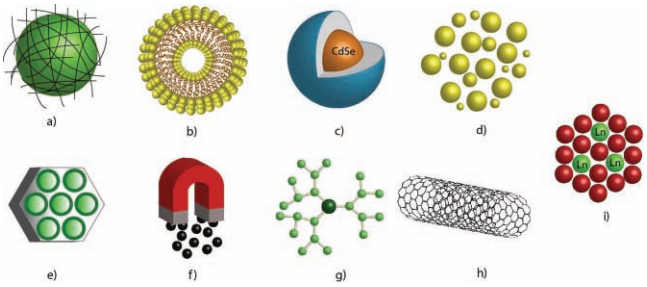

Figure 7. Examples of NPs used for cancer treatment and diagnosis. a) polymeric nanoparticles, b) liposomes, c) QDs, which usually posses a CdSe core, d) gold NPs, e) zeolite L, f) magnetic NPs, traditionally iron oxide, g) dendrimers, h) nanotubes, i) upconverting nanophosphors.

Based on their structural features, polymeric NPs can be further classified into nanocapsules and nanospheres (**Figure 8**).[81] In nanocapsules the shells are usually filled with an aqueous or oil solution, which can contain a solubilized drug. Nanospheres consist of a solid mass, which may be impregnated with an anticancer agent.[78,80–83] Other classes of polymeric NPs include polymeric micelles, which consist of amphiphilic block copolymers that self-assemble into micelles in aqueous solutions,[67,80] and dendrimers, which will be treated later in this review.

Polymeric NPs can be synthesized either by polymerization of monomer units or from a preformed polymer.[84] Monomer polymerization may be achieved using emulsion (aqueous or organic) or interfacial methods.[84] In the former, an emulsion of the polymer is formed and the polymer then isolated by precipitation or gelation.[81] Interfacial polymerization occurs at the interface of two non-miscible phases, with different monomers dissolved in each phase.[81] One-step procedures, based on the spontaneous formation of the polymer or self-assembly of the macromolecules, are used to form nanogels or polyelectrolyte complexes.[81]

4.1.2. Polymeric Nanoparticles for Drug Delivery

Drugs can be loaded into polymeric NPs by physical entrapment or chemical conjugation.[79] In nanocapsules, the drug can be physically loaded in the interior if soluble in the liquid phase contained in the nanocapsule, or via conjugation to the polymer chains. In nanospheres, the drug can also be dispersed or covalently bound to the polymer matrix, while in micelles, hydrophobic drugs are generally encapsulated in their hydrophobic interior. Four types of covalent drug–polymer conjugates have been described for potential combination therapies (two or more drugs administered simultaneously or use of a combination of two types of therapy): i) a polymer–drug conjugate plus non-conjugated free drug; ii) a polymer–drug conjugate plus a second polymer–drug conjugate; iii) a single polymeric carrier of a combination of drugs; and

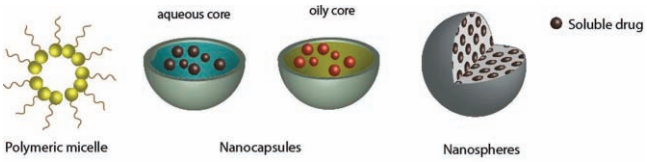

Figure 8. Main types of polymeric NPs.

iv) polymer-directed enzyme prodrug therapy.[85–87] Listings of polymer–drug and polymer–protein conjugates and micelles that are on the market or in clinical phases have been previously published.[79,87–89]

Recently, Grinstaff et al.[90,91] developed expansile polymeric NPs, which release drugs by expanding in response to a change in pH. The hydrophobic crosslinked NPs are based on a monomer containing a 2,4,6-trimethoxybenzaldehyde protecting group, which is stable at neutral pH but can be hydrolyzed at pH 5. The protecting groups are cleaved at low pH (5 in the endosome), causing the NP to change from hydrophobic to hydrophilic in character and to expand from nanometer to micrometer size. The NPs were loaded with paclitaxel, a non-water soluble anticancer drug. Testing against Lewis lung carcinoma in vivo showed that only NPs loaded with paclitaxel reduced the incidence of tumor growth and tumor burden.[90]

Zhang et al.[92,93] incorporated a lipidic component into polymeric NPs in order to increase drug retention in the NP. A lipid layer of lecithin was introduced at the interface between the hydrophobic polymeric core (consisting of PLGA) and the NP hydrophilic shell (consisting of PEG), through the use of a lecithin–PEG conjugate. This was found to slow down diffusion of the encapsulated drugs and to reduce water penetration into the core, enhancing the drug-encapsulation yield and slowing down drug release from the NPs by avoiding hydrolysis of the PLGA chains. Two anticancer drugs, paclitaxel and docetaxel, were loaded into the NPs, using A10 RNA and anti-CEA monoclonal antibodies as targeting ligands for the paclitaxel-loaded and docetaxel-loaded NPs, respectively.

The past decade has seen increasing attention focussed on the development of NMs capable of delivering short RNA duplexes, called small interfering RNA (siRNA), into the cytoplasm of tumor cells as a potential means of combating cancer. Once inside a cell, one of the siRNA strands (the guide strand) is recruited by an exogenous multiprotein complex, called the RNA-induced silencing complex (RISC), to initiate the catalytic cleavage of complimentary mRNA sequences. This process, known as *RNA interference* (RNAi), provides a powerful tool for suppressing gene expression and thereby halting the growth of tumor cells. Since siRNAs are highly negatively charged, however, modification with and/or linkage to other molecules or small particles that promote cellular uptake is necessary to affect RNAi.[94,95] A variety of polymeric materials have been explored for this purpose. For example, Davis[96] has reviewed the development of a polymer-based delivery system (CALAA-01), which consists of a cyclodextrin-containing polymer (CDP), PEG, and human transferrin (Tf) as a targeting agent. This is the first ever NP-based siRNA delivery system to enter a phase I clinical trial. Katas et al.[97] designed a delivery system for siRNA based on chitosan polysaccharide NPs and studied the association of siRNA to chitosan using methods such as simple complexation, ionic gelation (entrapment), and adsorption onto the surface of preformed chitosan NPs. Entrapment of siRNA in the NPs was found to give the best in vitro gene silencing activities, probably because of protection of the siRNA molecules from degradation by nucleases. Self-assembled polymeric NPs and micelles have also been used to encapsulate siRNA and shown to be effective for transporting the siRNA across cellular membranes.[98,99]

4.1.3. Polymeric Nanoparticles for Imaging

Polymeric NPs have been loaded with gadolinium complexes or magnetic NPs in order to image cancer by MRI. Traditionally magnetic NPs (magnetite) have been encapsulated in the core of polymeric micelles. For example, Gao et al.[100,101] encapsulated magnetic NPs together with doxorubicin in micelles formed from amphiphilic block copolymers of maleimide-terminated poly(ethyleneglycol)-block-poly(D,L-lactide) and methoxy-terminated poly-(ethylene glycol)-block-poly(D,L-lactide) copolymer. These micelles were functionalized with agents such as cRGD or a lung-cancer targeting peptide (LCP) for active targeting. Sohn et al.[102] recently designed magneto-fluorescent polymeric NPs based on glycol chitosan conjugated to *N*-acetyl histidine and bombesin, for targeting GRPRs overexpressed in prostate cancer cells. Magnetic NPs coated with oleic acid were incorporated into the polymeric matrix, and the NPs labeled with the near infrared fluorophore, Cy5.5.

Gadolinium, a positive magnetic resonance imaging (MRI) agent, was recently incorporated into polymeric NPs as Gd metal organic frameworks (MOFs), constructed from Gd^{3+} ions and organic bridging ligands, such as 1,4-benzenedicarboxylic acid. This material offers exceptional MRI capabilities over traditional methods for incorporating gadolinium into NPs using Gd_2O_3, $GdPO_4$, GdF_3, etc. The surface of MOFs is modified by covalent attachment of polymer chains to obtain the polymeric carriers.[103,104]

4.2. Liposomes

4.2.1. General Characteristics and Synthetic Aspects

Liposomes are spherical vesicles that consist of one or more phospholipid bilayers encapsulating water in their interior.[39,66] The phospholipids are arranged so as to form a closed sphere, shielding their hydrophobic tails from the water, thus leaving water in the liposome interior. Drugs can be encapsulated within the liposomes, not only in the aqueous volume but also within the bilayer, which allows drugs of different hydrophilicities to be carried (**Figure 9**).[39,105,106] Three types of liposomes have been described: i) multilamellar vesicles (MLVs) composed of a number of concentric bilayers; ii) multivesicular vesicles (MVVs) consisting of many small, non-concentric vesicles inside one lipid bilayer; and iii) small and large unilamellar vesicles (ULVs), which posses only a single lipid bilayer.[107–109]

Traditionally, liposomes have been synthesized by hydration of a thin film of a phospholipid mixture with a solution containing the desired drug and/or a chelating ligand for radiolabeling applications. The chelating ligand can also be incorporated into the phospholipid mixture that will assemble into the liposome membrane. After vortexing, the mixture is extruded under high pressure or sonicated, forming the unilamellar liposomes that can be purified by either column chromatography or ultracentrifugation.[110] Other methods for synthesis of liposomes include the Mozafari method, which avoids the use of organic solvents and hazardous substances. This method involves hydration of the liposome components in an aqueous media, followed by heating of the mixture in the presence of glycerol.[107,111]

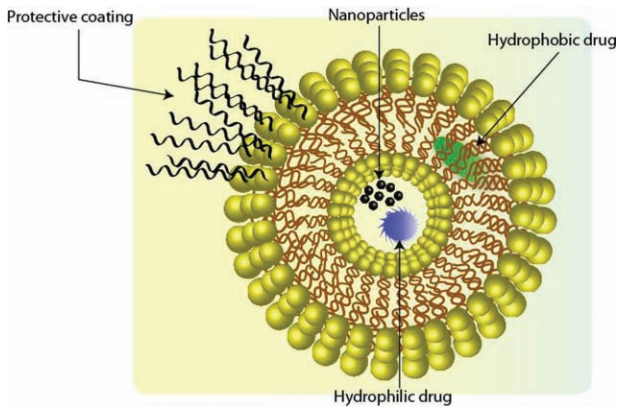

Figure 9. Structure of a carrier liposome. Hydrophilic drugs and NPs can be loaded in the liposome interior, while hydrophobic drugs can be loaded between the hydrocarbon tails of the phospholipids.

4.2.2. Liposomes for Drug Delivery

Liposomes are widely applied as delivery systems due to their ability to pass through cell membranes and lipid bilayers, their ease of preparation, biodegradability, and generally low toxicity.[110,112,113] Methods for loading drugs into the liposome include formation of the liposome in a saturated solution of the drug, use of organic solvents and solvent exchange mechanisms for lipophilic drugs, and the use of pH gradient methods.[114] Formulations produced by incorporating anticancer drugs such as doxorubicin into liposomes are in wide use.[115,116] Doxorubicin-loaded liposomes target tumors through passive accumulation based on the EPR effect. Attempts to increase their specificity have involved attaching targeting agents such as folate derivatives, the RGD-peptide and anti-HER2 monoclonal antibody fragments.[117] Other drugs loaded into liposomes include daunorubicin (Leukemia treatment), lurtotecan (ovarian cancer), annamycin (doxorubicin-resitant tumors), platinum compounds such as, and genes such as the *E1A* gene.[39,118–121] Due to their size and surface properties, traditional liposomes are usually cleared easily by the RES or accumulate in the liver and spleen.[122] A new range of stealth liposomes, e.g., liposomes coated with PEG, avoid RES clearance and have prolonged circulation times compared to traditional liposomes.[123,124]

Compounds other than anticancer drugs have been encapsulated in liposomes.[125] Recently, liposomes containing gadolinium in the lipid layer were developed for bimodal imaging (labeling and imaging of HeLa cells by magnetic resonance and fluorescence imaging).[126] A Gd-DOTA complex was conjugated to *N,N*-distearylamidomethylamine, which forms the lipidic layer of the liposome, and a fluorescent dye. Conjugation of the gadolinium complex to the liposome avoided leakage of the metal complex before the site of action was reached. Iron oxide nanoparticles have also been incorporated into liposomes (magneto-liposomes), to prevent the interaction of iron oxide with biological media and leakage into the blood stream.[127–129] Magneto-liposomes can be used for magnetically induced targeting of specific cells or organs, controlled release of certain drugs, and in cancer treatment by hyperthermia.[130,131]

Drug release from liposome cavities can be achieved by changes in pH,[132,133] mechanical stress,[134,135] light,[136,137] temperature,[138,139] redox reactions,[140] and the action of enzymes on the liposome surface.[141,142] For example, Mallik et al.[143–145] studied the cleavage and release of the contents of liposome formulations by the matrix metalloproteinase, MMP-9, which is found in high levels in metastatic tumors. Liposomes incorporating a cleavage site for MMP-9 were prepared and shown to release their contents after exposure to MMP-9. Wu et al.[146] have developed liposomes that are sensitive to NIR light. The liposomes incorporate hollow gold NPs (HGNs) as NIR absorbing entities, which are either encapsulated or tethered to the liposome membrane. The liposomes were shown to release their contents within seconds when exposed to pulses of NIR light due to collapse of transient vapor bubbles formed during collapse of the HGNs into gold NPs. While the gold NPs reached their melting temperature, the temperature increase observed in the surroundings was less than 1 °C.

4.2.3. Liposomes for Radio-Imaging and Therapy

Liposomes have also been studied as radionuclide carriers for tumor imaging and radiotherapy.[147–149] For imaging, labeling with ^{67}Ga, ^{111}In, ^{99m}Tc, and ^{64}Cu(II) has usually been achieved by conjugation of the radionuclide with an anchor molecule present inside the cavity of the liposome or incorporated in the phospholipid bilayer.[122,123,150] For example, ^{64}Cu(II) has traditionally been loaded into liposomes by complexation with azamacrocycles, such as 1,4,8,11-tetraazacyclotetradecane-*N,N′,N″,N‴*-tetraacetic acid (TETA), which confers stability and avoids leakage of the radionuclide.[151,152] For radiotherapy, liposomes labeled with β-emitters, such as ^{32}P, ^{90}Y, ^{188}Re, ^{67}Cu, ^{131}I, α-emitters, such as ^{223}Ra, ^{224}Ra and ^{225}Ac, and Auger electron emitters can be utilized.[122] Nuclisomes, which are tumor-targeting liposomes containing radionuclides coupled to DNA-intercalating molecules, are novel nanoobjects developed in the last few years.[153,154] Fondell et al.[155] loaded liposomes with a daunorubicin derivative labeled with ^{125}I, an Auger electron emitter. The liposomes target the epidermal growth factor receptor (EGFR) expressed in cultured U-343MGaCl2:6 tumor cells. On internalization of the liposomes into cells, their contents are released and the radionuclide-containing drug accumulates in the cell nucleus, where it binds to DNA and causes double-strand breakage, leading to substantial inhibition of tumor cell growth.

4.3. Dendrimers

4.3.1. General Characteristics and Synthetic Aspects

Dendrimers are a class of polymeric macromolecules that consist of repeating branching units emanating from a central core.[156–161] The unique properties of these almost monodisperse polymers reflect their compact, treelike molecular structure, providing an arrangement of inner and outer molecular functionalities that is influenced by the solvent environment.[162] Dendrimers can be considered to comprise three structural components: i) the core, which in larger dendrimers is almost completely shielded from the outside by the dendritic branch, ii) the outer shell, which possesses a well-defined microenvironment and is protected by (iii) the multivalent surface, which usually bears a high number of reactive sites.

Dendrimers are generally prepared via one of two main strategies. The first – *divergent synthesis* – begins from a multivalent core unit and involves the attachment of successive "layers" of branching units, each new layer producing a new *generation* of dendrimer. The second – *convergent synthesis* – involves preassembly of the complete wedge-shaped branching units, followed by coupling of these to the central core moiety in the final step. Dendrimers have also been synthesized applying novel concepts such as the dual-convergent-divergent method, "lego" chemistry, divergent click chemistry, solid-phase supported pathways, and self-assembly of Janus dendrimers into uniform dendrimersomes.[163]

Depending on the generation, the type of branching unit, and the moieties grafted onto their periphery, dendrimers can be prepared with sizes ranging between 1 to 10 nm. Lower generation dendrimers have a flat starfishlike shape. As the generation number increases, dendrimers become more spherical in shape. Importantly, in a physiological environment, higher generation dendrimers are stabilized as compact balls. Sixth generation polyamidoamine (PAMAM) dendrimers resemble proteins in size and shape.[156–163]

Due to the many variations possible in the basic framework and the peripheral substituents, dendrimers can be tailor-made for numerous applications, which include diagnosis and therapeutic agents.[164–171] Thus, the development of new, high-resolution contrast agents, synthetic vaccines, and novel drug transport systems based on dendritic structures are in progress. Moreover, dendrimers are intriguing as enzyme and liposome models with regard to the development of synthetic cells in molecular and cell biology.

4.3.2. Dendrimers for Drug Delivery

One very intriguing application of dendrimers is the encapsulation and controlled release of drugs. This principle was first demonstrated by Meijer and co-workers using the so-called *dendritic box*, which allows for shape-selective liberation of encapsulated guests.[172,173] Drugs are usually loaded into dendrimer structures by noncovalent interactions (of which ionic interactions seem most appropriate for medical applications) or by chemical conjugation to functional groups located on the surface or at focal points of the dendritic carriers.[174–176] In addition to influencing the rate of drug release, modification of the dendritic surface also allows the bio-distribution and pharmacokinetics of drug-loaded dendrimers to be tailored for specific purposes. PEGylation of dendrimers is often utilized for pharmaceutical applications.[174,177–179] Using this approach, anticancer drugs, such as camptothecin, doxorubicin, dimethoxycurcumin, etoposide, 5-fluorouracil, and paclitaxel, have been transported by PEGylated dendrimers into cancerous tissue. Another promising strategy is the encapsulation of drug molecules into dendritic sugar-containing biohybrids.[180–182] Recently, we showed that sugar-capped dendrimers can encapsulate and slowly release nanosized cluster molecules that selectively inhibit an enzyme involved in cancerogenisis.[183]

Drugs can be attached to dendrimer surfaces through hydrolyzable (amide or ester bonds) or biodegradable linkages, which offers the opportunity to release the drug after reaching the site of action by means of enzymatic action, pH differences, etc.[184–187] 5-Fluorouracil, cisplatin, carboplatin, methotrexate, doxorubicin, epirubicin and paclitaxel have all been covalently attached to dendrimers using appropriate linkers. The therapeutic index of such "dendritic drugs" can be significantly higher than the drug alone. For active targeting, folate has been the most applied vector for the accumulation of dendrimer–anticancer-drug conjugates in tumor tissue.[168,188] Dendrimers functionalized with a variety of tumor targeting peptides, such as RGD and neurotensin, are also gaining in importance due to metabolic stabilization of the peptides as well as enhanced affinity to cancer cells.[189–191]

4.3.3. Dendrimers for Imaging

Dendrimers have been widely studied as MRI contrast agents. Dendritic structures incorporating many paramagnetic centers are ideally suited for medical use due to their appropriate pharmacokinetic properties in combination with shortening of proton relaxation times and signal intensification. In the main, PAMAM dendrimers have been functionalized with Gd^{3+} complexes of diethylenetriamine pentaacetic acid (DTPA) and 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) derivatives.[192] Variation of the chelating agent does not appear to affect the biodistribution of the dendritic Gd^{3+} complexes.[193] Such dendritic contrast agents have been used to visualize tumor tissue and sentinel lymph nodes with high resolution and to gain information about the state of angenionesis.[194–196] A multigadolinium complex (24 Gd-DTPA units, Gadomer17, Schering) consisting of a polysine dendrimer with trimesic acid as the core element shows very fast and complete renal elimination, allowing the visualization of peripheral arterial deseases.[197]

Dendrimers provide an ideal platform to realize agents with dual/multimodality imaging capability combined with active targeting (**Figure 10**).[192,198] Pioneering work by Wiener et al.[199] resulted in the development of folate-conjugated PAMAM dendrimers capable of both MRI and fluorescence imaging. Boswell et al.[189] designed a PAMAM dendrimer containing RGD peptides for interaction with the integrin receptors of $\alpha_v\beta_3$-expressing tumor cells, which utilized Alexa Fluor 594 dye molecules for fluorescence imaging and Gd^{3+} complexes for MRI. A multimodal nanoprobe for radionuclide and five-color NIR optical lymphatic imaging based on a generation 6 PAMAM dendrimer has been developed to permit dual-modality scintigraphic (^{111}In) and optical imaging in mice.[200] Despite the fact that SPECT and PET provide convenient and broad information about biodistribution, pharmacokinetics, and functional imaging, relatively few examples of radiolabeled dendrimers have been reported so far. In this regard, ^{64}Cu, ^{67}Cu, ^{76}Br, ^{99m}Tc and ^{111}In have been used as radiolabels for dendritic structures.[190,200–205]

4.3.4. Dendrimers for Therapy

In the early-1990s, the potential of dendrimers to transport DNA building blocks into cells was recognized by Szoka and Kukowska-Latallo *et al.*, triggering a fast-paced development of gene transfection agents.[206–209] The combination of artificial non-viral dendritic carriers as pharmacologically active transfection agents

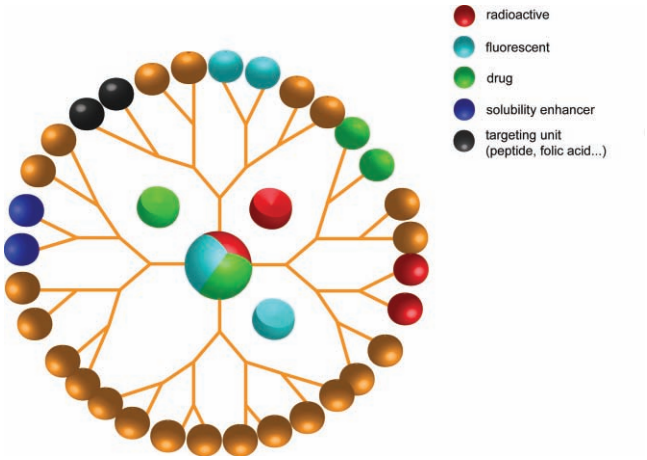

Figure 10. Multifunctional dendritic carrier capable of acting as both an imaging and therapeutic agent.

and appropriate transcriptionally targeted antitumor genes may create an efficacious gene medicine for the systemic treatment of tumors and their metastases. Dendrimers with a positively charged skeleton, e.g., polyamidoamino (POPAM), polypropylene amine (POPAM), and polylysine dendrimers, are predestined to form stable complexes with the negatively charged backbone of DNA, affording nanosized toroidal DNA/dendrimer associates (dendriplex, 50–200 nm) capable of penetrating into cells, and to reach the cell nucleus.[210,211] Both the structure and generation of dendrimers influence transfection efficiency. Thus, high-generation PAMAM dendrimers show a significant enhancement in activity.[212] The same holds true when PEG units or cyclodextrin are grafted on the surface of dendrimers or when terminal amino groups are replaced by arginine, ornithine, or spermine.[213,214] Novel strategies, e.g., phototriggered gene transfection, developing dendritic agents with vectors showing natural anti-cancer properties, and manufacturing inorganic-organic hybrids consisting of magnetic NPs decorated with PAMAM dendrons, are being evaluated to improve the effectiveness of gene medicine.[215–217]

Dendrimers have been used for boron neutron capture therapy (BNCT), unveiling the potential to non-invasively treat incurable forms of cancer. BNCT is based on the delivery of a high amount of the ^{10}B isotope to tumor cells, which, when exposed to thermal neutrons, emit short-range radiation capable of killing cells.[218,219] Initial studies showed that up to 120 boron atoms can be attached per dendrimer,[220,221] but pharmaceutical targeting was poor. Meanwhile, many boronated-dendrimer–antibody conjugates have been developed for BNCT which can target tumor vasculature by interacting with EGF/VEGF receptors.[222–225] Such conjugates contain up to 1100 boron atoms, affording enhanced tumor accumulation and improved effectiveness for BNCT, cf. simple boronated dendrimers. NIR imaging using Cy5 dye molecules attached to boronated-VEGF–dendrimer conjugates revealed internalization by receptor-mediated endocytosis and localization in regions of active angiogenesis.

Dendrimers are now being widely studied for photodynamic therapy (PDT). PDT is based on the in situ formation of singlet oxygen when a photosensitizer is irradiated with light.[226] The photosensitizer (drug) is ideally non-toxic under non-irradiating conditions, only becoming toxic when exposed to light. Due to their unique topology and the arising possibilities to influence solubility properties, pharmacokinetics and pharmacodynamics, dendrimers may remarkably improve PDT. Induced endogenous formation of protoporphyrin IX (PpIX) by 5-aminolaevulinic acid (ALA) has been one of the most popular forms of PDT. Higher tumor accumulation and enhanced PDT efficiency have been obtained with ALA-containing dendritic carriers in comparison to the free prodrug.[227–229] An immunoconjugate of fullerene-cored dendrimers containing pyropheophorbide as a photosensitizer was found to specifically bind to malignant cells.[230] Both enhanced cellular uptake and PDT efficiency for lung carcinoma cells have been obtained with Zn-porphyrin-cored polybenzylether dendrimers.[231] The photocytotoxicity was dramatically improved when applying supramolecular assemblies of such anionic dendritic porphyrins and cationic PEGylated polylysines.[232] PEGylated PAMAM and POPAM dendrimers are capable of encapsulating protoporpyrin IX and transporting the photosensitizer to the mitochondria of HeLa cells, where singlet oxygen is generated.[233] Enhanced photodynamic cancer treatment has been achieved with dendritic encapsulated phthalocyanines.[234] Other novel methodologies to improve PDT effectiveness[235] include the use of dendritic two-photon-absorbing chromophores to allow for a more efficient generation of singlet oxygen.[236,237]

Photothermal therapy has been recognized to be a very efficient and selective methodology to treat cancer.[237] By exposing tumors to a NIR diode laser, the photothermal effect of gold and silver NPs may be used to selectively destroy tumor tissue with minimum damage to the surrounding healthy tissue. Very recently, dendrimers have been applied as targeting vectors for cancer tissue. PAMAM dendrimers containing folic acid form stable associates with gold NPs and specifically target cancer cells overexpressing folate receptors.[238]

Dendrimer–antibody conjugates labeled with ^{90}Y and ^{212}Bi (emitting beta and alpha particles) have been reported as potential vehicles for use in radioimmunotherapy.[205] However, despite the potential for dendrimers to improve endoradionuclide therapy, e.g., through enhancement of specific activity, selective targeting of tumor cells and improved stability of radionuclides by encapsulation, this area is still very much in its infancy. One reason may be the non-specific accumulation of larger dendritic ensembles in the RES. Smaller dendritic structures and pretargeting approaches can potentially circumvent this problem.[190,240–242]

4.4. Quantum Dots

4.4.1. General Characteristics and Synthetic Aspects

QDs are highly fluorescent semiconductor nanocrystals, which typically range from 1 to 10 nm in size.[243–245] They are usually composed of semiconductor elements from group II–VI, such as CdSe, CdS, and CdTe, group IV–VI, such as PbS, PbSe, PbTe and SnTe, or group III–V, such as InAs and InP.[246] QDs possess broad absorption and narrow emission spectra, and their emission maxima can be tuned between 450 to 850 nm by changing their size (**Figure 11**). They are extremely bright due to their high extinction coefficient in the visible spectrum (ε up to 10^6 $M^{-1}cm^{-1}$; 10–100 times greater than for organic fluorophores)[244,247,248]

Figure 11. Quantum dots' fluorescence emission properties can be modulated by changing their size.

and high quantum yields (typically >50%).[249] They are resistant to photobleaching and chemical degradation, and more stable than conventional organic fluorophores and fluorescent proteins. Additional attractive properties for imaging and PDT of cancer include a high surface area, a large two-photon absorption cross-section, and the possibility of NIR photoluminescence.[250,251] There are concerns about the use of QDs for medical purposes because of the high toxicity of cadmium and selenium and their instability to photolysis and oxidative conditions, which could result in dissolution of the QD core. It should be noted, however, that cytotoxicity has not been observed in several in vivo and in vitro studies of QDs coated with ZnS,[252] and that coating with ZnS and other agents has been shown to reduce the cadmium toxicity and the production of free radicals from photo- and air oxidation.[253] Notwithstanding this, there has been considerable research into the development of cadmium-free QDs, such as $CuInS_2$,[254] as safe and non-toxic probes for biological use.

The traditional synthesis of CdSe and other QDs uses trioctylphosphine oxide (TOPO) and trioctylphosphine (TOP) as coordinating solvents to stabilize the particles and prevent agglomeration. A solution of organometallic precursors is injected into the solvents at temperatures from 290 to 350 °C. The reaction mixture is left for the desired time, cooled, and the QDs are purified.[246] In recent years, efforts have been made to avoid the use of toxic heavy metals, such as cadmium, lead, and mercury in the synthesis of QDs. Research has also been directed towards the use of group III–V semiconductors nano-crystals, which have a more robust structure due to the presence of covalent bonds within their matrix.[255–258] For example, InP and InAs QDs are being developed into diagnostic agents for cancer and other diseases.

In order to protect QDs from degradation and oxidation, a thin shell (also a semiconductor, but with a higher band gap) is formed around the QD core, which, as well as increasing their stability, also enhances their photoluminescence. The quantum yield of the nanocrystal core (typically 10%) can increase to ca. 80% after the high-bandgap-semiconductor shell is epitaxially grown around it.[259] Traditionally, ZnS, and ZnSe have been used as protector shells. The shell is formed at a temperature below that used to prepare the QDs in order to avoid nucleation of the shell particles. The QDs obtained by this method are only soluble in non-polar solvents, making it necessary to further modify their surface to achieve water-solubility. This is achieved by surface ligand exchange, where the coordinating ligands are displaced by bifunctional ligands or silanes, or by coating with amphiphilic polymers.[254,260]

4.4.2. Quantum Dots for Photodynamic Therapy

Although QDs are poor photosensitizers (PS) for PDT, due to a low efficiency of production of reactive oxygen intermediates/species (ROI/ROS), QD-PS conjugates can provide an efficient way to produce ROI. The poor efficiency of QDs stems from the dominance of radiative carrier relaxation over energy transfer to oxygen when the QD is photoactivated. Energy transfer from a QD to a conjugated PS is a more efficient strategy for the production of ROI, as QDs are excellent donors in fluorescence (Förster) resonance energy transfer (FRET). Indirect photoactivation of a PS conjugated to QDs enables prolonged imaging and PDT without photobleaching.[250,261–264] Photosensitizers (e.g., metallo-phthalocyanines, Rose Bengal (RB) and Chlorin e6) and the chemotherapeutic drug, Merocyanine 540 (MC540), have been conjugated with QDs for PDT.[265–268] UV irradiation of green and red CdTe QDs capped with mercaptopropionic acid generated ROS in human pancreatic carcinoma cells (PANC-1), which was attenuated by the antioxidant NAC. Cytotoxicity of these QDs increased with UV irradiation time, QD concentration, and post-exposure time.[269] Yang et al.[270] found a linear relationship between radiation dose rate and number of visible photons generated from QD-Photofrin NPs excited by 6-MV X-rays. FRET from the QD to the PS increased, approaching 100% as the number of conjugated Photofrin molecules was increased.

4.4.3. Quantum Dots for Fluorescence Imaging and Diagnostic Assays

QDs have been widely studied for fluorescent imaging of cancer and even proposed for personalized medicine.[271] For example, Li et al.[272] have developed CdSe QDs coated with a generation 4 PAMAM dendrimer conjugated to an aptamer (GBI-10), for the targeting and imaging of U251 glioblastoma cells in vitro. Simultaneous imaging, therapy and sensing of the release of a conjugated drug can be achieved by activation of the QD's fluorescence. For example, a QD-aptamer(Apt)-doxorubicin (Dox) conjugate [QD-Apt(Dox)] was prepared for specific delivery of doxorubicin to prostate cancer cells.[273] The double-stranded A10 PSMA aptamer intercalates Dox but also recognizes the extracellular domain of the prostate specific membrane antigen (PSMA). When Dox intercalated into the aptamer, quenching of fluorescence from both the QD and Dox was found to occur via a Bi-FRET mechanism. Binding of Dox to the QD (donor–acceptor) diminished QD fluorescence, while the binding of Dox to the aptamer (donor–quencher) decreased Dox fluorescence. Release of Dox from the NPs by physical dissociation from the conjugate, or biodegradation of the PSMA aptamer by enzymes present in lysosomes, induced recovery of fluorescence from both the QD and drug.[273] Aptamers are proving useful in the active targeting of cancer cells as they are comparable to antibodies in terms of their specificity and affinity. Cheng et al.[274] have designed an aptamer-based assay with QD-based fluorescence readout for detection of mucin 1 (MUC1), a cell surface-associated glycoprotein expressed on most epithelial surfaces that serves as a useful biomarker for early cancer diagnosis.

QDs have been used to develop multimodal imaging probes for detection of tumors via fluorescence-MRI, by conjugating a ligand for Gd^{3+} complexation[275,276] or directly doping the QD with Mn^{2+} (as $CdSe/Zn_{1-x}Mn_xS$),[277] or via fluorescence-PET imaging, by conjugation of ligands for complexation of ^{64}Cu[278,279] and other radioactive elements. Manganese (Mn)-doped NIR-QDs were recently used to image pancreatic tumors in mice by fluorescence imaging and MRI.[280] Mn-doped CdTeSe/CdS nanoparticles with a fluorescence emission around 822 nm were

prepared in a one-pot synthesis from manganese acetylacetonate ($Mn(acac)_3$) and were covered with lysine to enhance water solubility. The QDs were further functionalized with the antibodies anti-claudin 4, anti-mesothelin, or anti-PSCA, which are overexpressed in primary and metastatic pancreatic cancers and used for Panc-1 and MiaPaCa pancreatic cancer cell staining and in vivo fluorescence imaging. In addition, QDs radiolabeled with ^{125m}Te (as $Cd^{125m}Te/ZnS$) have been produced and used to asses biodistribution of the QDs with specific targeting agents.[281,282]

Multicolor imaging with QDs is a significant recent development that could aid the diagnosis of cancer and other diseases, as it permits simultaneously tracking of multiple molecular targets.[283,284] The utility of simultaneous detection of different tumor markers was demonstrated using QDs with different emission maxima (525, 565, 605, 655, and 705 nm) conjugated to a variety of primary antibodies. These were employed to detect the markers in MCF-7 and BT-474 breast cancer cells. Multispectral confocal microscopy established the spatial distribution of the different markers in the BT-474 and MCF-7 cells, cell membrane, cytoplasm, or cell nuclei. The markers were also quantified by single-cell spectroscopy and the expression of the markers in human breast cancer specimens was evaluated and quantified.[285]

Quantum rods (QRs) are another type of fluorescent particle, which have been gaining increasing attention, as they exhibit stronger fluorescence, larger Stokes shifts, and larger absorption cross-sections compared to QDs. They produce linearly polarized emission, unlike the plane polarized emission from a single QD, and have large surface areas, which can be useful for conjugation to biomolecules. Furthermore, QR emission can be reversibly switched on and off by externally applied electric fields. Biotin, folic acid, and the cyclic RGD peptide have been conjugated to QRs for tumor targeting and in vitro and in vivo imaging.[286–289]

4.5. Iron Oxide Nanoparticles

4.5.1. General Characteristics and Synthetic Aspects

Iron oxide NPs are the most commonly explored members of a broader class of NPs referred to as magnetic NPs, which have attracted great interest because of their potential use in a broad range of applications, including catalysis, data storage, bioseparations, and MRI. Magnetite (Fe_3O_4, ferrimagnetic, superparamagnetic when the size is smaller than 15 nm) and maghemite (γ-Fe_2O_3, ferrimagnetic) have proven particularly popular for biomedical applications because of their great biocompatibility. Despite this, there are recent reports that naked iron oxide NPs could be toxic for neuronal cells and that they may potentially induce oxidative stress processes in the body.[290,291]

Monodispersed magnetite and maghemite NPs can be synthesized by methods that allow control of size, size distribution, shape, and solubility. These techniques include co-precipitation from aqueous solutions of iron salts and microemulsion as well as hydrothermal and high temperature reactions. Coprecipitation from ferric and ferrous salts in aqueous media is most commonly used to obtain large quantities of magnetite NPs (typical size 2–17 nm).[292,293] Several factors affect the properties of the NPs obtained, such as the Fe^{2+}/Fe^{3+} ratio (ideally between 0.4 and 0.6), the iron concentration (typically 40–80 mM), the solution pH and ionic strength (with smaller NPs being produced at higher pH and ionic strength), the temperature, and whether the reaction is carried out under an inert atmosphere. The main disadvantage of the coprecipitation method is the wide size distribution obtained.[294] Maghemite NPs can be obtained by oxidation of the Fe^{2+} centers in magnetite to Fe^{3+} with nitric acid.[295]

In the microemulsion method, NPs are prepared within micelles (1–10 nm diameter) or water–oil emulsion nanoreactors (10–100 nm), where encapsulation of the iron salt precursors controls the nucleation and growth of the NPs.[292] This method produces NPs with narrow size distributions, however, the removal of the surfactant is difficult and not amenable to large scale synthesis.[295] In the hydrothermal synthesis, water is used as reaction media at high temperature and pressure and is postulated to promote nucleation over particle growth of the NPs.[292,296] Surfactants such as sodium *bis*(2-ethylhexyl)sulfosuccinate (AOT), *n*-decanoic acid or *n*-decylamine are added to improve the shape and size distribution of the NPs.[292,297]

The thermal decomposition of iron pentacarbonyl ($Fe(CO)_5$) and iron acetylacetonate ($Fe(acac)_3$) in high temperature organic solvents and surfactants is another widely used method for producing magnetite NPs. This yields NPs with a narrow size distribution and the surfactant surrounds the NPs, acting as a stabilizing agent. Whilst high-quality NPs are obtained, their large-scale production presents safety issues, mainly due to the toxic and flammable nature of the reactants. The NPs obtained by this method are not dispersible in water and phase transfer into aqueous solution is necessary for biomedical applications.[292,296,298,299] Other synthetic methods include sol–gel, gas phase, polyol, and electrochemical syntheses.[292,300]

The protection, stabilization, and functionalization of iron oxide NPs is important for medical applications. Stabilization in water has been achieved using carboxylates, phosphates, sulphonates, silicon compounds, gold, and polymers sucha s dextran, PEG and polyvinyl alcohol (PVA) (**Figure 12**).[292,295] Citric acid is the most common carboxylate-stabilizing agent used. Upon electrostatic binding to the surface via one carboxyl group, the surface becomes negatively charged, increasing the hydrophilicity of the NPs.[292,301] Silica and siloxane derivatives are also widely used for coating and derivatization of iron oxide NPs. Silica can stabilize NPs by shielding magnetic dipole interactions between NPs, and by introducing a negative surface charge that increases inter-particle electrostatic repulsion,

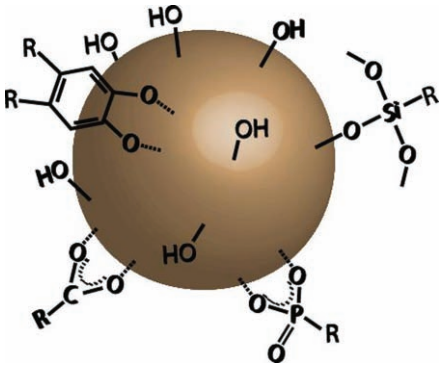

Figure 12. Iron oxide NPs offer a wide range of possibilities for their stabilization and functionalization with different molecules such as silanes, carboxylic acids, catechols, and phosphonates.

avoiding aggregation.[292] Silica can be deposited on the NP surface by deposition from silicic acid or the hydrolysis of tetraethylorthosilicate (TEOS) with ammonia.[292,302] Triethoxysilane derivatives can be attached by hydrolysis on the naked NP surface, or on the silanol-rich silica coating, and subsequently used to functionalize the NPs for biomedical applications.

Different polymeric materials have been used to increase the stability of magnetic NPs in water and to avoid their clearance by the RES. These are typically biocompatible PEG and PEG derivatives, which can be easily functionalized with various biological molecules. Xie et al.[303] recently developed iron oxide NPs with four PEG polymers (PEG_{600}, PEG_{3000}, PEG_{6000}, and PEG_{20000} with sizes ranging from 40 to 90 nm) covalently bound to the surface via the chelating *o*-catechol moiety of dopamine. The NPs coated with PEG_{600} exhibited the greatest macrophage uptake, while uptake of the others was negligible. PEG has also been attached to magnetic NPs via a trichloro-s-triazine unit, yielding particles with an average hydrodynamic diameter of 40 nm, which were stable in buffered saline solutions at pH 7.[304] Other polymeric materials used for particle coating include dextran, starch, and PVA. Dextran consists of R-D-glucopyranosyl units, making it highly compatible with the human body. Dextran-coated NPs are synthesized by coprecipitation of the iron oxide from ferrous and ferric salts in the presence of dextran.[292,305,306] Starch, which consists of a mixture of amylose and amylopectin, and PVA have typically been used to coat NPs by coprecipitation.[307–310]

4.5.2. Iron Oxide Nanoparticles for MRI and Multi-Modal Imaging

Iron oxide NPs have been extensively studied as contrast agents for imaging of tumors by MRI. They generally produce enhanced proton relaxation rates at significantly lower doses than paramagnetic ions (Gd^{3+}) because of their larger magnetic moment, and they provide negative (dark) contrast by enhancing T_2 relaxivity of water protons. Although passive targeting of iron oxide NPs to tumors can be achieved through the EPR effect, a range of molecules have been attached to their surface to improve tumor targeting for MRI (and other) applications, including proteins, antibodies, peptides, and oligosaccharides.[269] The simplest method exploits electrostatic interactions between molecules and the surface of the NPs. Hildebrandt et. al.[311] attached a small peptide, IELLQAR, known to inhibit sialyl Lewis X binding to the E-selectin receptor, to the surface of negatively charged dextran-coated magnetic NPs by appending a positively charged polylysine chain to the end of the peptide. Xie et al.[312] bound the small peptide, c(RGDyK), specific for targeting integrin $\alpha_v\beta_3$-rich tumor cells, to magnetite NPs via a Mannich reaction with 4-methylcatechol bound to the oxide surface. Very small NPs were obtained (diameter ≈ 8.4 nm) that were stable as a water dispersion, and could be used as contrast agents, as they localized in tumors with limited uptake by macrophages. Herceptin, an antibody specific for the HER2/*neu* receptor that is overexpressed in breast cancer cells, has been attached to water-stable magnetite NPs via conjugation to a dimercaptosuccinic acid coating.[313] In an in vivo experiment, these NPs accumulated in an induced tumor within 5 min, reducing the T_2 value by about 20%.

Iron oxide NPs have been popular materials for the preparation of multimodal tumor imaging/therapeutic agents. For diagnosis, radionuclides such as ^{18}F or ^{64}Cu, have been loaded on magnetic NPs by complexation to surface-bound organic ligands (DOTA in the case of $^{64}Cu(II)$[314]). Iridium complexes have been loaded into magnetic NPs for dual-modal luminescent and magnetic resonance imaging, as well as photodynamic therapy. Lai et al.[315] used silane chemistry to coat magnetite NPs with an iridium complex. The complex was reacted with IECTS (silane), mixed with TEOS, and the mixture hydrolyzed over the surface of the NPs. The resulting water-dispersable, multimodal system was used for phosphorescent labeling and to simultaneously induce apoptosis of cancer cells by production of $^1O_2$.

4.5.3. Iron Oxide Nanoparticles for Drug Delivery

Iron oxide NPs can also be used as drug-delivery systems due the vast opportunities that their surface provides for modification and subsequent incorporation of drugs and targeting agents; doxorubicin being a classical example of this. Jain et al.[316] have developed iron oxide NPs that can carry water-insoluble drugs, releasing them in a controlled fashion via interaction with pluronic acid bound to the NP surface. The NPs were coated with oleic acid and then with pluronic acid by mixing solutions of the acids and NPs and stirring overnight. Doxorubicin was loaded onto the NPs by mixing a solution of the drug with the pluronic-NP suspension, resulting in encapsulation of 82% of the drug, which was then available for slow release. Chen et al.[317] covalently bound doxorubicin to iron oxide NPs using silane chemistry. A thin shell of silica was formed around the NPs, followed by a shell of aminopropyltriethoxysilane, which was then reacted with glutaric anhydride, leaving free carboxyl groups on the surface that were used to link doxorubicin molecules by amidation. The loaded doxorubicin could be easily detached by the hydrolytic action of proteases at low pH, releasing the free drug inside the tumor cells only.

Functionalized iron oxide NPs have been also used for simultaneous delivery of siRNA and imaging of tumors. Lee et al.,[318] for example, prepared manganese-doped iron oxide NPs coated with bovine serum albumin and PEG and functionalized with RGD peptide and siRNA labeled with Cy5. They demonstrated that the expression of green fluorescence protein (GFP) was inhibited. Medarova et al.[319] used dextran-coated magnetic NPs, which were modified with Cy5.5 and siRNA for the inhibition of GFP expression. Additionally, this probe was modified with myristoylated polyarginine peptides (MPAP), which serve as membrane translocation modules.

Recently, Agrawal et al.[320] designed *dendriworms* consisting of linear chains of iron oxide NPs (*nanoworms*) surface-coated with generation 4 PAMAM dendrimer molecules for the delivery of siRNA into cells. The dendriworms facilitated the in vitro and in vivo delivery of the siRNA into cells, leading to reduced EGFR expression in glioblastoma (GBM) tumors. Functionalization of the dendrimers with NIR fluorophores enabled the uptake of the dendriworms to be visualized by fluorescence.

4.5.4. Iron Oxide Nanoparticles for Hyperthermia Therapy

Iron oxide NPs have considerable potential for use in hyperthermia treatment of cancer. In this therapy, the heat generated when NPs are exposed to an oscillating magnetic field is used to kill the tumor cells. Heating occurs by

two particle-size-dependent mechanisms; for NPs less than 100 nm in dia-meter (single domain NPs), heat is produced mainly through Brownian modes, which produces heat due to friction between oscillating particles, whilst for larger particles, Neél modes produce heat by rotation of the magnetic moment with each field oscillation.[321,322] The use of biocompatible NPs for hyperthermia treatment is increasing. Magnetic NPs coated with pullulan acetate, a polysaccharide used as a food additive and shown to be safe for human use, were used for in vitro hyperthermia treatment of KB cells, producing therapeutic efficacies of 56% and 78% at 45 °C and 47 °C, respectively.[323] Chitosan-coated magnetite NPs with a high heating capacity useful for hyperthermia have also been produced.[324] Recently, Bruners et al.[325] reported the use of magnetic NPs for thermal ablation of malignant kidney tumors (followed by computed tomo-graphy). Zhang et al.[326] reported that core/shell iron/iron oxide NPs coated with a biocompatible phospholipid layer provide an increase in magnetization, and are more effective for hyperthermia treatment due to improved local heating than for iron oxide NPs alone.

Another potential treatment for cancer involves attaching magnetic NPs, via targeting ligands, to free-floating cancer cells in the circulatory system, allowing them to be captured and removed from the body with a permanent magnet. Such a strategy might help to improve the long-term survival rates of cancer patients by dimininshing the probability of cancer-cell migration (metastasis) throughout the body.[327]

4.6. Gold Nanoparticles

4.6.1. General Characteristics and Synthetic Aspects

Among the metal NPs, gold NPs are receiving greatest attention, mainly because their properties lend themselves to multiple applications, such as labeling, delivery, heating, and sensing. Gold NPs are made by simple reduction of metal salt precursors with reducing agents under controlled conditions, in either water or organic solvents (**Figure 13**). Addition of a stabilizing agent (surfactant), which is typically charged to increase the repulsion between particles, is also required during the synthetic procedure.[328–330] Seed-mediated growth of gold NPs is another widely used method that involves growing the NPs from small seed particles of gold. Initially, very small, uniformly spherical seed particles are formed. The reaction conditions are then altered, adding more gold ions, a different reducing agent, and a shape-templating surfactant, to obtain specific particle morphology.[329,331] The conventional methods for the synthesis of gold NPs are the citrate reduction of $HAuCl_4$ in water and the Brust–Schiffrin method, in which thiol ligands that strongly bind to the surface of the particles are used.[332]

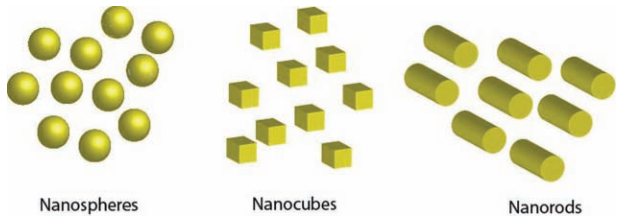

Figure 13. Representation of gold NPs. Different shaped NPs exhibit different properties that maybe useful for different applications.

Gold nanorods are typically prepared by seed-mediated synthesis and by the template method, in which gold is electrochemically deposited within the pores of nanoporous polycarbonate or alumina template membranes.[333] Gold nanocages have been synthesized by galvanic replacement reactions against silver nanocubes in the presence of $HAuCl_4$; the Ag nanocubes are obtained by reducing Ag(I) salts in the presence of a polyol (e.g., ethylene glycol).[331,333]

4.6.2. Gold Nanoparticles for Photothermal Therapy

Gold NPs have been widely studied for photothermal cancer therapy (PTT), also called photothermal ablation therapy (PAT). PTT is based on the surface-plasmon resonance (SPR) property of the particles. Gold NPs are much more efficient photon–thermal-energy converters than typical organic dyes due to the much higher photon-capture crosssection of gold NPs and nanoshells around the SPR absorption band (4–5 orders of magnitude greater than for photothermal dyes). Gold NPs absorb and convert the photon energy into thermal energy when a laser beam is used to irradiate them at the SPR wavelength. The NP temperature can suddenly increase, even above their melting point, releasing the energy to the surroundings or destroying the NPs.[334–336] Photoinduced hyperthermia treatment with gold NPs works better for tissue close to the skin due to the limited penetration of visible and IR light into the tissue; treatment deep inside tissues works better with hyperthermia induced by magnetic NPs.[328] Dickerson et al.[337] used plasmonic photothermal therapy (PPTT) with PEGylated plasmonic gold nanorods and a NIR laser. After accumulation of the nanorods in subcutaneous squamous cell carcinoma xenografts, grown in nude (*nu/nu*) mice, they were exposed to NIR light, which elevated the rod temperature and suppressed tumor growth. Other work has revealed the possibility of using gold NPs for PAT after aggregation is induced by mild acidic in the intracellular environments similar to those found in tumors. Aggregation of the particles inside the tumor allows the application of an external light source.[338] A NP system was designed that included citraconic amide molecules bearing thiol groups for anchoring to the surface of NPs. The amide bonds remained stable under neutral or basic conditions, but were easily hydrolyzed at pH < 7, producing citraconic acid and changing their charge from negative to positive under acidic conditions. B16 F10 mouse melanoma and HeLa cells were incubated with the NPs, and pH-induced accumulation was confirmed, which shifted their absorption to the far- and near-IR spectral regions. This was used to establish the efficacy of photothermal treatment after aggregation.[338] Release of drugs induced by the thermal effect produced by irradiating gold NPs with NIR could become a major application. You et al. electrostatically loaded doxorubicin onto hollow Au nanospheres and used them for doxorubicin delivery and dual treatment of cancer by PAT.[339] Drug release in MDA-MB-231 cells was induced by heat produced after PAT with NIR light.

4.6.3. Gold Nanoparticles for Imaging

Gold NPs provide a convenient way to image tumors as they can be detected by simple methods (e.g., using an optical microscope equipped with a dark-field condenser), due to the ability of noble

metal NPs to scatter light very strongly at their localized SPR frequency.[336,340] Other NPs usually require sophisticated instrumentation such as lasers, optical components, and detectors, or complex image processing methods or dark-field imaging.[340] SPR is based on the interaction of light and the conduction electrons in gold NPs. When light interacts with the metal, the conduction electrons oscillate at a resonance frequency relative to the lattice of positive anions. Some of the photons are released at the same resonance frequency in all directions, a process known as scattering.[341,342] Tuning of the optical properties of the gold NPs is possible because the SPR effect is very sensitive to changes in size, shape and the dielectric constant of the surrounding medium.[343,344] Usually, 30–100 nm particles scatter intensely and can be detected by a conventional microscope under dark-field conditions.[336] The SPR peak can also be tuned by using gold shell NPs (gold nanoshells) and changing the shell thickness-to-particle dia-meter ratio.[341,343] Gold nanocubes are useful for cancer imaging as they possess the highest photoluminescence quantum yield among gold NPs. This effect is due to the SPR-involved local field enhancement and can be used for phototherapy using visible light.[345]

Gold NPs have been also decorated with different targeting agents for specific uptake by tumors and tumor cells. For example, NPs modified with human transferrin,[346,347] folic acid,[348] methotrexate (MTX),[348] and also loaded with drugs such as doxorubicin, placlitaxel, and kahalalide F (a cyclodepsipeptide with antitumoral activity isolated from a marine mollusk, *Elysia rubefescens*) are being developed for specific delivery and release.[349,350]

4.7. Upconverting Nanophosphors

4.7.1. General Characteristics and Synthetic Aspects

Upconverting nanophosphors (UCNPs) are an exciting new class of fluorescent probes for biomedical imaging that are essentially lanthanide (rare earth)-doped ceramic materials. In contrast to organic fluorophores and semiconductor QDs (so-called "down-converters"), UCNPs convert longer wavelength radiation (typically NIR) into shorter wavelength luminescence, i.e., they exhibit anti-Stokes emission (**Figure 14**).[351] Presently, the two major types of inorganic host matrices used to prepare UCNPs are rare earth fluorides (e.g., $NaYF_4$ and LaF3) and oxides (e.g., Y_2O_3 and Y_2O_2S).[352] These are co-doped with Yb^{3+} and Ln^{3+} (Ln = Er and Tm) ions to tune their emission spectra.[353–355] UCNPs featuring Er^{3+} as the emitter display two predominant emission lines, one in the green (ca. 540 nm) and one in the red (ca. 650 nm),[342] whilst Tm^{3+}-doped UCNPs exhibit an intense NIR emission around 800 nm and two weak visible emission peaks (ca. 480 and 650 nm).[355]

UCNPs are commonly prepared via surfactant-mediated hydrothermal synthesis.[353,356,357] Additives such as polyethylenimine (PEI)[358] and poly(vinyl pyrrolidone)[359] have been used to control the growth of UCNPs. Recently, a facile microwave-based method for preparing high-quality UCNPs has been described.[360] The spectral properties of UCNPs can be tuned not only by varying the dopants and their relative proportions,[352] but also by controlling the temperature during thermolysis.[361]

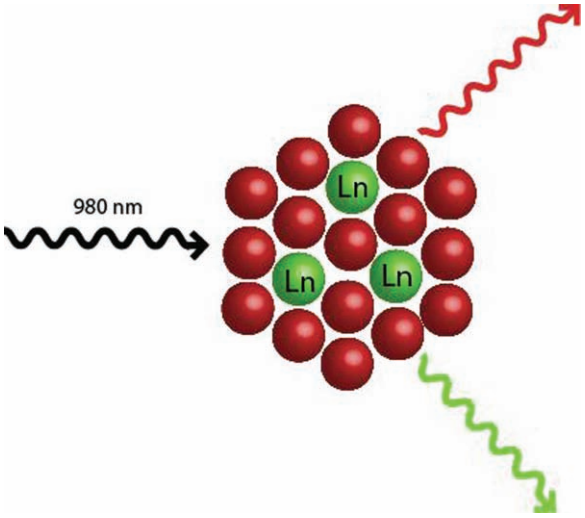

Figure 14. Schematic representation of energy upconversion by an UCNP.

As with many other NPs, the majority of methods produce either uncoated or hydrophobic, oleic acid-coated UCNPs that aggregate strongly or do not suspend in water. Several methods to render them water-dispersable have been described, including coating with polymers such as polyacrylic acid,[362] ligand exchange with molecules such as PEG-phosphate and mercaptopropionic acid,[355] and silica coating (using aminopropyl-trimethoxysilane),[363,364] followed by conjugation to the biomolecule. The addition of PEI during hydrothermal synthesis of UCNPs allows for direct surface functionalization, circumventing the need for an additional coating or ligand exchange step.[358] Active targeting of UCNPs has been achieved by conjugating a range of bioactive molecules to their surface, including antibodies,[364] folic acid,[364,365] and peptides.[363,366]

4.7.2. UCNPs for Fluorescence Imaging

The major appeal of UCNPs for tumor imaging is that relatively inexpensive low-power NIR diode lasers may be used as the excitation source, which allows for deeper tissue penetration compared to traditional fluorescence imaging as well as higher-contrast optical imaging due to an absence of autofluorescence and decreased light scattering.[367] Unlike many organic fluorophores, UCNPs are extremely resistant to photobleaching and their rare earth components are approximately one-thousand-fold less toxic than the heavy metals within QDs.[368] Moreover, the NIR wavelengths that UCNPs are excited at are less cytotoxic than the radiation used to excite most other fluorophores.[367]

UCNPs functionalized with the cyclic RGD peptide have been successfully used for imaging of integrin $\alpha_v\beta_3$-positive tumor cells.[363,365] Zako et al.[363] prepared silica-coated Er^{3+}-doped Y_2O_3 NPs and modified their surface by treatment with a heterobifunctional PEG derivative and then the RGD peptide. Upconversion emission was observed from the NPs for U87MG cancer cells (high integrin $\alpha_v\beta_3$ expression), but not for MCF-7 cancer cells (low integrin $\alpha_v\beta_3$ expression), confirming integrin $\alpha_v\beta_3$-specific binding/uptake. Xiong et al.[366] reported similar in vitro findings for RGD-labelled $NaYF_4$ UCNPs that had been tri-doped with Yb^{3+}, Tm^{3+}, and Er^{3+} to produce multicolor upconversion luminescence (green, red, and NIR). These

workers also reported the successful in vivo and ex vivo imaging of U87MG tumors within nude mice. Luminescence imaging of tissue slices showed no autofluorescence, even at penetration depths as high as 600 μm, and a high signal-to-noise ratio (ca. 24) between tumor and background tissue.

Hu et al.[364] have produced folate-displaying, silica-coated $NaYF_4$:Yb/Er UCNP composites incorporating fluorescein isothiocyanate. These display good water solubility, photostability, and biocompatibility and can be monitored by down- and upconverting luminescence simultaneously. Using confocal microscopy and localized spectroscopy, receptor-mediated delivery of this nanocomposite to targeted FR(+) cell lines was demonstrated, whereas there was almost no uptake in FR(–) cell lines, after short incubation times (<1 h). The use of $NaYF_4$:Yb/Tm UCNPs for imaging of tumor cells has been reported.[355,369] A major advantage offered by Tm^{3+}-doped particles is that both the excitation wavelength (980 nm) and upconverted emission (ca. 800 nm) fall within the region of the electromagnetic spectrum, where human tissue is relatively transparent (the so-called biological window), which is ideal for deeper tissue penetration. Boyer et al.[369] prepared PEG phosphate-coated NPs of this type, and successfully employed them for upconversion imaging of an ovarian cancer cell line (CaOV3). Nyk et al.[355] demonstrated in vitro cellular uptake of mercaptopropionic acid-coated $NaYF_4$:Yb/Tm NPs into Panc 1 human pancreatic cancer cells. Animal imaging studies were also performed using Balb-c mice injected intravenously with the UCNPs. The luminescence signal at 800 nm was readily detectable through the skin (without hair removal) and provided a high contrast image.

4.7.3. UCNPs for Photodynamic Therapy

The ability of UCNPs to convert low-energy radiation to higher-energy emissions provides an elegant tool by which to extend the tissue penetration range of therapies based on the use of high-energy light. Zhang et al.[370] and Chaterjee and Yong[371] have proposed that UCNPs with surface-bound photosensitizers could be potentially used as nanotransducers for photodynamic therapy of cancer in deep tissues, a possibility otherwise precluded by the poor tissue penetration of the high-energy light required to form cytotoxic oxygen species. Zhang et al.[370] produced silica-coated $NaYF_4$:Yb/Er UCNPs with a photosensitizing molecule (Merocyanine 540, M-540) doped into the silica layer, and a mouse monoclonal antibody (anti-MUC1/episialin) highly specific toward MCF-7/AZ breast cancer cells covalently attached to the surface. In vitro tests confirmed the photodynamic cytotoxicity of the M-540-coated UCNPs toward MCF-7/AZ cells. Chaterjee and Yong[371] produced $NaYF_4$:Yb/Er UCNPs modified with a zinc phthalocyanine photosensitizer, which targeted folate receptors on human colon cancer cells. As well as enabling tumors in rats to be imaged with high signal-to-background ratio, NIR excitation of the particles after deep intramuscular injection led to the release of singlet oxygen and significant cell destruction. Austin and co-workers[372] have produced three-layer composite NPs with an UCNP interior, a coating of porphyrin photosensitizer, and a biocompatible PEG outer layer to prevent clearance by the RES. These generate millimolar amounts of singlet oxygen under NIR radiation.

4.7.4. UCNPs for Drug Delivery

Another area of recent application of UCNPs is the fluorescent imaging and targeted delivery of siRNA into cancer cells. To facilitate uptake into SK-BR-3 cells with high Her2 receptor expression, Jiang et al.[365] prepared silica-coated $NaYF_4$:Yb/Er UCNPs conjugated to anti-Her2 antibodies and electrostatically bound siRNA onto the protonated amine-bearing surface of the NPs. Successful uptake of the UCNPs was established via confocal microscopy, whilst delivery of the siRNA was confirmed by the down-regulation of expression of the target gene (luciferase) in the SK-BR-3 cells, but not in MCF-7 cells with low Her2 receptor expression.

4.8. Other Classes of Nanomaterials

In addition to the various types of NMs described above, several other types of nanoscalic agents are gaining relevance in the field of oncology research. These include carbon nanotubes (CNTs) (single- and multi-walled), which have been investigated as drug-delivery systems due to their low cytotoxicity, high surface area, and easy surface modification.[373] Multi-walled carbon nanotubes (MWCNTs) can be used for photothermal cancer ablation, as they release substantial vibrational energy on exposure to NIR radiation.[374] Also, the possibility of using CNTs as nanocontainers makes them very attractive for drug delivery; they can be filled with metals, semiconductors, salts, organic materials, fullerenes, etc.[375,376] MWCNTs functionalized with polyethylenimine have been modified with QDs of different sizes to produce a multicolor fluorescent nanoprobe for tumor imaging.[377] CNTs have also been coated with PAMAM dendrimers functionalized with imaging and targeting agents.[378]

Small silicon NPs (1–10 nm) hold great potential in emerging applications of diagnostic imaging due to their unique properties, such as, for example, tunable light emission, high brightness, stability against photo-bleaching, and low toxicity.[379,380] Surface functionalization with, for instance, amino groups opens the way for grafting specific biomolecules and, consequently, to achieve active targeting. Initial bioimaging results have shown that these NPs are readily taken up by murine cells, allowing for efficient staining.[381]

Nanocontainers based on zeolite L represent a novel class of NMs that could be of potential use in cancer diagnosis and therapy because they can be heavily loaded with luminescent molecules, photosensitizer and/or radiometals without leakage after locking with stopper moieties (**Figure 15**). Efficient functionalization of the zeolite surface can be achieved by direct coupling of appropriate molecules via silanol groups or, for example, using click chemistry, allowing for selective targeting of desired systems. The potential use of zeolite L nanomaterials for scintigraphic imaging (loaded with ^{111}In) and PDT (grafted with phthalocyanines) has been demonstrated.[382,383] Preliminary results suggest the possibility of developing zeolite L nanocontainers suitable for detecting and curing neoplastic tissue. Lo and co-workers very recently described mesoporous silica nanoparticles (MSNPs) for the controlled release of anticancer chemotherapeutics, which feature doxorubicin (Dox) conjugated to the MSNPs channels via acid-labile hydrazone linkages. Upon exposure to the acidic environment of endosomes/lysosomes, Dox is released intracellularly, resulting in efficient apoptotic cell death.[384]

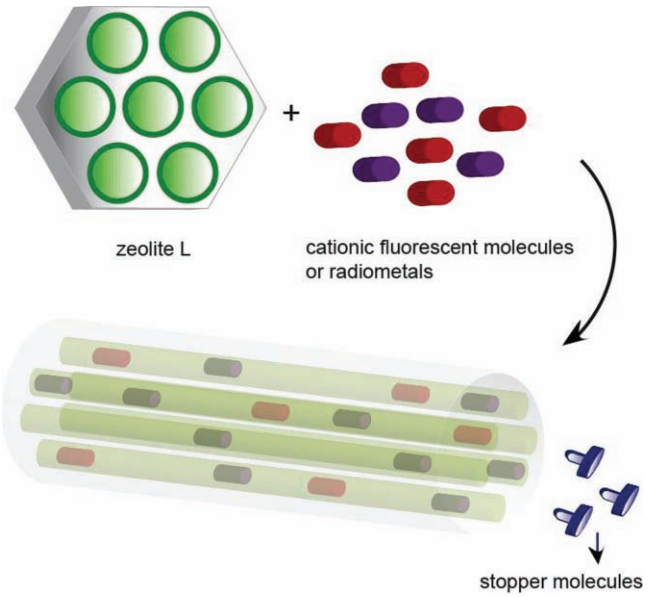

Figure 15. Representation of zeolite L loaded with fluorescent molecules or radiometals.

5. Conclusion and Outlook

The past decade has witnessed an explosion of interest in the use of NMs in oncology research because of their potential to revolutionize the way cancer is diagnosed and treated. Significant advances have been made in synthetic methodology, such that it is now possible to prepare a variety of NPs with highly controlled size, shape, surface charge and physicochemical characteristics, and to decorate their surface with polymers and bioactive molecules in order to improve biocompatibility and achieve active targeting. As this review demonstrates, this has facilitated the development of a diverse range of nanometer-sized objects that are able to recognize cancer tissue and enable visualization of tumors, delivery of anti-cancer drugs, and/or the destruction of tumors by different therapeutic techniques.

Future research will undoubtedly see the discovery and exploration of new types of NM. Increasing attention will be paid to the development of hybrid systems, incorporating multiple types of NMs (nanocomposites), since this will enable greater multifunctionality to be achieved. Recent research has, for example, seen the emergence of silica nanostructures containing both embedded QDs and magnetic NPs, enabling both magnetic guidance and optical tracking.[385] Even more sophisticated designs will no doubt emerge over time.

There is considerable scope for further development of stimulus-responsive nanoagents (smart nanoparticles) designed to enhance the localization and efficacy of therapeutic payloads, and new strategies for controlled drug release are continually being proposed. For example, Carling et al.[386] very recently presented evidence that UCNPs can be employed for NIR-triggered release of surface bound molecules. Previously, the release of caged molecules, or photoconversion of inactive compounds to active forms, has required direct excitation with high-energy light, limiting in vivo application. Although not yet demonstrated, it is foreseeable that UCNPs might find future application as nanotransducers for the highly spatially and temporally controlled, photo-triggered release of caged cancer therapeutics. The development of such systems would enable higher drug doses to be used, whilst reducing side-effects and systemic toxicity.

The application of different types of NPs *in combination* to achieve improved therapeutic outcomes presents some further exciting opportunities. Park et al. recently demonstrated that gold nanorods could be used to improve the delivery of magnetic nanoworms or doxorubicin-loaded liposomes exhibiting the peptide LyP1, which binds specifically to the p32 protein or gC1qR receptor, overexpressed in tumor-associated cells undergoing stress.[387] Heating of the gold nanorods with NIR light not only induced local tumor heating, but also accelerated the accumulation of the targeted NPs, leading to faster rates of drug delivery. Other combinations of NPs might similarly display synergistic effects that prove useful for cancer therapy.

Notwithstanding the significant advances made in the field of NP-based cancer diagnostics and therapeutics, our overall understanding of NP pharmacokinetics (adsorption, uptake, distribution, metabolism, and excretion) is quite limited at present. Further in-depth exploration of the physicochemical and physiological processes that NPs are subjected to within biological environments is required before the biodistribution of NPs can be predicted from their physicochemical properties. Moreover, careful studies need to be done on investigating and improving the safety profile of these systems before they will find extensive application in the clinic. In this context, the role of protein-nanoparticle interactions has recently been recognized as the key to nanomedicine and nanotoxicity.[388,389] A deep understanding of the biological effects of NPs thus requires knowledge of the equilibrium and kinetic binding properties of proteins (and other biomolecules such as lipids and polysaccharides) that associate with the particles, and especially under competitive binding conditions, such as occur *in vivo*. Consequently, reliable methods have to be developed and introduced for the characterization of the true risks of new NMs.[390]

Currently, there is an emerging rush to commercialize NMs for therapeutic purposes.[42,391,392] So far, Doxil,[393] a liposomal system for doxorubicin delivery and treatment of ovarian carcinoma, and Abraxane,[393,394] an albumin-taxol NP for the treatment of metastatic breast cancer, are the only two NPs that have achieved FDA approval for use in cancer therapy. That said, promising pre-clinical results have seen many NP systems moving rapidly to clinical trials recently, including: i) CALAA-01, a cyclodextrin containing NP for siRNA delivery (phase I),[395,396] ii) INGN-401, a liposomal formulation for metastatic, non-small cell lung cancer (phase I),[396] iii) SGT-53, a liposome for treatment of cancer tumors (phase I),[396] iv) Auroshell, gold NPs for solid tumors (phase I),[396] v) XMT-1001, a campothencin based prodrug (phase I),[396] and vi) Aurimmune CYT-6091, a colloidal gold formulation for solid tumors (phase II).[396] It is expected that there will be significant progress in improving our understanding of the structure–activity relationships for NPs in the coming years.

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