

Nanoparticles in Cancer

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Abstract: Nano-engineered particles have been developed to reach specific molecular targets on diseased cells and have been used in various experimental and clinical conditions. The medical application involves diagnostic and therapeutic applications and a large deal of this research concerns malignant disease. Various approaches have been tried to effectively reach the cancer cell and PEGylated liposomes have demonstrated targeting and controlled release of antineoplastic drugs. For cancer diagnostics nanoparticles have been engineered to optimize magnetic resonance imaging, ultrasound imaging and nuclear medicine imaging. Radiolabeled nanoparticles can also be used for therapeutic purposes when tagged with appropriate radionuclides. This article aims to provide an overview how nanomedicine is presently influencing drug design and, more specifically, the development of radiopharmaceuticals for cancer management.

Keywords: Nanoparticles, nanocarriers, oncology, therapy, imaging.

INTRODUCTION

Cancer is on the increase in the developing world as well as in the industrial world. The latest world cancer statistics report that the number of new cancer cases will increase to more than 15 million in 2020 [1]. It is evident that enormous challenges lie ahead for all those that are involved in cancer research and patient management. It should be mentioned that medical research has achieved ground-breaking progress in the field of tumor biology and molecular genetics. This will undoubtedly lead to the development of new and more effective drug therapies in the foreseeable future. In parallel with this progress, on the molecular scale, remarkable developments in medical imaging have kept pace with the other advances. Cancer imaging (ultrasound, magnetic resonance, nuclear medicine and computer tomography) is now recognized as a major modality to measure the effect of treatment. Both the field of drug design and cancer imaging are going to gain from a development that is generally regarded as a fundamental breakthrough in medicine, namely the introduction in nanotechnology.

The integration of nanotechnology with biotechnology and medicine means the ability to uncover the structure and function of biosystems, which intrinsically have an organizational level at the nano-scale. In other words: nanotechnology provides the tools to measure and understand biosystems [2]. More specifically, nanotechnology maybe translated into nanomedicine thereby referring to treatment and curing of diseases at a molecular scale. Indeed, the use of nanoparticles (100 nm or smaller) for delivery and targeting of therapeutic and diagnostic agents is at the forefront of projects in cancer medicine. The targeting and accumulation of drugs to specific sights where the agent is released provides a means to reach high drug concentration at a designated area with far less systemic side effects. Likewise, medical imaging may make a gigantic step forward with the use of nanoparticles with superparamagnetic properties for magnetic resonance imaging (MRI). These developments harbor favorable prospects for diagnostic and therapeutic applications in oncology. This article presents an overview of the possible applications of nanoparticles in medicine, highlighting its utilization in oncology as far as therapeutics and imaging is concerned.

GENERAL FEATURES OF NANOPARTICLES

The pharmaceutical perspectives of nanotechnology are tremendous. Its medical use as drug delivery system and for (non

invasive) imaging has been effected already and the proof of the principle has been delivered.

Summarizing, the following characteristics offer advantages over conventional pharmaceutical agents:

- Nanotechnology produces the smallest functional units by building groups of atoms.
- Nanoparticles are smaller than 100nm and are in the similar size-range as biologicals like viruses, DNA and proteins.

Nanoparticles developed on a platform of biotechnology, nanotechnology and information technology can be used to take part in molecular, biochemical and biological processes, e.g. genetics and pharmacogenomics:

- The surface of nanoparticles can be decorated with various molecules in order to avoid being recognized by the immune system, enabling them to reach their target more efficiently.
- Nanoparticles may be designed to overcome physiological barriers like the blood brain barrier and dermal tight junctions.
- Due to the leaky constitution of neovasculature in malignant tumors, nanoparticles may penetrate the lesion.
- Nanoparticles may carry drugs and be designed to release their contents at a site of disease.
- Nanoparticles may consist of an inorganic core of superparamagnetic materials coated with polymer such as dextran. These particles are used as contrast agents in magnetic resonance imaging for diagnostic applications and therapy monitoring.
- More specific tissue targeting ("functionalizing") can be achieved by the conjugation of the nanoparticle with a ligand e.g. monoclonal antibodies.

DRUG DELIVERY WITH NANOPARTICLES

Various drug delivery and drug targeting systems are currently applied or under development. Drug carriers include synthetic polymers, microcapsules, liposomes, dendrimers and many others and have one thing in common, namely the increased drug bio-availability and increased accumulation at the pathological site. This is especially important for anticancer drugs, which should preferably be delivered locally with minimal or no effect onto normal tissue. Drug containing nanoparticles can be made slowly biodegradable, thus delivering their pay-load at a controlled rate. Furthermore, nanocarriers are ideal entities to deliver poorly water-soluble agents at the desired site [3]. These factors may

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contribute considerably to the drug performance and clinical acceptance.

PASSIVE TARGETING BY NANOPARTICLES

In order to acquire an efficacious therapeutic level of pharmaceuticals at a pathological site, nanoparticles should move from the circulation to the lesion. A prerequisite for this process is that the blood carrier stays in the blood long enough to slowly accumulate in the tissue of interest with affected and leaky vasculature. In cancer, this process of passive targeting takes place in a non-specific way through gaps into the tumor interstitial space. These gaps between adjacent endothelial cells with a diameter up till 800nm exist in neoangiogenic blood vessels which serve to supply the tumor with nutrients. Tumor tissues use permeability factors as vascular endothelial growth factor (VEGF) to increase the permeability of tumor blood vessels [4]. Nanoparticles, carrying encapsulated drugs, extravasate into the tumor interstitial space and passively target the tumor tissue. Once the drug is locally released the local concentration becomes many times larger than after conventional systemic intravenous administration. The altered lymphatic drainage of the tumor contributes to this effect [5]. This type of passive targeting is known as "enhanced permeation and retention" (EPR) [6].

ACTIVE TARGETING OF NANOPARTICLES

Larger tumors show poor vascularization, especially inside the necrotic areas, which prevents the localization of the nanoparticles and makes local drug deposition impossible.

Because of these inherent limitations the next generation of nanoparticle delivery systems, smart nanocarriers, is being developed. The ultimate goal is to deliver therapeutic agents and contrast agents to the single cancer cell. This can be achieved by conjugating the nanocarrier with biological recognition moieties which recognize cell surface antigens or receptors. Experimental evidence suggests that such a route may be successful. An example is the nanoparticle that targets the folic acid receptor which is overexpressed in various human carcinomas [7]. The nanoparticle conjugated with folic acid as a receptor seeker and containing methotrexate as a chemotherapeutic agent has been demonstrated to enter the cancer cell and inhibit its growth [8]. A widely investigated modality for the recognition of cancer cells is the use of monoclonal antibodies. The recognition of cell surface antigens has been successful in various instances, especially in non-solid tumors as Non-Hodgkin's Lymphoma [9]. However, therapeutic antibodies target their intended cancer cells only in the 0.01-0.001% range [10] and heterogeneity of antigenic expression, antigenic modulation and cross-reactivity with other tissues than the tumor present limitations which are difficult to overcome.

NANOPARTICLE ARCHITECTURE

Nanoparticles are designed as multifunctional diagnostic and therapeutic devices. For pharmaceutical purposes and more specifically for cancer drug delivery and cancer imaging various carrier types have been developed. Most of these nanoparticles are created in such a way that a variety of molecules (drug pay-loads) can be attached to the surface or encapsulated in the interior of the particle. For a better understanding of this exciting field, we provide a short overview of nanoparticles which appear to be important for cancer medicine (*casu quo*: drug treatment and imaging both in pre-clinical research and clinical practice).

Nanocrystals consist of aggregates of around hundreds of drug molecules combined in a single crystal with a thin coating as a surfactant. Nanocrystals are produced according to a technique called nanonization using dispersion in an aqueous surfactant solution

[11, 12]. Nanocrystals are advantageous as an oral delivery system for various drugs as problems as poorly drug solubility are solved in this way with no or virtually no potential toxicity. Effective treatment with these type of carriers of gastrointestinal tract disorders has been reported [13].

Carbon nanotubes are sheets of atoms in the form of tubes of which the size mimics the physical dimensions of nucleic acids [14]. They are considered optimal vehicles for the delivery of genes, peptides and proteins, but at this time only used under experimental conditions [15].

Liposomes are closed vehicles in which drug molecules are entrapped in a central aqueous space surrounded by a membranous lipid bilayer of phospholipids. The drug may also be intercalated into the lipid bilayers, depending on the way of production. For already two decades these particles have proven their value in basic research and human application for drug delivery. A special modification is the surface attachment of polyethylene glycol (PEG) to the bilayer, producing the so-called stealth liposomes, in order to alter the pharmacodynamic profile and improve the immunologic biocompatibility [16]. These "Trojan Horses" have been shown of minimal systemic toxicity and specific designs prevent early degradation [17]. Liposomes are, up till now, the most used nanocarriers for targeted drug delivery in the clinical setting [18].

Solid lipid nanoparticles are colloidal carriers consisting of a monolayer of phospholipid coating around a solid hydrophobic core containing the drug in a high melting fat matrix. The drug, being in a solid state, is released slowly from the particle and this delivery system may offer advantageous in terms of biodegradation and tolerance [19].

Polymeric nanoparticles typically consist of polylactic acid, polyglycolic acid or acrylates. These colloidal carriers, prepared as nanospheres or nanocapsules, contain the drug in an entrapped or encapsulated form. Their design allows for the highly concentrated drug delivery at a desired location, but the main obstacle for human use is their cytotoxicity for macrophages [20].

Dendrimers are highly branched macromolecules with a controlled three-dimensional architecture [21]. The branches are structured around a designed central core and, like a tree, expand outward *via* polymerization reactions, which allow for exact shaping of the nanoparticle. The branched structure makes it possible to attach other molecules like drugs and contrast agents to the surface. Toxicity is minimized by "hiding" the drug molecules in the interior of the dendrimer. These carriers may be made as an uniform population of molecules with a size ranging from 1 to 10 nm in diameter, resembling that of biomolecules like proteins. Their low immunogenicity make *in vivo* applications within reach, especially for targeting of tumors by transversion through vascular pores. The abundant reactive sites provide numerous possibilities for surface modification to improve targeting [22].

Viruses have the capacity to target cancer cells and deliver drugs in both the cytosol and the cell nucleus. The functionality of several nanocarriers can, at least theoretically, be improved by constructing hybrid virus nanoparticles which could use the targeting virus characteristics to selectively invade cancer cells, using a similar mechanism as proposed for gene therapy [23].

CANCER IMAGING WITH NANOPARTICLES

Optical Imaging with Quantum Dots

Nanocrystals possess unique properties which make them suitable for optical imaging. Optical fluorescent imaging is achieved using these nanocrystals made of cadmium selenide, cadmium sulfide or cadmium telluride, surrounded by an inert polymer coating. These semiconductor crystals or quantum dots ("qdots") are used as fluorescent labels of live cells, receptors and oncologic markers [24].

Qdots absorb white light and re-emit the light a few nanoseconds later. This light has a tunable wavelength, depending on the size and nature of the nanocrystal, ranging from ultraviolet till far-infrared, including the visible spectrum. The particle can be functionalized ("active targeting") by way of molecular recognition systems like monoclonal antibodies attached to the outer coating against a specific target or via receptor interactions with specialized ligands. The intrinsic limitation of qdots, however, is the low penetration depth of light through tissues, which hampers external *in vivo* imaging. In an attempt to overcome this disadvantage near-infrared fluorescent qdots have recently been developed which enabled the visualization of e.g. lymph nodes in mice and xenografted tumors [25,26]. Although these refinements offer interesting possibilities for biological investigations, including oncological research, human applications are not foreseeable because the visualization of deeper situated organs is not possible.

Magnetic Resonance Imaging (MRI)

Whereas X-ray based modalities offer information on attenuation and blood flow, MRI offers a wealth of information on local biology and pathology based on nuclear magnetic resonance signals, received from hydrogen nuclei present in the organism under different (patho) physiological conditions. MRI lends itself exquisitely to creating three dimensional images of the body and can be used to characterize many types of tissues. Tumors can be highlighted with contrast agents (e.g. gadolinium chelates) which help to enhance the image contrast for diagnosis and the assessment of treatment response.

For MRI much attention has been devoted to the development of superparamagnetic nanoparticles for contrast enhancement. These particles consist of an inorganic core of iron oxide (Fe^{2+} or Fe^{3+} salts), often coated with polyethylene glycol or dextran. These nanoparticles possess large magnetic moments and disturb a homogeneous magnetic field. The contrast enhancement is due to this effect which results in signal reduction on T2-weighted images, also known as "negative contrast". Their size affects their plasma half life and biodistribution properties and on that basis they are usually categorized in two groups:

SPIOs (superparamagnetic iron oxides) with an average size greater than 50 nm and usually subjected to uptake by Kupffer cells (reticuloendothelial system).

USPIOs (ultra small paramagnetic iron oxides) with an average size lower than 50 nm allowing longer circulation and final removal by the lymphatic system.

Commercially available SPIOs as MRI contrast enhancers include Lumirem® and Endorem®, respectively in clinical use for imaging of the gastro intestinal tract and for liver and spleen tissue. In the latter application the increased contrast between healthy and diseased tissue facilitates the detection of space occupying lesions like primary or secondary liver tumors [27].

A commercially available USPIO is Sinerem® which can be used for bloodpool visualization with MRI. Experimental and clinical tumor imaging may occur on the basis of neovascularization and discriminates tumor tissue from the surrounding normal tissue [28,29]. The selective extravasation at pathological sites requires the engineering of the surface characteristics of these particles to prolong their circulation time also MRI contrast agents have been made "invisible" to macrophages by surface coating with PEG [30,31]. After intravenous interstitial administration USPIOs can be used for the detection of lymph node diseases [32]. In this important application the contrast agent is slowly transported to lymph nodes by way of the lymphatic vessels. Subsequently the contrast agent is internalized by macrophages. In this way the normal lymph nodes accumulate the USPIOs, whereas areas invaded by tumor tissue do not accumulate this contrast agent. The iron-containing nanoparticles

disturb the magnetic field generated by the imaging device and demonstrate signal drop-out on the MRI scan. The affected lymphnodes, not accumulating the USPIOs, appear as bright areas as they do not change the magnetic field homogeneity.

Commercial contrast agents do not have active targeting characteristics and during recent years there has been an increasing interest to functionalize the particles in order to enhance and widen its diagnostic utility. For active targeting a second generation of superparamagnetic nanoparticles (also known as cross-linked iron oxides, CLIOs) have been generated. They can be conjugated to monoclonal antibodies or receptor-seeking agents. This principle of active targeting is well known in diagnostic and therapeutic nuclear medicine [33] in which field radiolabeled biomolecules with unique biological properties have found widespread applications. An example is the experimental study on the targeting ability of G-250 antibody conjugated to MRI contrast agents wrapped in a neutral liposome against renal cell carcinoma [34]. Also, Herceptin® conjugated MRI probes have been shown to allow the successful monitoring of the *in vivo* behaviour of human cancer cells in a mouse model [35]. Of particular interest would be active targeting with MRI contrast agents covalently linked to monoclonal antibodies recognizing epitopes with internalizing properties, thus allowing the magnetic labeling of specific cancer cells [36].

With an eye on the future, folate targeting is an interesting concept as it offers advantages over the use of monoclonal antibodies. Various tumors, including ovarian, colorectal, and lung malignancies, possess folate receptors since DNA methylation is dependent on folate. In contrast to the large majority of antibodies, folate is not immunogenic. Moreover the folate receptor is absent in most human normal tissues. Last but not least, nanoparticles decorated with folic acid are internalized through receptor mediation [37-39].

Nuclear Medicine Imaging and Therapy

To our knowledge the making of nanoparticles did not have a large impact on the development of radiopharmaceuticals in the field of diagnostic and therapeutic nuclear medicine. In a short review Lucignani has summarized a number of relatively new technological approaches for the synthesis of radiotracers [40]. He points out that micro-reactor technology in the use of radioactive tracer synthesis might lead to the use of smaller quantities of expensive chemical precursors. This is of value for the production and quality control of the tumor-agent ^{18}F -fluorodeoxyglucose and other agents like iodinated Annexin V as an apoptosis marker and iodinated doxorubicine for the study of the biodistribution of this anticancer drug. In the field of therapeutic nuclear medicine an interesting development comes from the synthesis of lanthanide nanoparticles in the form of clusters of ^{153}Sm -atoms, meant for peptide mediated tumor treatment [41].

Liposomes can be used to carry radioactive compounds as radiotracers can be linked to multiple locations in liposomes. One option is the hydrated compartment inside the liposome, another the lipid core into which especially hydrophobic conjugates can be attached, and the third option is the outer lipid leaflet where molecules can be bound by covalent linkage. Delivery of agents to the reticuloendothelial system (RES) is easily achieved, since most conventional liposomes are trapped by the RES. For the purpose of delivery of agents to target organs other than RES, long-circulating liposomes have been developed by modifying the liposomal surface. Understanding of the *in vivo* dynamics of liposome-carried agents is required for the evaluation of the bioavailability of drugs encapsulated in liposomes [42].

Non-labeled peptide-targeted liposomes have been used in experimental cancer therapy, and augmented killing (approximately 4-fold) of U937 leukemia and HT1080 sarcoma cells was obtained by the peptide-targeted delivery of doxorubicin-containing liposomes,

compared with control liposomes administered without the peptide (43). Gamma camera imaging of an experimental tumor has been applied to double-labelled liposomes, which were coated with ^{99m}Tc -peptide and encapsulated with ^{125}I -albumin. Targeting of liposomes to the lungs of tumor-bearing mice indicated the existence of non-visible lung micrometastases [44].

In the drug development, the biodistribution and pharmacokinetics of ^{111}In -DTPA-labeled PEGylated liposomes has been studied in 17 patients with locally advanced cancers [45]. Positive tumor images were obtained in 15 of 17 studies (4 of 5 breast, 5 of 5 head and neck, 3 of 4 bronchus, 2 of 2 glioma, and 1 of 1 cervix cancer). The levels of tumor liposome uptake estimated from regions of interest on gamma camera images were approximately 0.5-3.5% of the injected dose at 72 h. The greatest levels of uptake were seen in the patients with head and neck cancers, $33.0 \pm 15.8\%$ ID/kg. A significant localization of the liposomes was seen in the tissues of the reticuloendothelial system (liver, spleen, and bone marrow). Samples of the tumor, adjacent normal mucosa, muscle, fat, skin, and salivary tissue were obtained at operation. The levels of tumor uptake were 8.8 and 15.9% ID/kg, respectively, with tumor uptake exceeding that in normal mucosa by a mean ratio of 2.3:1, in skin by 3.6:1, in salivary gland by 5.6:1, in muscle by 8.3:1, and in fat by 10.8:1.

Molecular imaging with radionuclides gives broad possibilities for drug development [46]. Radionuclides have been used in nanoparticle related drug delivery research in a limited extent. Among nanocarriers for contrast agents, liposomes and micelles draw a special attention because of their easily controlled properties and good pharmacological characteristics. General approaches have been used to prepare liposomes for gamma imaging where the signaling metal atom is chelated into a soluble chelate and then included into the interior of a liposome. Alternatively, DTPA or a similar chelating compound may be chemically derivatized by the incorporation of a hydrophobic group anchoring the chelating moiety onto the liposome surface. ^{111}In and ^{99m}Tc -liposomes have been prepared to try different chelates and hydrophobic groups [47].

For tumor imaging, attention has focused recently for design of polymer nanostructures such as shell cross-linked nanoparticles (SCKs) [48]. In a recent study were ^{64}Cu -radiolabeled folate-conjugated SCKs evaluated as candidate agents to shuttle radionuclides and drugs into tumors, which are over expressing the folate receptor. According to this study ^{64}Cu labeled SCKs showed in mice that functionalized SCKs are promising drug-delivery agents for imaging and therapy of early-stage solid tumors [49].

Applications using no-carrier added ^{64}Cu (half-life = 12.7h) for PET imaging and radiotherapy may have potential to deliver radionuclide to solid tumors together with cytotoxic drugs improving the therapeutic efficacy and imaging at the same time [49].

For thermoablative cancer therapy monoclonal antibody (Mab)-linked iron oxide nanoparticles escaping into the extravascular space binding to cancer cell membrane antigen were followed by In-111 labelling [50].

Penetration Through Blood-Brain-Barrier

Penetration through blood-brain-barrier is a prerequisite in some malignant conditions, such as in treatment of gliomas and brain metastases. There is some experimental evidence, that convention enhanced delivery method can be used in administration of PEGylated liposomal doxorubicin in experimental brain tumor model. The intrathecally administrated drug increased the survival time from 30 days up to 90 days [51]. Coated nanoparticles can be designed for penetrating through blood-brain-barrier (BBB), apolipoprotein A-I on nanoparticle surface acts on scavenger receptor B1 in the BBB [52].

PEG-coated liposomal doxorubicin is known to penetrate the BBB and have additive effects with vincristin and trastuzumab [53].

Radiolabelled PEG-coated liposomal doxorubicin was investigated in 15 patients who had glioblastoma multiforme (5 pts) or brain metastases (10 pts). The drug concentration was 13-19-fold higher in brain tumor than in normal brain tissue and 7-13-fold higher in metastases as compared to normal brain tissue [54].

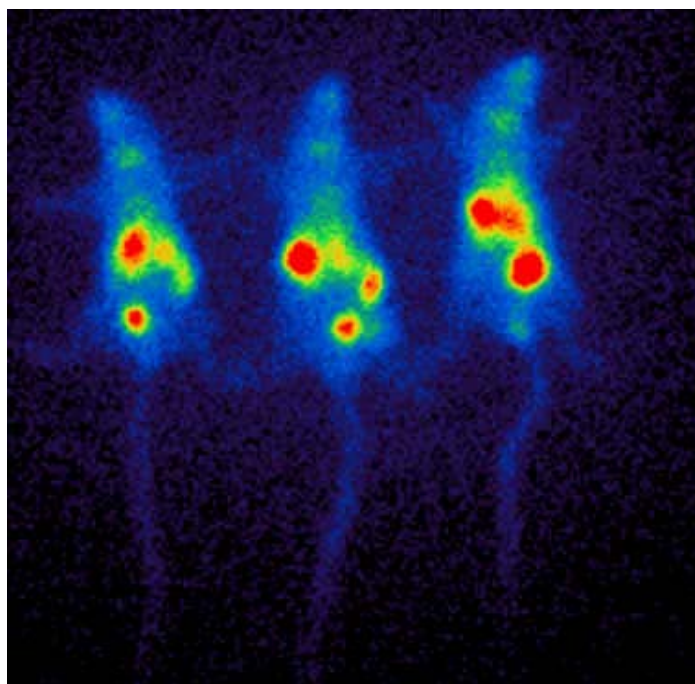


Fig. (1). Mice injected with ^{125}I -labelled peptide targeted PEGylated liposomes.

On the left the mouse was preinjected with large amount cold control peptide (displacement study), the tumor uptake is weak, whereas sufficient tumor uptakes are seen in mice with no peptide blockade (in the middle, on the right).

Ultrasonography

The growth of nanotechnology has opened the way to improve the quality of ultrasound methods for medical diagnosis. Microbubble contrast agents are miniature gas bubbles, which can remain suspended in the circulation for an extended period. During scanning the energy produced by the ultrasound beam causes rapid contraction and expansion of the bubble leading to contrast enhancement. The utilization of these microbubbles includes plaque detection [55, 56], the assessment of hepatic artery patency following liver transplantation [57] and the study of tumor angiogenesis [58]. However, ultrasound examinations suffer from operator dependency, which could influence the results, especially in longitudinal studies. Apart from gas-filled bubbles, also solid nanoparticles have been used to enhance the ultrasonic images. In an experimental setting Liu *et al* [59] have shown that these particles allow the visualization of mouse livers. Whether these solid particles dispersed in agarose have a future for clinical ultrasound diagnosis is not certain.

TOXICITY OF NANOCARRIERS

A common concern with regard to the clinical use of these elegant nanocarriers of all types is the biocompatibility of these agents, which translates into short- and long- term toxicity. It is obvious that cytotoxicity depends for a great deal on the local concentration of the nanocarrier used as well as the degree of exposure of the agent during the cellular contact. Therefore, *in vitro* imitation of the physiological conditions is not sufficient and scientifically unsatisfying. This is especially true for nanocarriers designed to overcome drug solubility, stability and drug-induced side-effects. These nanoparticles may cross the cellular membrane and penetrate the nuclear envelope of the normal cell. Particle uptake by normal endothelial cells [60], pulmonary epithelium [61] and intestinal epithelium [62], as well as the uptake of nanoparticles by macrophages has been described, but a definite answer to the ultimate fate of the nanocarriers themselves has not yet been given [63]. For non-biodegradable particles and their coating agents this question is even more important: inherent to the cellular penetration and possible entrance into the cellular nucleus is the interference with the cellular biochemistry and the possibility of altered gene expression. Therefore, engineered polymeric compounds may have a number of unexpected effects and severe consequences for normal molecular biology [64]. Toxicity issues are of even greater importance in the search for non-viral gene-delivery vehicles which should overcome the severe immunogenic and genetic consequences of the random integration of nucleic acids in the host genome by viral vectors [65].

Moghimi *et al* [66] have reviewed the health risks of the use of nanoparticles in medicine. In their paper hypersensitivity to

nanocarriers as an idiosyncratic reaction is also mentioned as a potential pitfall associated with the use of engineered particles in nanomedicine, presumably secondary to complement activation [67].

In summary, the toxicity-profile of nanoparticles needs special attention and for each type and each application safety tests are required [68]. At the same time it should be realized that nanoparticles open unexpected ways on research on microbiological level as well as for diagnosis and treatment for malignant disease. Nanotechnology is presently neither a plague nor a panacea. The challenge is to uncover the possibilities with an eye on the potential risks.

Another way to go is the use of nanoparticles with hyperthermia. Already in the 1960's hyperthermia was recognized as a means for cancer therapy. These investigations have come so far as realizing the concept of intracellular hyperthermia inducing heat-controlled cellular necroses based on heat production by magnetic nanoparticles under an alternating magnetic field [69]. To achieve complete tumor regression intracellular temperatures have to be higher than 44 centigrades and Japanese researchers have achieved promising results in this field [70-72].

CONCLUSIONS

Summarizing, biotechnical developmets have provided us with nanoparticles of different composition enabling the solubilization of poorly soluble anticancer drugs and, at the same time, increasing their bio-availability. Nanomedicine may help to increase drug resistance to stomach acid and enzymes, allowing better uptake from the small intestine. Specially designed carriers allow controlled biodegradability facilitating drug release at pathological areas, which can be reached *via* the so called enhanced permeability and retention effect due to leaky vasculature. More specific targeting to required areas can be achieved by the attachment of ligand molecules to the carrier surface. The folate receptor seeking ligand is a good example and is applicable in cancer eventually better than large proteins as monoclonal antibodies, which may limit access to tumors. In which way this new technology will transform medicine is , however, difficult to predict as a number of obstacles still need to be taken. One such an obstacle is the present limitation of nanovectors to be specific enough to deliver their payload to the cancer lesions without collateral effects on healthy tissue. As mentioned before, the conjugation with monoclonal antibodies does not provide an optimal way of targeting and the efficacious aiming of nanoparticles needs another approach. Another obstacle belongs to the class of biophysical barriers: once a cancer lesion becomes larger the increased osmotic pressure results in the ejection of the drug. Therefore, not only the specific delivery but also the penetration,

Table 1. Summary of the Differences Between Imaging Modalities and their Possibilities for Nanoparticle Applications. The Method Characteristics (Spatial Resolution, Depth Resolution, Temporal Resolution, Sensitivity, the Amount of Needed Molecular Probe are Modified from the Data in the Literature [77]

| Modality | Spatial resolution | Depth | Temporal resolution | Sensitivity (mol/L) | Molecular probe | Nanoparticle design |
|------------------|--------------------|----------|---------------------|-----------------------|-----------------|---|
| PET | 1-2 mm | No limit | 10 s-min | $10^{-11} - 10^{-12}$ | ng | Label outside, in the membrane, or inside (radionuclide) |
| SPECT | 0.5-1 mm | No limit | min | $10^{-10} - 10^{-11}$ | ng | Label outside, in the membrane, or inside(radionuclide) |
| Bio-luminescence | 3-5 mm | 1-2 mm | sec-min | $10^{-15} - 10^{-17}$ | g-mg | Label inside (or outside), luminescent compound |
| Fluorescence | 2-3 mm | <1 mm | sec-min | $10^{-9} - 10^{-12}$ | g-mg | Label outside or inside, fluorescent compound |
| MRI | 25-100 μ m | No limit | min-hrs | $10^{-3} - 10^{-5}$ | g-mg | Label outside, in the membrane, or inside, paramagnetic atom, particles |
| CT | 50-200 μ m | No limit | min | $10^{-1} - 10^{-4}$ | N/A | Label inside (or outside), contrast media |
| Ultrasound | 50-500 μ m | mm-cm | sec-min | $10^{-1} - 10^{-4}$ | g-mg | Label inside, gas filled particles |

especially in solid tumors, is a major hurdle right now for therapy. To overcome these hurdles significant progress in virology may come to help: researchers are currently studying novel ways for gene delivery and in this context recombinant adeno-associated viruses may serve as low- immunogenic and cell- or tissue specific vectors. Already now the proof of the principle has been delivered in experiments with adeno-associated viruses which penetrate solid tumor tissue [73] and it is to be expected that these tailored viruses can be used as a platform for advanced drug and imaging nanotechnology [74]. Furthermore, double action can be achieved by combining oncolytic viruses with preferential replication in tumor cells with drug containing nanoparticles [75].

One obstacle is of a different kind and concerns the general public opinion, which expresses a skeptical view on such a new kind of technology. This is comprehensible as major breakthroughs are not easily visible to the public at large, focusing on the environmental consequences and toxicity issues. However, especially when cancer treatment is concerned well- directed toxicity is where researchers are looking for, whereas the environmental danger- if there is any- can be controlled by adequate measures.

Although some approaches are still at a basic level and not yet applicable in a clinical situation various nano-scale agents are presently under clinical testing. One successful agent is Abraxane®, consisting of nanoparticles coated with paclitaxel (Taxol®) in use for patients with metastatic breast cancer [76]. Furthermore the U.S. National Institutes of Health presently spends around \$100 million per annum on nanotechnology, a large deal of which is used for cancer research. In the end, safe and efficacious nanocarriers for drug delivery and medical imaging will certainly evolve out of these combined efforts, providing the cancer patients the best quality of life and greatest chances of survival.

We have summarized the differences between imaging modalities and their possibilities for nanoparticle applications (Table 1). The method characteristics (spatial resolution, depth resolution, temporal resolution, sensitivity, the amount of needed molecular probe) give the possibility for multiple options. For ultrasonography and MRI nanoparticle constructs may be essential in targeting biological processes.

In functional *in vivo* imaging the radionuclide methods seem to be most sensitive with subnanomolar amounts of molecular probes.

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