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The application of nanomaterials in cancer diagnostics and treatment

Oleksandr Turkin

TopMaster programme in Nanoscience
Zernike Institute for Advanced Materials
University of Groningen

Supervisor:
Prof. Antoine van Oijen

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Cancer nanotechnology is an interdisciplinary area of research in science, engineering, and medicine with broad applications for molecular imaging, molecular diagnosis, and targeted therapy. Recently the application of nanomaterials to tumour and biomarker imaging as well as delivery and targeting of therapeutics for cancer treatment have received significant attention. A wide range of materials may be used to construct nanoparticles that can encase or solubilize chemotherapeutics to increase the capability of delivery or to provide unique optical, magnetic, electrical and structural properties for imaging and therapy. Several functional nanoparticles have already been demonstrated, including some clinically approved liposome drugs and metallic imaging agents. The next step is to develop multifunctional nanoparticles that may ultimately further the realization of individual therapy. These multiplexed nanoparticles may be able to identify malignant cells by means of molecular detection, visualization of their location in the body by providing enhanced contrast in medical imaging techniques, killing tumour cells with minimal side effects through selective drug targeting, and monitoring treatment in real time.

1. THE IMPORTANCE OF THE PROBLEM

Cancer. People associate this word with everything bad and evil. In the Dutch language 'cancer' is also a part of numerous swearing words. But what exactly is cancer? A commonly used scientific definition classifies it as a number of diseases in which a healthy cell of the human body gets modified due to genetic defects and starts to grow in an uncontrolled way, invading the adjacent tissues and spreading throughout the whole body as metastasis. The defects in cells may be caused by chemicals – carcinogens – or may randomly occur due to errors in DNA replication, repair and recombination processes. People of all ages can be affected by cancer and the risk for most types increases with age. The key facts[1] about cancer are summarized below:

- Cancer is one of the leading causes of death worldwide: it accounted for 10 million deaths (around 13% of all deaths) in 2007.
- Lung, stomach, liver, colon and breast cancer cause the most cancer deaths each year.
- Cancer arises from a change in one single cell. The change may be started by external agents and inherited genetic factors.
- Deaths from cancer worldwide are projected to continue rising, with an estimated 12 million deaths in 2030.
- Currently nearly 65% of persons diagnosed with cancer are expected to live at least five years after the cancer is discovered

The main causes of death[2] estimated in the regions of World Health Organization for 2002 are listed below (Table 1).

Table 1. List of causes of death by rate

Cause	Percent of death
Cardiovascular deseases	29.34
Infectious and parasitic diseases	23.04
Malignant neoplasms (cancers)	12.49
Respiratory diseases	6.49
Unintentional injuries	6.23
Perinatal conditions	4.32
Digestive diseases	3.45
Intentional injuries (Suicide, Violence, War, etc)	2.84
Neuropsychiatric disorders	1.95
Diabetes mellitus	1.73
Diseases of the genitourinary system	1.49
Other	6.63

Currently, medicine's main weapons against cancer are chemotherapy, radiation and surgery. Their improvement that these techniques have seen over the past few decades is striking but they are still far from optimal. That is why the improvement of these treatments as well as diagnostic methods remains the main goal of current cancer research. A new branch of science, nanotechnology, has shown tremendous promise in cancer diagnostics and treatment and has the potential to revolutionize the field in the near future[3,4]. New optical, electronic, magnetic and structural properties of the materials present only on the nanometer scale offer significant opportunities for *in vivo* applications. A number of nanoparticles have already successfully been used clinically as targeted chemotherapeutics and imaging contrast agents[5,6].

The development of new technologies in nanomedicine may enable the following possibilities[7]:

- cancer detection at the earliest stages;
- assessment of therapeutic efficacy at real time;
- targeting and bypassing of the biological barriers to deliver multiple therapeutic agents directly to cancer tissues;
- identification of molecular changes in cells that further become cancerous;

In the following chapter we mention some of the nanoparticles, provide their general description, common features and briefly discuss the advantages they have over conventional materials. Afterwards, in chapter 3 we deal with the use of nanomaterials in present imaging techniques. We provide a description of the phenomena responsible for imaging contrast enhancement and also underline peculiar chemical and physical properties of imaging

nanoparticles. In chapter 4 we discuss the multiplexed quantum dot staining of cancer biomarkers which may become a powerful personalized tool in early cancer diagnostics.

Treatment methods are discussed in chapters 5 and 6: targeted drug delivery and hyperthermia based therapy correspondingly. The former one is one of the most promising nanoparticle applications and that is why most scientific efforts are concentrated around drug delivery. To summarize this review we provide an outlook for the future of nanomedicine in general as well as cancer imaging/therapy with nanoparticles in particular.

2. GENERAL NANOPARTICLE INFORMATION

There are many different nanoparticles existent: liposomes, dendrimers, carbon nanotubes, quantum dots, magnetic nanoparticles, etc. Their size lies in the range of 1–100 nm, a size regime that gives rise to new, unique physical and chemical properties. These properties, together with the high surface-to-volume ratio of nanoparticles and their size being comparable to those of biomolecules make nanomaterials a powerful tool for imaging, diagnosis and therapy.

A good example of the use of nanomaterials in medical diagnostics is their application as contrast agent in Magnetic Resonance Imaging (MRI). MRI is one of the most important imaging techniques in modern medicine but its applications are still limited at low concentrations of contrast agents. That is why so much effort is made to increase the sensitivity of MRI imaging with the help of new imaging agents, nanoparticles in particular. Superparamagnetic iron oxide particles have already successfully been used to significantly improve the imaging contrast[8].

Another example of nanoparticles surpassing conventional materials may be the use of quantum dots in fluorescence imaging. Their advantages include multicolour optimization, absence of photobleaching and other possible degradations, high quantum yield and possibility to simultaneously identify multiple markers[9].

Poor solubility, unfavourable pharmacokinetics, half-life, therapeutic efficiency and target specificity impose significant restrictions on the use currently available anticancer drugs. The situation can be rectified by the use of nanomaterials, which can provide control of the therapeutic effect, better bioavailability, prevention of drug deactivation before it reaches a tumour.

Another important matter is that of localization. A simple-minded assumption is that nanoparticles are uniformly distributed throughout a human body. However, there are numerous locations that are not that easily reached. For example, the brain is protected by the blood–brain barrier. By understanding the size and surface property requirements for reaching specified sites within the body, localization of nanoparticles to these sites can be accomplished. Ultimately, localization and targeting is done via the addition of special targeting ligands that provide nanoparticle–cell interactions that drastically influence the final location of the nanoparticle. For example, an addition of a moiety such as a small molecule, peptide, protein or antibody to the nanoparticles can target the latter to cancer cells[10].

Table 2. Representative examples of marketed nanoparticle-based drug delivery and imaging contrast agents[7]

Commercial name	Nanoparticle carrier	Active agent	Approved indications
Drug delivery			
Doxil Caelyx	PEGylated liposome	Doxorubicin	Ovarian cancer, AIDs-related Kaposi's sarcoma, and recurrent breast cancer. Combinatorial therapy (with bortezomib) of multiple myeloma
Myocet	Liposome	Doxorubicin	Combinatorial therapy (with cyclophosphamide) of recurrent breast cancer, ovarian cancer and AIDs-related Kaposi's sarcoma
DaunoXome	Liposome	Daunorubicin	Kaposi's sarcoma
Onco TCS	Liposome	Vincristine	Relapsed aggressive non-Hodgkin's lymphoma
Abraxane	Albumin	Paclitaxel	Metastatic breast cancer
Abelcet Amphotec	Liposome	Amphotericin B	Treatment of invasive fungal infections in patients who are refractory to or intolerant of conventional amphotericin B
AmBisome	Liposome	Amphotericin B	Empiric therapy for presumed fungal infections in febrile neutropenic patients. Treatment of visceral leishmaniasis. Treatment of invasive fungal infections in patients who are refractory to or intolerant of conventional amphotericin B
Imaging contrast agents			
Lumiren Gastromark	Silica	Superparamagnetic iron oxides	Imaging of the gastrointenstinal tract and abdomen with MRI
Endoderm Feridex	Dextran	Superparamagnetic iron oxides	Detection of liver and spleen lesions associated with metastases, primary liver cancer, cysts and various benign tumors, adenomas and hyperplasia with MRI
Resovist	Carbodextran	Superparamagnetic iron oxides	Detection and characterization of especially small focal liver lesions with MRI
Sinerem	Dextran	Ultra-small paramagnetic iron oxides	Blood pool visualization and differentiation of metastatic and non-metastatic lymph nodes in patients with confirmed primary cancer who are at risk for lymph node metastases with MRI

3. CANCER IMAGING

The possibility to detect and diagnose cancer and other human diseases at earlier stages than with current imaging methods caused a drastic increase in interest in nano-imaging technology. Nanoparticles have been developed with better distribution throughout the body and better tumour targeting than standard contrast agents. Different kinds of nanoparticles can potentially improve imaging techniques such as X-ray imaging, computed

tomography (CT), near-infrared (NIR) fluorescence imaging, positron emission spectroscopy (PET) and magnetic resonance imaging (MRI).[11-13]

3.1. Quantum Dots

Quantum Dots (QDs) are 2-8 nm semiconductor nanocrystals typically composed of cadmium selenide (CdSe). They have a spherical shape with dimensions that are smaller in size than the exciton Bohr radius (<100 nm).[14] This latter property results in the physical confinement of conduction band electrons. Change of solvent, precursor or temperature can be used to tune the size of QDs during their synthesis from organometallic precursors. Being tiny objects, they can be introduced into the organism via injection, subsequently excited *in vivo* using visible light and their fluorescence is imaged using a sensitive charge-coupled device (CCD) camera.

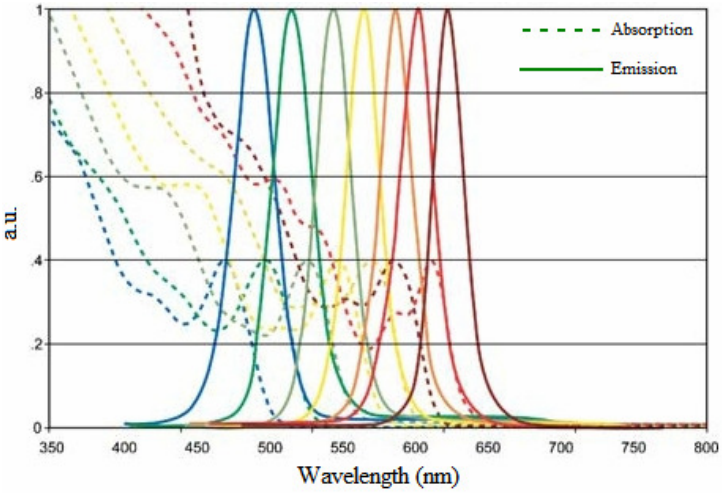

Fig. 3.1 Absorption and emission properties of ZnS-shelled, CdSe-cored quantum dots. Tunable optical properties demonstrate increasing 1st exciton absorption and peak emission wavelengths with increasing CdSe diameters, ranging from 1.9 nm (blue curve) to 5.2 nm (red curve), by EvidentTech

QDs represent such an exciting new class of fluorescent dyes for imaging since their optical properties can be easily tuned (Fig. 3.1) by changing their size, shape and composition. For example, a 2 nm CdSe QD emits at 543 nm (green), while a 4 nm diameter QD of the same material emits at 655 nm (red). Thus simply by varying the size of the nanoparticle, the fluorescence can be tuned over a continuous spectral range. In contrast, to achieve a different emission wavelength for organic dyes one has to change the chemical structure of the compounds. The selection of organic dye emission wavelength is restricted as well because of the non-continuity of their fluorescence spectrum as it is for a QD. QDs are highly photostable[15] in comparison with the organic dyes that have a property of photobleaching.

The most remarkable property of QDs is that they have a very broad absorption spectrum and very narrow emission bands (Fig. 3.1). This allows for a single wavelength of light to excite a set of QDs of various sizes, which will then in turn emit light for multiplex imaging at several different wavelengths. Such systems have been used to image five different lymphatic basins simultaneously (Fig. 3.2).[16]

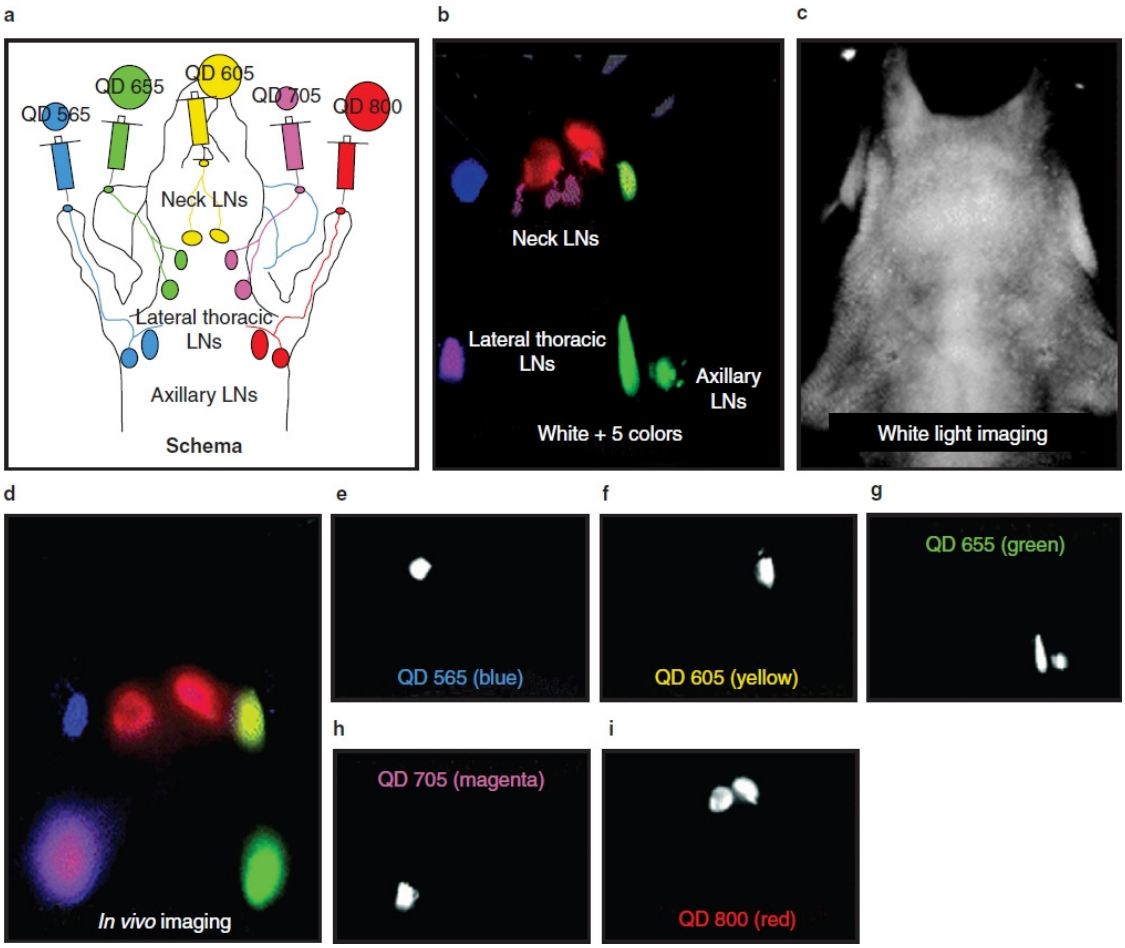

Fig. 3.2 Visualization of five distinct lymphatic drainages in a mouse by *in vivo* five-colour spectral fluorescence imaging using quantum dots (QDs). **(a)** Schema showing location of intracutaneous injections of five carboxyl QDs (565, blue; 605, yellow; 655, green; 705, magenta; 800, red) into the middle digits of the bilateral upper extremities, the bilateral ears, and at the median chin; **(b)** white and five-colour results of the spectral fluorescence imaging; **(c)** white light imaging; **(d)** *in vivo* image showing five primary draining lymph nodes (LNs) simultaneously visualized with different colors through the skin; **(e–i)** individual QD color results from the spectral fluorescence imaging

A number of QD systems have already demonstrated efficacy in living systems. For example, glioblastoma tumours implanted in mice were successfully imaged using QDs that were labeled with the arginine-glycine-aspartic acid (RGD) peptide sequence.[17]. Further, different types of cancer were targeted using different agents: hepatocellular carcinoma with a QD-alpha-fetoprotein antibody conjugate[18], prostate cancer with a QD-antibody construct[19], and brain cancer with phagocytized QD-polyethylene glycol (PEG) conjugates[20].

The toxicity of QDs is one of the main concerns in medical applications. For protection of the body against metal poisoning, QD surfaces have to be covered with a passivating agent, typically zinc sulfide (ZnS) or cadmium sulfide (CdS). Different biocompatible agents, such as PEG, proteins, sugars, or other bio-recognition molecules are often added to these outer-surfaces. A significant increase in the effective particle size caused by these add-ons, may badly affect renal elimination of QD materials.[21]

3.2. Metal Nanoshells

Nanometer-sized metal nanoshells represent another class of nanoparticles that are more frequently used in biomedical applications. Typical metal nanoshell preparation is performed *via* coating a dielectric core with an ultra-thin layer of metal. Many different metals can be used for this purpose, but gold ones received the most attention for medical applications because they are resistant to corrosion, physiologically inert and non-toxic.[22] The silica core of a nanoshell is synthesised via the Stöber method,[23] namely tetraethylorthosilicate chemical reduction in ethanol under basic conditions. The thus obtained silica nanoparticles are then functionalized with amine groups and subsequently coated with a thin layer of gold by the chemical reduction of $HAuCl_4$.[24] The final nanoparticle size may ranges from 50–500 nm in diameter, though the gold layer may be only a few nanometers thick.

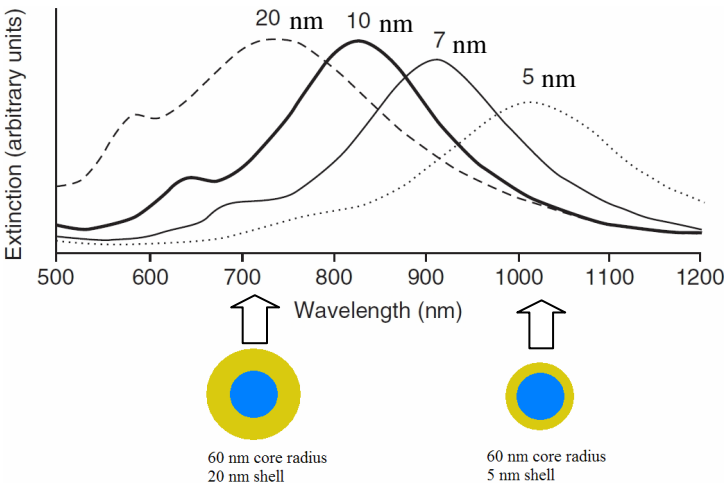

Fig. 3.3 Optical resonances of gold shell–silica core nanoshells as a function of their core/shell ratio

Nanoshell properties are based upon a different phenomenon than observed in QDs. This phenomenon is called surface plasmon resonance (SPR)[25]: a collective oscillation of metal shell conducting surface electrons in an electromagnetic field, leading to the emission of a strong visible light (Fig. 3.3). SPR can be accurately tuned by particle size and shape variations[26].

Gold nanoshells have been used as contrast agents in optical coherence tomography, photoacoustic tomography, NIR tomography, confocal imaging, iridotomy, and photothermal coagulation. Fu *et al.* studied nanoshells as light-scattering contrast agents as well as the influence of surface fictionalization on the SPR.[27] By conjugating gold nanoshells to antibodies for human epidermal growth factor receptor 2 (HER2), a biomarker of breast carcinoma, HER2 has been targeted and imaged by C. Loo *et al.*[28]

3.3. Superparamagnetic iron oxide (SPIO)

If one reduces the size of a paramagnetic material so that it no longer contains multiple domains but only a single domain in which all spins are mutually aligned, the particle will display a large magnetic moment known as superparamagnetism. An iron oxide particle with this property is known as superparamagnetic iron oxide (SPIO). These nanoparticles are chemically and structurally similar to gold nanoparticles and QDs. They use a highly superparamagnetic iron oxide (SPIO) as a core material, and biocompatible polymers such as dextran as a coating.[29] Large magnetic moments of SPIO nanoparticles make them appropriate contrast agents in MRI. These nanoparticles are successfully used clinically for more than two decades and different fictionalization agents such as antibodies, nucleosides, proteins or enzymes may be used to direct them to tumour sites.[30]

The most attractive SPIO for medical applications is magnetite (Fe_3O_4), which has mixed oxidation state of iron.[31] Amongst a variety of methods to synthesize Fe_3O_4 particles,[32] the most widely used is co-precipitation of $Fe(OH)_2$ and $Fe(OH)_3$ suspensions[33] or the use of microemulsion technique[34]. Particle sizes range from several nanometers to several hundred nanometers in diameter.[32]

There are several commercial SPIOs developed in order to improve the contrast in MRI imaging. The advantages of the SPIO nanoparticles are biocompatibility, and easy detection at moderate concentrations. They also possess a high saturation magnetization and loss of magnetization in the absence of magnetic field, and are less toxic than typical optical imaging agents. Most often liver tumours are targeted with SPIO nanoparticles.[35] The extensive study of the relation between pharmacokinetics, particle size, and surface modification is yet to be done.

Biological markers can be covalently attached to the surface of SPIOs. As examples of the use of SPIO-labeled antibodies may serve the imaging of rectal carcinoma,[36] breast cancer,[37] and vascular cell adhesion molecule 1 (VCAM1).[38] Another example is derivitizing SPIOs with HIV-tat peptide that showed 100-fold increase in accumulation within lymphocytes than nonmodified particles.[39] Conroy Sun *et al.* studied the cellular uptake and MRI contrast enhancement by SPIOs conjugated with folic acid.[40]

3.4. Carbon Nanotubes

The discovery of carbon nanotubes (CNTs) in 1991 in the soot of an arc discharge apparatus gave birth to an extensive research of carbon-based nanotechnology.[41] A CNT can be regarded as a monolayered graphite sheet rolled into a tube with the length range of 300–3000 nm, with a diameter of approximately 1.0 nm. CNTs possess exceptional properties such as high electrical and thermal conductivity and great tensile strength which might find their implications as field emission devices, tips for scanning microscopy, nanoscale transistors, or components for composite materials. Possible biomedical applications of CNTs are currently being investigated. For instance the unique NIR fluorescence properties[42] of single-walled carbon nanotubes (SWNT) may find their applications as imaging contrast agents[43] and biological sensors.[44] Multi-walled carbon nanotubes (MWNTs) with surface functionalization are also reported as a successful bioimaging contrast agent.[45]

There is a number of methods to obtain CNTs, for instance arc discharge and evaporation[41], laser ablation[46] and chemical vapor deposition (CVD)[47]. Even though the most widely used one is CVD, the highest purities ~90% can be achieved with laser ablation. However, this technique is not available on industrial scale. The easiest and most common procedure is the arc

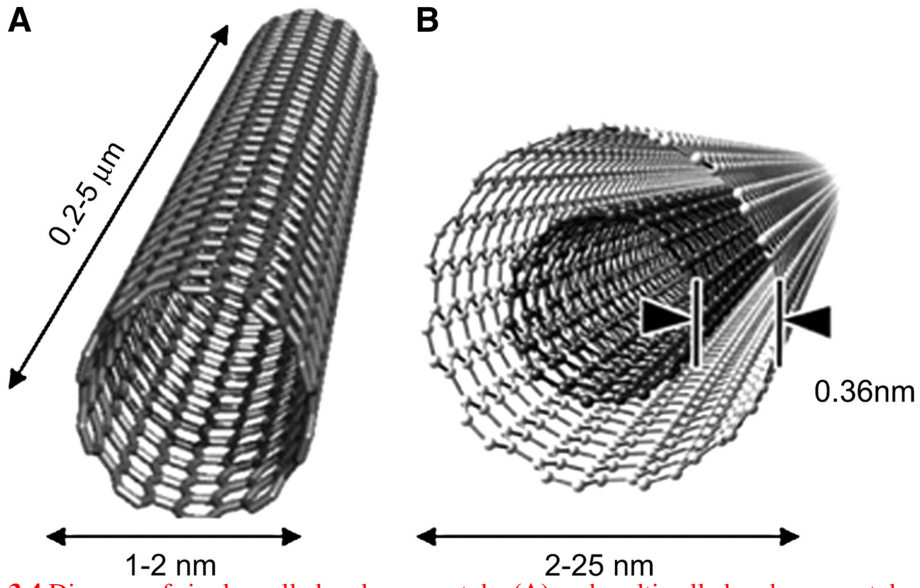

Fig. 3.4 Diagram of single-walled carbon nanotube **(A)** and multiwalled carbon nanotube **(B)**, showing typical dimensions of length, width, and separation distance between graphene layers in MWNTs.)

discharge method in which two carbon rods are evaporated by electrical current and a deposition of tube-like structures is observed on the cathode.

The requirements for the use as biomedical imaging agents include solubility so that the particles can be delivered to the target. Furthermore, the particles need to be free of any potential harmful side-effects. CNTs do not quite fulfil these requirements however surface functionalized CNTs do. It is reported that the latter ones are soluble in water and have reduced cytotoxicity.[48, 49]

Here are a few examples of SWNT application studies for bioimaging so far: confocal microscopy imaging in cells of SWNTs covalently linked to visible-light fluorophores,[43] internalization of SWNTs and SWNT-streptavidin conjugates into human leukaemia and T cells;[43] investigation of the possibility of building a nanobioelectronic device that could be specific to the receptors of the cancer cell surface using fluorescently labelled and immunoglobulin G functionalized CNTs and confocal microscopy.[50]

4. MOLECULAR CANCER DIAGNOSIS: CORRELATION OF BIOMARKERS WITH CANCER BEHAVIOUR

The relation between the conventional histopathology and molecular signatures of the biomaterial can be established using nanoparticle probes for example by measuring a set of biomarkers on intact cancer cells or tissue specimens (Fig. 4.1). Two important features of nanoparticles, namely an enhanced binding affinity and high specificity are crucial in the imaging of cancer biomarkers that are present only in small number of cells or found at very low concentrations. Live or fixed cells as well as freshly harvested tissues are typical objects for fluorescent QD tagging studies. It is however important to note that the prevailing clinical material is archived, namely formalin-fixed paraffin-embedded (FFPE). These samples can be several decades old but they still remain very important since their clinical outcome is known and it means that these tissues can be used to correlate the molecular profile and clinical outcome.

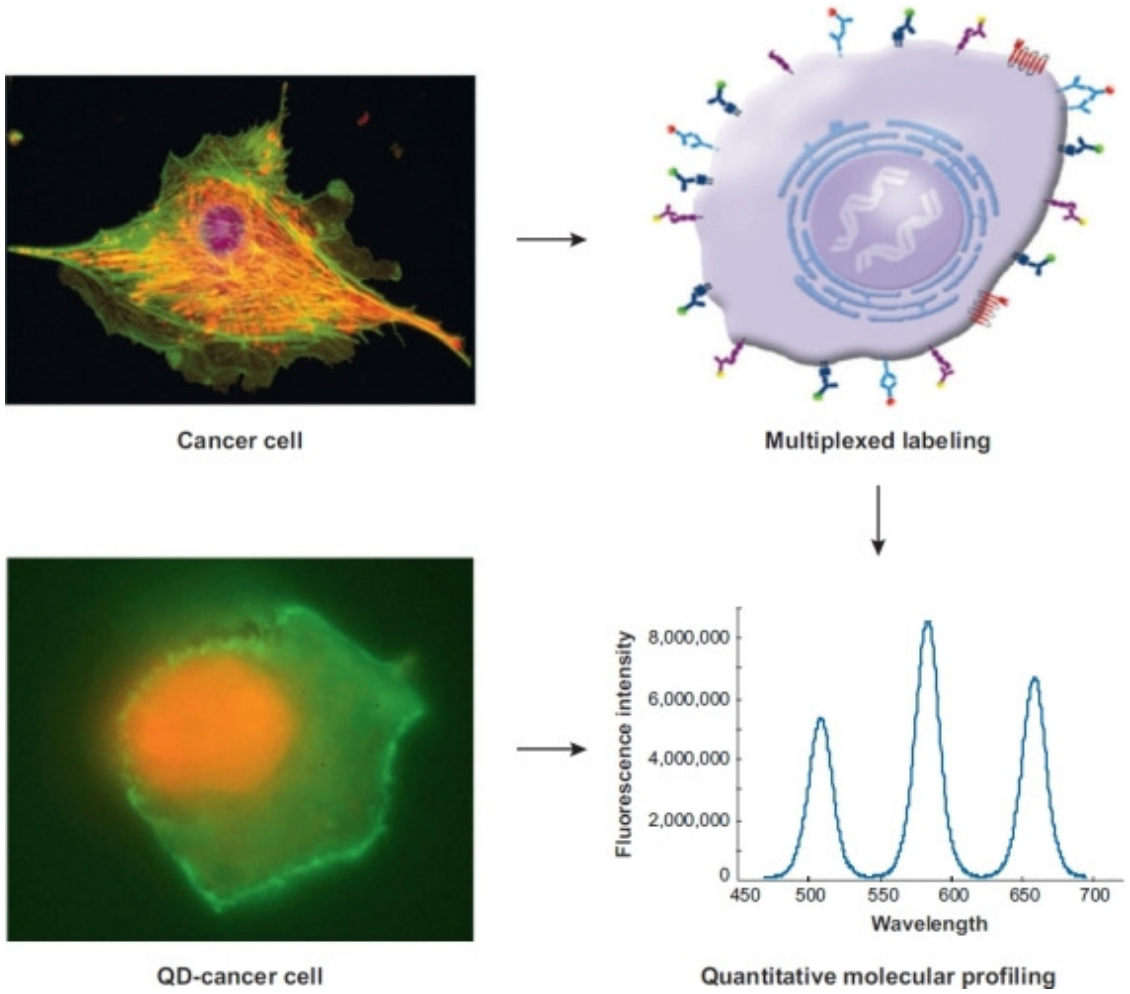

Fig. 4.1 Schematic illustration of multiplexed detection and quantification of cancer biomarkers on intact cells or tissues with multicolor nanoparticle probes. The left-hand images show cancer cells labeled with quantum dots, and the right-hand drawings suggest how wavelength-resolved spectroscopy or spectral imaging could quantify surface and intracellular biomarkers.

As an illustration of a highly successful procedure of QD staining may serve QD staining of FFPE tissue specimens conducted by the group of Prof. Dr. Nie.[51] The overgrowth of prostate cancer to the bone as a result of epithelial-mesenchymal transition (EMT) can be studied using QD tagging. EMT is a typical process for malignant cells which is characterized by the increase in cell mobility caused by the changes in cellular adhesion molecules, in particular, an increase of N-cadherin and a loss of E-cadherin. Amongst the other important markers one can name the cytoskeleton proteins vimentin, cytokeratin 18, and RANKL. The two phenotypes characteristic of prostate cancer progression are represented by two cell lines (ARCaPe and ARCaPm). The first one defines the onset of the disease and this line is more epithelial-like and less invasive, while the second is more invasive and has more mesenchymal characteristics, indicating a fully ongoing cancer. Nie *et al.* analyzed these two FFPE prostate cancer cell lines using QD-conjugated antibodies.

Simultaneous staining of four different biomarkers was achieved with profiles consistent with Western blot data (Fig. 4.2). But unlike Western blot, QD staining provides inter- and intracellular localization information.

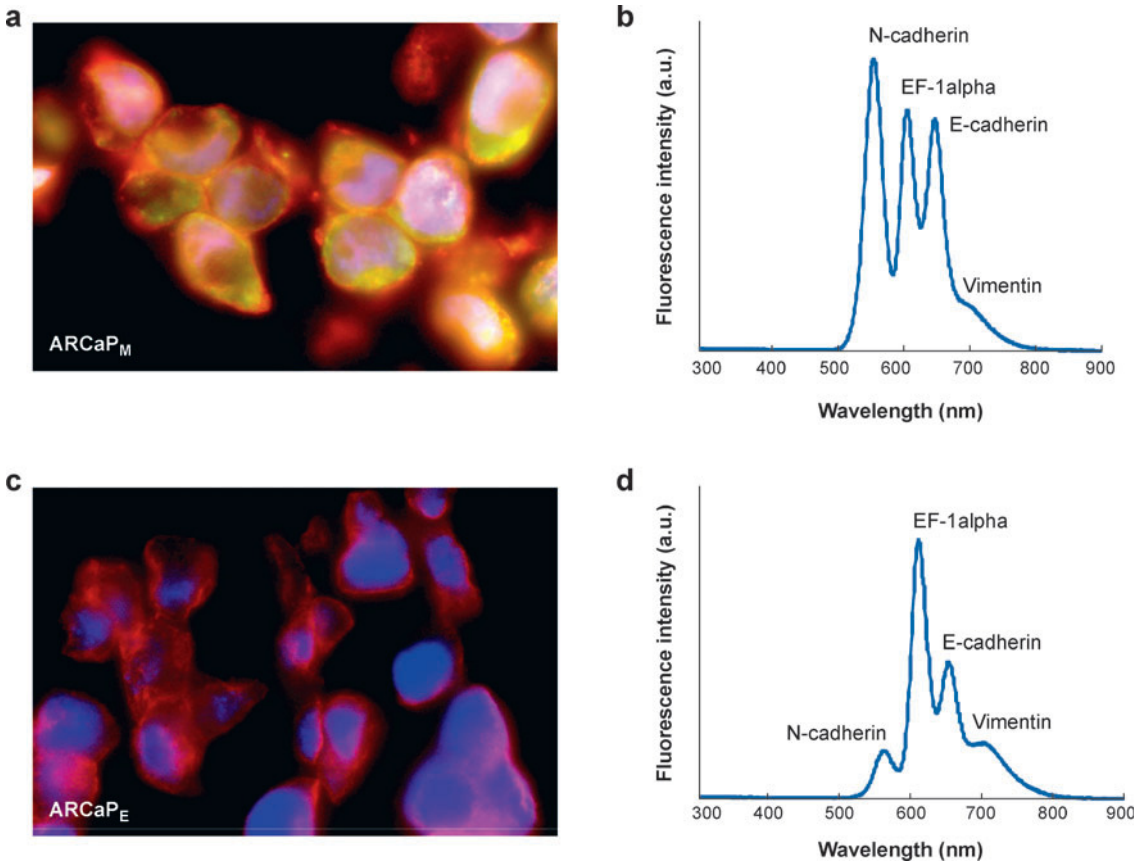

Fig. 4.2 Multiplexed QD profiling of four tumor biomarkers using two FFPE prostate cancer cell lines (ARCaPe and ARCaPm) with distinct bone-metastasis behaviors. The four markers, all associated with epithelial-mesenchymal transition (EMT), are N-cadherin, EF (elongation factor)-1alpha, E-cadherin, and vimentin, and their corresponding QD colors are 565 nm, 605 nm, 655 nm, and 705 nm, respectively. The cell nuclei were counterstained blue by DAPI, and the spectra were captured under blue excitation. **(a)** Color fluorescence image of highly metastatic prostate cancer cells (clone ARCaPm); **(b)** single-cell fluorescence spectrum obtained from image **(a)**; **(c)** color fluorescence image of benign prostate cancer cells (clone ARCaPe); **(d)** single-cell spectrum obtained from image **(c)**. The relative abundance of these markers is consistent with previousWestern blot data. Note that individual cancer cells have heterogeneous expression patterns, and that the single-cell data in **(b)** and **(d)** are representative of a heterogeneous cell population.

Nie *et al.* chose four tumour behaviour correlated antigens (mdm-2, p53, EGR-1, and p21) for molecular profiling of clinical FFPE samples. These four markers are important in prostate cancer diagnosis. The biomarkers were indeed detected in the tissue specimens (Fig. 4.3) but they had a higher autofluorescence than that of FFPE cells. However, autofluorescence can be desirable as it acts as tissue morphology counterstaining. If desired one can deliberately illuminate the sample to bleach out the autofluorescence while leaving the QDs bright enough for imaging and spectral analysis. From the abovementioned results one can clearly see the practicability of QD tagging of FFPE clinical specimens for molecular profiling, which makes QDs a promising tool for multiplexed molecular profiling of clinical tissue specimens and for the studies of biomarkers and cancer behaviour correlation.

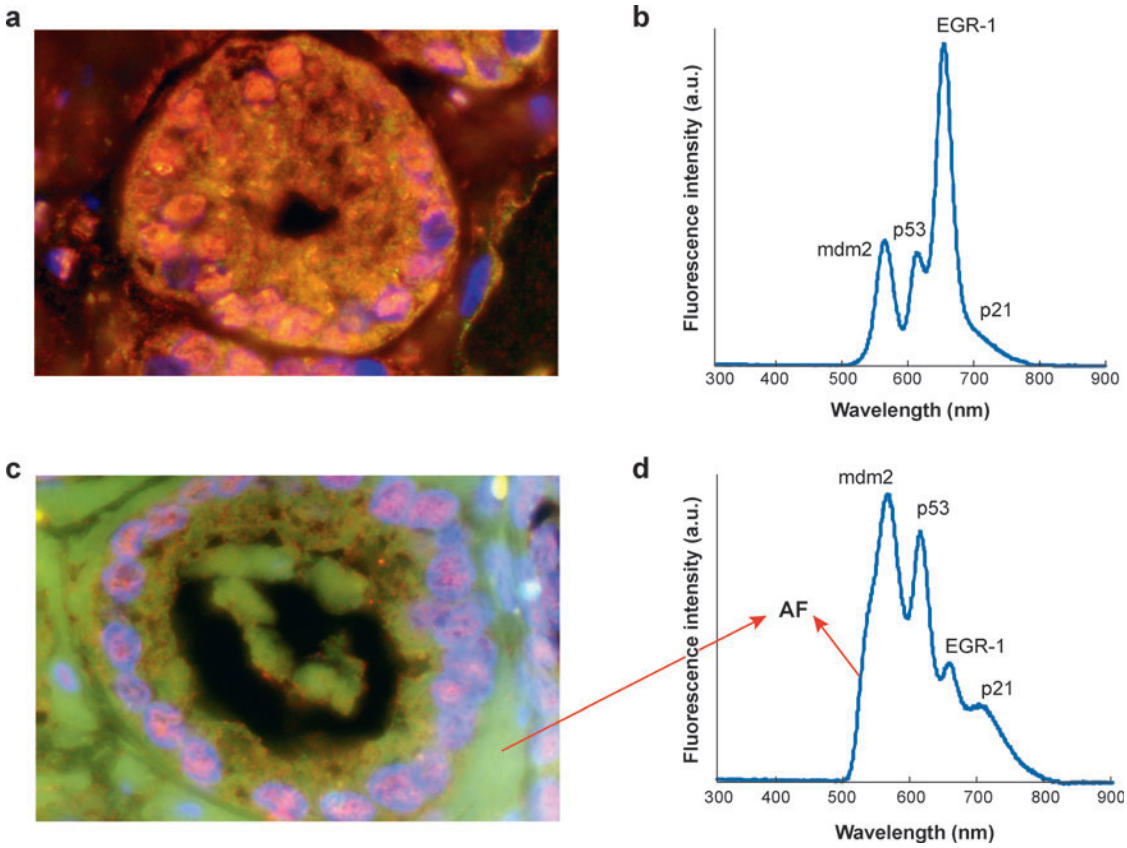

Fig. 4.3 Multiplexed QD staining of archived FFPE clinical specimen from human prostate cancer patients and comparison between two different glands on the same tissue specimen. Four tumor biomarkers (mdm-2, p53, EGR-1, and p21) were labeled with four colors of QDs emitting at 565 nm, 605 nm, 655 nm, and 705 nm, respectively. **(a)** Color fluorescence image of QD-stained tissue specimens showing one prostate gland; **(b)** representative fluorescence spectrum obtained from individual cells in the gland (image **a**); **(c)** color fluorescence image of the same QD-stained tissue specimens showing a different gland; **(d)** representative fluorescence spectrum obtained from single cells in the second gland (image **c**). Note the distinct biomarker profiles for these two prostate glands, demonstrating the ability to resolve cellular populations in highly heterogeneous human tissue specimens. AF stands for autofluorescence and provides information on tissue morphology

5. TARGETED CANCER THERAPY

Present anticancer drugs are known to be systematically toxic to the human body. Further, they possess a number of adverse effects since they do not greatly distinguish between normal and tumour cells. These agents have a number of side effects, such as bone marrow suppression, cardiomyopathy, and neurotoxicity etc., and that is why the maximal drug dose is greatly limited. High concentrations of cytostatics are however needed since they quickly become widely spread into the organs where the presence of anticancer drug was not required. All these reasons impose economical and toxicological limitations on current cytostatic drugs. A problem of targeted drug delivery can be solved by nanotechnology and thus it would be beneficial to cancer patients. Targeted nanoparticles application, namely for drug delivery is in fact the most exciting and clinically important subdivision of nanomedicine. Different targeting mechanisms and most important drug delivery agents are discussed in the following chapter.

5.1. Passive *vs*. active targeting

All malignant tumours are characterized by permeable blood vessels and poor lymphatic outflow. These features give rise to two useful effects: enhanced permeability and retention (EPR) effect[52] (Fig. 5.1) which allows a nanocarrier to escape directly into the tumour tissues via these leaky vessels; and the second is retention of nanocarriers since they cannot escape via non-functioning lymphatic drainage and thus they can continuously release drugs around the tumour cells. Liposomes of different mean sizes were used to determine the threshold vesicle size for escape into tumours and it was found to be around 400 nm. Other studies showed that particles with diameters <200 nm are more effective.[53]

Obviously, passive targeting has a number of drawbacks. Some drugs cannot diffuse efficiently to the targeted cells within a tumour and it is difficult to control the process which may give rise to multiple-drug resistance – a failure of chemotherapy treatments because cancer cells become resistant the drugs. Further limitations of the passive strategy arise in case a tumour does not have the EPR effect, and the permeability of blood vessels may change throughout a tumour.

An obvious solution of these problems is to somehow force the nanocarriers after escaping from the blood flow to selectively bind to particular cells. This binding may be accomplished by supplying the surface of nanocarriers with targeting moieties such as ligands (molecules that bind to specific receptors on the cell surface). The interaction between a ligand and a receptor will make nanocarriers identify and bind to specific cells, become internalized by them before the drug is released inside the cell (Fig. 5.1). It goes without saying that the targeting moiety of the nanocarrier must possess a high selectivity to the surface molecules of the targeted cell.

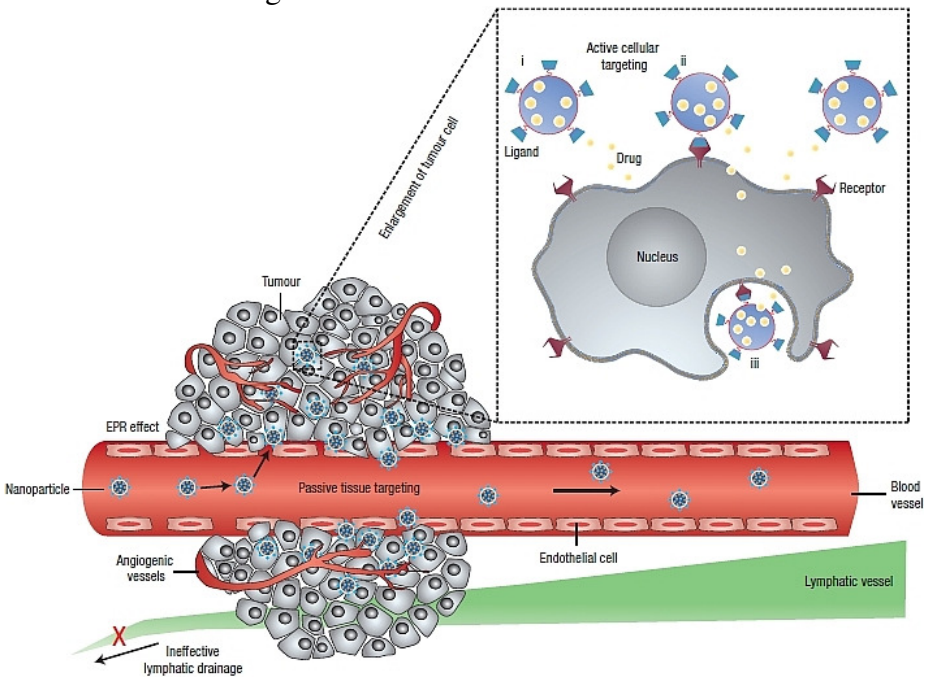

Fig. 5.1 Schematic representation of different mechanisms of drug delivery to tumours. Polymeric nanoparticles are shown as representative nanocarriers (circles). Passive tissue targeting is achieved by means EPR effect. Active cellular targeting can be achieved by functionalizing the surface of nanoparticles with ligands that promote cell-specific recognition and binding. The nanoparticles can **(i)** release their contents in close proximity to the target cells; **(ii)** attach to the membrane of the cell and act as an extracellular sustained-release drug depot; or **(iii)** internalize into the cell.

5.2. The arsenal of nanocarriers

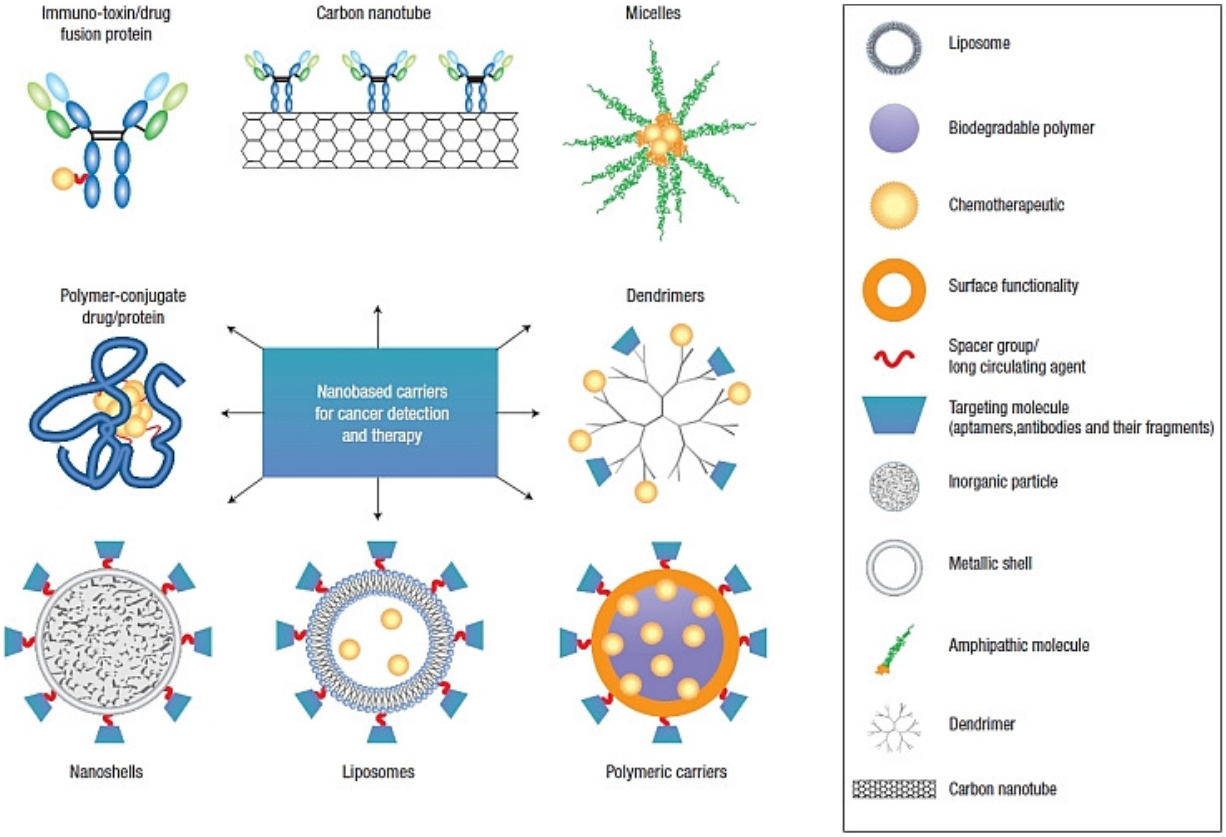

Fig. 5.2 Examples of nanocarriers for targeting cancer. A whole range of delivery agents are possible but the main components typically include a nanocarrier, a targeting moiety conjugated to the nanocarrier, and a cargo (such as the desired chemotherapeutic drugs).

5.2.1. Polymer nanoconjugates

Polymeric nanoconjugates represent a novel technology in nanomedicine. These agents can be supplied by numerous tumour directing biochemically active groups by covalently binding them to the numerous functional groups.[54] One can simultaneously interfere in several tumour pathways by functionalizing nanoconjugates with more than one group, thus accomplishing the delivery of optimal drug concentrations to the cancer site, and the reduction of side-effects on healthy tissues. The accumulation of nanoconjugate drugs in nontargeted organs is obviously not desired and therefore biodegradable nanoconjugates are preferred to non-biodegradable ones. Biodegradability implies eventual nanoconjugate disintegration into water and carbon dioxide.

Comparing to physical entities, represented by unconjugated nanodelivery vehicles (micelles, liposomes, etc.), nanoconjugates are totally chemical entities of targeting and other functional molecules. Nanoconjugates are also less immunogenic, chemically more stable in plasma smaller in size than micelles and liposomes. This class of carriers accumulates at the tumour site and is removed slower because of their high molecular weight. Drug resistance, toxicity and other disadvantages of present chemotherapy can be overcome by specifically targeting the nanoconjugates to tumour cells, enhancing the drug uptake by cancer cells, and bypassing multidrug resistance transporters. Whereas all the privileges of multi-functional

and biodegradable drugs could drastically improve cancer treatment, relatively few multi-functional drug delivery systems have been introduced.

Phase I and II clinical trials of around a dozen polymer–drug conjugates intended for tumour blood-vessel targeting are now being conducted. Here are a few examples of them: anti-endothelial immunoconjugates, fusion proteins,[55] and caplostatin, the first polymer-angiogenesis inhibitor conjugates.[56] Since the pharmacokinetic profile of polymers changes upon the chemical functionalization, they are often regarded as new chemical entities. Four drugs (doxorubicin, camptothecin, paclitaxel, and platinate) and four polymers (N-(2-hydroxylpropyl)methacrylamide (HPMA) copolymer, poly-*L*glutamic acid, poly(ethylene glycol) (PEG), and Dextran) are the only that have successfully been used polymer–drug conjugate development.[56]

5.2.2. Micelles

Micelles are spherical structures, composed of amphiphilic block copolymers, which self-assemble to form a core/shell structure in aqueous media (Fig. 5.3).[57] The hydrophobic core region can obviously act as a storage for hydrophobic drugs, whereas the outer hydrophilic shell acts as a stabilizer of the hydrophobic core and makes the micelle water-soluble, which allows their intravenous administration.[58] There are two ways of loading the drug into a polymeric micelle: encasing the drug physically or covalently attaching it.

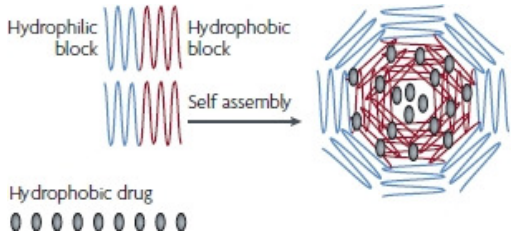

Fig. 5.3 The formation of a polymeric micelle

Polymeric micelles exhibit exceptional stability and biocompatibility, and they can solubilize various poorly soluble anti-cancer drugs; drug-loaded micelles of different types are currently undergoing preclinical and clinical trials. A phase I and pharmacokinetic study has been conducted in patients with advanced refractory malignancies.[59]

5.2.3. Dendrimers

Dendrimers are complex spherical molecules defined by core, branched units and end groups.[60] Well-controlled synthesis methods enable new classes of dendrimers that can be utilized to carry therapeutic or diagnostic agents. Hydrophobic/hydrophilic self-assembly causes dendrimers to form micelles. To make the release of dendrimer micelle payload site-specific, one needs to make it sensitive to pH change or an enzyme that would disrupt dendrimer micelles and lead to the release of a drug. Polyester dendrimers for intracellular release of doxorubicin after hydrolysis of hydrazone linkage were developed based on 2,2-bis(hydroxymethyl)-propanoic acid

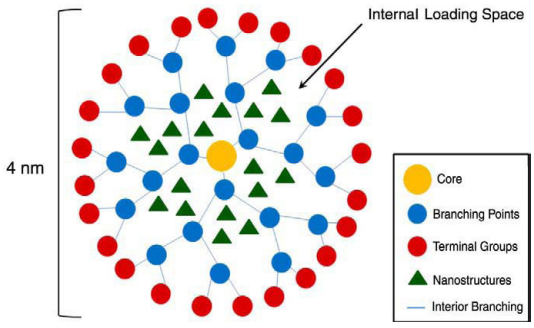

Fig. 5.4 A schematic of a third-generation dendrimer. There is space within the dendrimer to load molecular cargo or other nanostructures. The terminal groups provide the surface functionalities and can include dyes, markers, and target directing groups

monomers.[61] Polyester dendrimers appear to be rather challenging as drug delivery systems and that is why extensive studies of structure and molecular weight optimization are currently being conducted. It was shown that higher molecular weights lead to longer circulation times and that the degree of branching affects the circulation time as well as renal clearance of the drug. The treatment of in mice C-26 colon carcinomas efficiency of polyester dendrimer drug conjugate were proven to be simimilar to that of Doxil.[62]

A remarkable dendrimer is polyamidoamine (PAMAM) dendrimers. It displays biocompatibility, monodispersity and pH responsivity. A number of dendrimers is now commercially available, differing by cores (diamino derivatives and ethylenediamine) and surface functional groups (alcohol, amine, succinic acid, and carboxylic acid). Outstanding *in vitro* and *in vivo* results were achieved using multifunctional PAMAM dendrimers with an imaging agent (fluorescein isothiocyanate FITC), a cancer cell targeting molecule (folic acid) and a therapeutic drug (Taxol).[63]

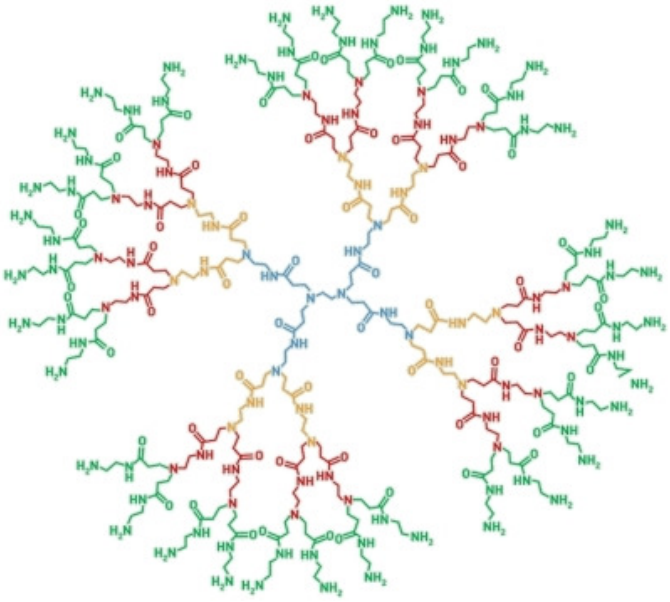

Fig. 5.5 Third generation PAMAM dendrimer

5.2.4. Liposomes

Nanoparticles formed by an amphiphilic lamellar membrane of lipids are liposomes.[60] Possessing a hydrophilic head group and a hydrophobic tail, lipids assemble into bilayers as a result of hydrophobic interactions, thus forming liposomes. The unique property of them is that they are able to carry hydrophilic and hydrophobic molecules simultaneously therefore opening possibilities for combined cancer treatment therapy. Furthermore, through introduction of some modifications one can increase liposome circulation times and enhance their accumulation within a tumour.[64]

First FDA approved liposome was Doxorubicin encapsulated liposome (Doxil) in 1995. It is highly active against numerous neoplasms including Kaposi's sarcoma[65] and ovarian cancer[66] and it has now acquired a routine use in medicine. The circulation of the Doxil in the blood stream is prolonged with the help of surface functionalization with methoxypolyethylene glycol. Doxil is highly successful clinically and this resulted in FDA approval of a number of other new liposomes, such as DaunoXome (daunorubicin liposomes), DepoDur (morphine liposomes), and Ambisome (amphotericin B liposomes). DaunoXome is a 45 nm liposome that is active against Kaposi's sarcoma and other tumours.[67] Though being a powerful tool in cancer treatment, liposomes still lack sufficient stabilities and drug release profiles *in vivo*.

5.2.5. Nanoshells

Oppositely charged polymers form a thin multilamellar structure via the self-assembly and thus form polymeric nanoshells (20 to 60 nm) that can further assemble into a core/shell structure.[60] They can be effectively loaded with drugs since nanoshells comprise layer-by-layer assembled nanoparticles and it means that polymer properties and drug diffusion coefficient define the therapeutic release rate. An advantage of polymer nanoshells is that they can easily be targeted to the tumour since their surface functionalization presents no difficulties.

Another interesting class of nanoshells includes metallic nanoshells (~20 nm). They have a dielectric core which is coated with a thin layer of metal for refining upon their biocompatibility and optical absorption properties. The latter property may be used for photothermal therapy in the near IR spectrum with the help of gold nanoshells.[68] In a similar way IR sensitivity may be exploited using polymeric hydrogels or optically active nanoshells to accomplish photothermally modulated drug delivery. For example the controlled release of 5-fluorouracil was achieved using the nanoshell particles with a magnetic core (carbonyl iron) and a biodegradable poly(butylcyanoacrylate) (PBCA) shell.[69] Magnetic nanoshells with a polymer encased drug can be targeted to specific locations in the body by applying an external magnetic field.

6. HYPERTHERMIA-BASED THERAPY

One of the conventional treatments for solid tumours is its surgical removal which can be limited for poorly localized tumours.[7] That is why in that case a hyperthermia-based therapy may be an alternative. Hyperthermia-based therapy implies tumour destruction by elevated temperatures. The new candidates for this purpose are noble metal nanostructures which can be tuned for optimal susceptibility in the near IR region by varying their size, shape and aggregation state. These highly IR absorbing materials make use of a surface plasmon resonance that efficiently produces desease site localized heat upon laser irradiation. Feasibility of this method was successfully demonstrated in animal models.[70]

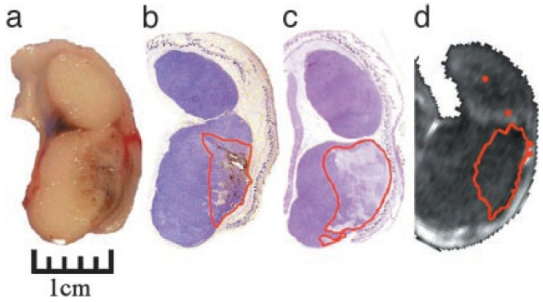

Fig. 6.1 Gold-nanoshell-mediated photodermal ablation of human breast carcinoma tumors implanted in mice. **(a)** Gross pathology after in vivo treatment with nanoshells and NIR laser reveal hemorrhaging and loss of tissue birefringence beneath the apical tissue surface. **(b)** Silver staining of a tissue section reveals the region of localized nanoshells (outlined in red). **(c)** Hematoxylin_eosin staining within the same plane clearly shows tissue damage within the area occupied by nanoshells. **(d)** Likewise, MRTI calculations reveal an area of irreversible thermal damage of similar dimension to **a**, **b**, and **c**.

Photothermal therapy may also be achieved using another phenomenon, namely heat induction in iron oxide nanoparticles upon magnetic field application. Ivkov *et al*. proved that tumour cells are effectively destroyed if a temperature of > 42°C is kept for over 30 min.[71] Though being successful in animal models, these methods are still not used in humans. An obstacle here is the need of control heat delivery: it should not affect healthy tissues that side with the cancerous ones.

7. FUTURE

Nanotechnology industry has begun its successful expansion into biomedicine. The capabilities of traditional imaging, delivery, and sensing devices were surpassed within a very short time by the nanoparticle tools. Currently available dyes cannot provide a long-term imaging of numerous cell markers because of their photobleaching property. In comparison with previous micron-scale agents, nanoparticles are able to penetrate endothelial barriers to reach tumour sites. Nanotechnology is really promising but it surely still needs some time to develop so it is a question 'when', not 'if' nanoparticles will become a standard tool in biomedicine.

So far we mostly discussed numerous positive features of nanoparticle therapeutics for cancer, but there are surely drawbacks present. Nanoparticle behaviour in the human body is not yet extensively studied and that is why nanoparticle toxicity makes a valid issue to concern about since nanoparticle size and surface properties allow them to reach the locations that are not reachable for larger particles. Different mechanisms such as binding to proteins in the blood or removal by macrophages can have its influence upon nanoparticle biodistribution. The latter phenomenon was investigated in rat blood–brain barrier using differently charged nanoparticles and it appeared that those that were highly charged altered the integrity of the blood–brain barrier irregardless of the charge sign. We can conclude that nanoparticle biocompatibility is yet to be fully defined. Nanostructure harmfulness was studied in animals and it turned out that toxicities vary significantly depending on the nanomaterial itself. Some nanoparticles described in this review have nevertheless passed strict toxicity tests and are already used in humans for years. The more biocompatibility data are available, the better we understand what is required to tune the properties of nanoparticles to ensure their safety for systemic use.

Multicomponent nature of nanoparticles is likely to cause their manufacture to be difficult and expensive but nevertheless, a few intricate nanoparticles reached the clinic. For example, CALAA-01 has four components that assembles into a highly multifunctional siRNA containing nanoparticle that currently undergoes clinical studies. This example shows that complex nanoparticles can be manufactured at current Good Manufacturing Practice and fulfil the requirements for at least Phase I trials initiation. It is still an open question whether these extremely complex nanoparticles will ever be of a routine use and if so they will be very expensive.

Nanoparticles having both imaging and therapeutic agent are being extensively developed though there is also a number of situations where this combination is not desired. It is obvious that therapy does not necessarily every time have to be juxtaposed with imaging. There is surely no sense in targeting an expensive imaging agent and afterwards not making any use of it. A dual nanoparticle can obviously be substituted by individual nanoparticles: for imaging and therapy purpose separately. It is however important to keep the same size and surface properties for both nanoparticles so that their biodistribution would be the same and thus the imaging agent would localize similarly to the therapeutic agent. Therefore the existence of the tumour is verified before the treatment and in case it is needed the nanoparticle is supposed to get freely to the target since the size and surface properties of the imaging and therapeutic nanoparticles are similar. Nanoparticles that can carry numerous types of therapeutics and imaging agents can exploit this strategy thus a so-called personalized medicine would be achieved. Personalized medicine implies that no treatment is performed until it is definitely known that the target actually exists in the patient.

A wide application of nanoparticle therapeutics in the future is doubtless though there are still many challenges and problems on the way of nanomedicine to become approved and eventually routine clinical practice. However, numerous advantages of nanoparticles should fuel their meticulous investigation and ultimately result in a breakthrough in oncology.

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