Journal of Biomaterials and Nanobiotechnology, **2012, 3, 269-279**
http://dx.doi.org/10.4236/jbnb.2012.322033 Published Online May 2012 (http://www.SciRP.org/journal/jbnb)

In Vivo Distribution of Inorganic Nanoparticles in Preclinical Models*

Mariana Varna[1,2,3#], Philippe Ratajczak[1,2#], Irmine Ferreira[1,2], Christophe Leboeuf[1,2], Guilhem Bousquet[1,2], Anne Janin[1,2,3†]

[1]Inserm, Paris, France; [2]Université Paris Diderot, Sorbonne Paris Cité, Laboratoire de Pathologie, Paris, France; [3]AP-HP, Department of Pathology, Hôpital Saint-Louis, Paris, France.
Email: †anne.janin728@gmail.com

Received January 20th, 2012; revised February 28th, 2012; accepted March 16th, 2012

ABSTRACT

Ongoing progress in nanotechnologies has led to their implementation for *in vivo* diagnostic and therapy. Thus, the main applications of inorganic nanoparticles are imaging for diagnosis and cell tracking, photothermal and drug-delivery therapies. Following nanoparticles *in vivo* administration, the systemic circulation can distribute them to every body organ and tissue. Precise characterization of nanoparticles distribution and accumulation in the different body parts in preclinical models is required before any application in humans. The biodistribution of inorganic nanoparticles has been analysed in different preclinical models, particularly mouse, rat and rabbit. This review covers the *in vivo* biodistribution of different inorganic nanoparticles in preclinical models: gold nanoparticles, silica nanoparticles, iron oxide magnetic nanoparticles, quantum dots and carbon nanotubes.

Keywords: Nanoparticles; *In Vivo* Biodistribution; Animal Model; Biocompatibility

1. Introduction

Ongoing progress in nanotechnologies has led to their implementation not only for *in vivo* diagnosis but also for imaging, drug delivery and innovative therapies. Different types of nanoparticles have been developed for therapy or imaging. Nanoparticles are interesting because they are easy to synthesise and have numerous imagery applications. In addition, the surface of nanoparticles is fairly easily conjugated with antibodies [1], siRNA [2], or specific ligands for recognition and binding to target cells [3-5].

The ideal nanoparticle agent should be 1) resistant to aggregation, 2) not affected by solvent polarity, ionic strength, 3) resistant to reticuloendothelial system (RES) uptake, 4) it should have high sensitivity and selectivity for the cellular target, and 5) have prolonged circulation times in the blood if administered intravenously [6].

After *in vivo* nanoparticles administration, the systemic circulation can distribute them to all body organs and tissues. Precise characterization of nanoparticles distribution and accumulation in the different body parts in preclinical settings is required before any nanoparticles use, whether for diagnosis, photothermal therapy or drug delivery in humans.

In this review, we will discuss the *in vivo* biodistribution of a number of inorganic nanoparticles in preclinical models: gold, silica, and iron oxide magnetic nanoparticles, quantum dots and carbon nanotubes.

2. Types of Inorganic Nanoparticles and Their Applications

Different types of inorganic nanoparticles are currently being developed [7,8]. Their main applications are: imaging for diagnostic purposes [9,10], cell tracking [11], photothermal therapy [12-15], and drug delivery [14].

In addition, these nanoparticles can be engineered to become biocompatible and thus escape detection by the immune system [1].

There could be major translational applications for nanoparticles in the field of cancer.

When encapsulated in nanoparticles, drugs are protected from disintegration in the liver. They circulate in the blood for longer periods and concentration in tissues is higher [16] and more prolonged [17]. Drug encapsulation in nanoparticles can significantly improve drug biodistribution in tumors and reduce drug toxicity, when compared with the same non-encapsulated drug [16].

Apart from applications in drug encapsulation, nanoparticles can be conjugated with a ligand for active targeting

*The authors each declare no conflict of interest.
#Equally contribution.
†Corresponding author.

of tumor cells.

Gold nanoparticles and carbon nanotubes can also be used in photothermal therapy, because they are able to transform laser irradiation into heat [14,18,19].

3. Inorganic Nanoparticles

3.1. Gold Nanoparticles

3.1.1. General Presentation

Gold nanoparticles have emerged as promising tools because they possess a plasmonic resonance that can be shifted to the near-infrared (NIR) spectral region by shape engineering [20]. This spectral region, known as the "optical therapeutic window", is characterized by low absorption of biological materials, and it allows light to penetrate deeply (up to several cm) into the tissues. Their excellent optical properties make gold nanoparticles good candidates for imaging contrast agents and for innovative cancer therapies [21]. Because of the enhanced permeation and retention, microvessel networks in malignant tumors offer a preferential area of distribution and enhanced uptake for nanoparticles.

Gold nanoparticles can be synthesised into a variety of forms, including spheres, crescents, rods, cages, prisms, stars, using different strategies, the most common being chemical methods, or electrochemical reduction of gold (III) precursors. The chemical, optical and electromagnetic properties are influenced by particle size and shape [22].

Gold nanocrescents possess optical properties that are particularly well suited to biological applications. Their plasmon resonance is split due to the symmetry of the crescent shape. The axial plasmon mode is near 600 nm while the transverse mode is in the near infra-red region (typically around 800 nm). This mode is magneto-inductive and has the unique ability to redirect scattered light in a direction dependent on cup orientation, acting as a three-dimensional nano-antenna. This mode lies in the "optical therapeutic window" which is particularly suitable for *in vivo* application due to the penetration depth of the excitation light into the tissues (several centimeters).

For gold nanorods, their plasmon resonance absorption and scatter in the near infrared region makes them suitable for *in vivo* imaging applications [23]. The light energy, which penetrates tissues, is converted in part into heat, thus acting as an effective contrast agent for *in vivo* bioimaging, and as a thermal converter for photothermal therapy [24]. When functionalised with folates, they can accumulate on the surface of tumor cells and make them highly susceptible to photothermal damage from irradiation at the nanorod plasmon resonance [19]. Gold nanoshells also possess optical and chemical properties for biomedical imaging and therapeutic applications. They are formed of a silica core coated with gold [25]. By varying the size of the core and shell, the optical resonance of these nanoparticles can also vary from near-UV to near-infrared [26]. They can be used for contrast enhancement [27], magnetic resonance imaging [28], molecular imaging [13], and photothermal therapy [29]. In preclinical models, gold nanoshells have been successfully used to deliver tumor necrosis factor (TNF) to solid tumors [30].

3.1.2. *In Vivo* Biodistribution

Gold nanoparticles distribution in organs

Under inductively coupled plasma mass spectrometry (ICP-MS), polyethylene glycol (PEG)-coated gold nanoparticles show prolonged circulation times *in vivo* after systemic injection. Following intravenous injection into mice, 54% of injected PEG-modified gold nanoparticles were found in the blood at 0.5 h [31]. The PEG coating prevents or minimizes adsorption of the gold nanoparticles by macrophages, resulting in a prolongation of blood clearance (up to 48 h) and greater organ exposure [32]. The size of gold nanoparticles influences their *in vivo* distribution. Thus, using transmission electron microscopy, Zhang *et al.*, showed that 5 nm and 10 nm PEGylated gold nanoparticles accumulated in the liver and 30 nm PEGylated gold nanoparticles accumulated in the spleen. The 60 nm PEGylated gold nanoparticles did not accumulate in either of these organs in mice euthanized 28 days after intraperitoneal administration of gold nanoparticles at a dose of 4000 μg/kg [33].

Other studies have evaluated the bioaccumulation and effects of different doses (40, 200, and 400 μg/kg/day) of 12.5 nm gold nanoparticles by intraperitoneal administration in mice every day for 8 days. Using inductively coupled plasma-mass spectrometry, one team quantitatively evaluated biodistribution in tissue samples. The different doses administered did not change blood gold levels, but there was a significant relationship with gold accumulation in the spleen, brain, kidney, liver and lung [34]. The kinetics and distribution of gold nanoparticles with diameters of 10, 50, 100 and 250 nm have also been measured with ICP-MS after intravenous administration in rats. At twenty-four hours, the 10 nm nanoparticles were detected in the blood, liver, spleen, kidney, testis, thymus, heart, lung and brain, whereas the larger nanoparticles (50, 100 and 250 nm) were only detected in the blood, liver and spleen [35]. Another study, also using ICP-MS analysis, showed that gold nanoparticles accumulation depended on particle size. The 15 nm gold nanoparticles were found in the blood, liver, lung, spleen, kidney, brain, heart, and stomach, and were able to pass the blood-brain barrier [36]. In addition, 20 nm, but not 100 nm, gold nanoparticles were found on retinal layers using confocal laser scanning microscope [37]. In 2009,

gold nanoparticles were used as tracers in photoacoustic mapping of lymph drainage to identify sentinel lymph nodes in rats [38]. This study was performed with a deep-penetration photoacoustic microscopy system.

Photoacoustic imagery (PAI) is an emerging hybrid, noninvasive and nonionizing mode of exploration that combines spectral selectivity of molecular excitation by laser light with the high resolution of ultrasound imaging [39]. PAI has been shown to be a powerful tool for imaging blood vessels, but it has also been used to track the uptake, delivery, and excretion of nanoparticles *in vivo* [40,41]. Photoacoustic imaging with gold nanoparticles shows many advantages, including good resolution (50 μm) and low cost.

Gold nanoparticles distribution in tumor tissues

Photoacoustic imaging has also been used to visualize gold nanoparticles accumulated in mouse tumors [42]. The 3D distribution of gold nanoshells injected intravenously into mice, with subcutaneous xenografted human colon tumor, showed preferential accumulation in the tumor site. Two-photon-induced photoluminescence imaging showed a heterogeneous distribution in xenografted tumors [43], perhaps due to the enhanced permeability and retention effect of the abnormal tumor microvasculature [19]. When analysed in photoacoustic microscopy, nanoparticles were found to be more concentrated in the tumor cortex than in its core [44].

The size of the gold nanoparticle is important to consider, since a significantly higher tumor uptake and extravasation from tumor blood vessels was found with 20 nm gold nanoparticles compared to 40 and 80 nm gold nanoparticles using gamma imaging and dark field microscopy [45]. The molecular weight of the PEG chain is also important to consider since, using gamma imaging and dark field microscopy analyses, gold nanorods grafted with PEG 5 kDa and 10 kDa showed significantly greater tumor uptake, extravasation from the tumor blood vessels, and greater circulation stability in mice than those with smaller (2 kDa) or larger (20 kDa) PEG chains [8,45].

3.2. Silica Nanoparticles

3.2.1. General Presentation

Silica nanoparticles have emerged as a promising field for treatments because of their biocompatibility and low toxicity. They have interesting potential in drug delivery [46,47], DNA delivery [48] gene therapy and molecular imaging [49]. Their surface can be functionalized by different groups such as polyethylene glycol, amine, carboxyl or vinyl, rendering them useful for biomedical applications [50,51]. Mesoporous silica nanoparticles (MSNs) have large surface areas with controllable pore size making them attractive as drug-delivery devices, for diagnostic approaches [52-54] or for bone tissue regeneration [55].

3.2.2. *In Vivo* Biodistribution

Silica nanoparticles distribution in organs

The biodistribution of fluorescent-labelled silica particles was analyzed in one study *in vivo* in mice and *ex vivo* on excised tissues using the IVIS 200 imaging system. Twenty-eight days after administration of 70 nm silica nanoparticles to mice, they were detected in the skin, regional lymph nodes, parenchymal hepatocytes, cerebral cortex and hippocampus. In hepatocytes, the silica nanoparticles were distributed throughout the cytoplasm, and inside the nucleus and mitochondria [56]. Silica nanoparticles 20 - 25 nm in diameter, conjugated with near-infrared fluorophores and rendered radioactive with $^{(124)}$I, for optical and PET imaging, were used in another study to assess *in vivo* distribution in nude mice. 75% of the silica nanoparticles accumulated in the liver and spleen, whereas the lung, heart and kidney, accumulated less than 5%. The clearance studies carried out over a period of 15 days indicated hepatobiliary excretion of the nanoparticles. The absence of any adverse effect or any other abnormalities in the tissues was controlled by histological analysis [57].

The elimination time and organ accumulation levels of silica nanoparticles depend on the chemical surface modification. Thus, the biodistribution of three types of surface-modified 45 nm Silica nanoparticles, (OH-Silica nanoparticles, COOH-Silica nanoparticless, and PEG-Silica nanoparticles) were investigated using a fluorescence tracing imagery system. This study showed that after intravenous administration in mice, PEG-Silica nanoparticles had relatively longer blood circulation times and lower uptake by the reticuloendothelial system (RES) organs than OH-Silica nanoparticles and COOH-Silica nanoparticles [58]. Seventy nm silica nanoparticles modified with amine or carboxyl groups, or unmodified, were used to test cell toxicity. Unmodified nanoparticles showed a nuclear localisation, while the modified nanoparticles did not [59].

Different doses of MSNs (ranging from 10 $mg \cdot kg^{-1}$ to 200 $mg \cdot kg^{-1}$) were injected intravenously in nude mice once a day for 10 days by another team. Serological analyses of blood samples and fluorescent imaging of mouse tissues (liver, kidney, lung, heart, intestine, stomach, mesentery and spleen) both showed good tolerance [60]. The effect of surface charge on the uptake of mesoporous silica nanoparticles has also been analysed in 3T3-L1 cells and human mesenchymal stem cells without evidencing changes of viability, proliferation, or differentiation of the treated cells [61].

Silica nanoparticles distribution in tumor tissues

Biodistribution studies using human breast cancer cell MCF-7 xenografts were performed with *in vivo* and fluorescent microscopy imaging, as well as with inductively coupled plasma mass spectroscopy. MSNs preferentially

accumulated in tumors. Nanoparticle fluorescence intensity was greater in tumors than in other tissues and greater at 4 h than at 24 h [60]. Functionalization is important to target a specific cell. MSNs functionalised with folic acid thus exclusively enter cells expressing folate receptor [62]. In two different human pancreatic cancer cell lines (PANC-1 and MiaPaca-2) xenografted in mice, the researchers observed tumor-suppressing effects with silica nanoparticles concomitantly conjugated with camptothecin and folic acid. Measures of silica nanoparticles concentration in the urine with inductively coupled plasma optical emission spectrometry (ICP-OES) showed that most nanoparticles were excreted within 4 days [47].

3.3. Magnetic Nanoparticles

3.3.1. General Presentation

Magnetic iron oxide nanoparticles have been investigated for their magnetic capacities, very promising for magnetic resonance imaging (MRI) contrast enhancement [63], and hyperthermia [64]. A large number of functional groups to target tumors can be attached to their surface, such as antibodies, peptides, or small molecules for diagnostic imaging or drug therapy [65,66]. Moreover surface modifications in these nanoparticles have been shown to render them biocompatibles with long blood retention times and low toxicity [1]. Among the molecules used for coating magnetic nanoparticles to increase their stability and half-life circulation are dextran, polysaccharides, PEG and polyethylene oxide [8]. Superparamagnetic iron oxide nanoparticles (SPIONs) are composed of an iron oxide core of about 5 - 10 nm, and a surrounding layer of stabilising macromolecules, resulting in particle diameters of 30 - 80 nm [67]. The superparamagnetic property of iron oxide is due to a magnetic moment in the presence of an external magnetic field. The most widely used method for synthesis of SPIONs is alkaline co-precipitation of $Fe(OH)_2$ and $Fe(OH)_3$ suspension [68,69].

Ultrasmall superparamagnetic iron oxide (USPIO) nanoparticles have been used in MRI as contrast enhancement agents for clinical diagnosis. Their surface can be modified and different functional groups can be attached. USPIO nanoparticles provide a non-invasive method to detect and label tumor cells [70]. However it is important to determine the biodistribution, clearance, and biocompatibility of magnetic nanoparticles for *in vivo* applications.

3.3.2. *In Vivo* Biodistribution

Magnetic nanoparticles distribution in organs

The influence of magnetic nanoparticle size, composition and surface chemistry in intracellular uptake, biodistribution, macrophage recognition and cytotoxicity, was analysed by [71]. *In vivo*, injected magnetic nanoparticles are captured by macrophages, and this reduces the circulation time in the blood and leads to high levels of biodistribution in the liver (80% - 90%) and the spleen (5% - 8%) [72].

The iron levels in the serum of rats receiving 10 mg Fe/kg magnetic nanoparticles through the tail vein in one study showed a gradual increase for up to 1 week and a slow decrease thereafter. From study of the different organs, the presence of iron was detected in the liver (about 55% of magnetic nanoparticles injected were detected at 6 hours while only 20% were detected after day one), and the spleen, and in smaller quantities in the brain, heart, kidney, and lung. In this model the magnetic nanoparticles did not cause long-term changes in liver enzyme levels [73].

To evaluate lymph node accumulation of SPIONs, another team removed the lymph nodes of rats having received 200 μmoles of nanoparticles injected into the tail vein 24 hours previously. The iron concentration was determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES). In this study, the SPION particles showed a short blood half-life of approximately 13 min for large-size particles (60 - 90 nm), while small-size particles showed a half-life of 90 min [67]. Smaller particles circulate longer than larger particles and can be taken up gradually by lymph nodes and bone marrow [74]. USPIO nanoparticles have been analysed in rats to determine metabolic consequences on the kidney, liver and spleen after intravenous administration. It was shown that alterations of renal, hepatic and splenic function were reflected by changes in the metabolic pathways involved in energy, lipid, glucose, and amino acid metabolism. These effects were linked to their surface chemistry and particle size [75].

In a rabbit model of human atherosclerosis, SPIONs coupled to annexin V showed specific accumulations in the liver, spleen, kidneys and bladder under magnetic resonance imaging [76].

Magnetic nanoparticles distribution in tumor tissues

The bio-distribution of intra-tumoral-injected magnetic nanoparticles was studied in a mouse model. Most magnetic nanoparticles remained in the tumors and less than 1% of injected nanoparticles were detected in the liver and spleen [77]. In a rat model of 9L-glioma brain tumors, PEGylated magnetic nanoparticles were delivered and followed up using magnetic resonance imaging. A preferential accumulation in the tumor xenograft and not in normal tissues was shown in this preclinical model [72].

Magnetic nanoparticles can also be used for specific delivery of chemotherapeutic agents into tumors. The biodistribution of magnetic nanoparticles in the tumor, peritumoral area, different organs and body fluids (blood

and urine) were explored by another team. The concentration of magnetic nanoparticles in the tumor tissues and in the surrounding area was higher under influence an external magnetic field [66].

Numerous studies have used hyperthermia as a therapeutic approach without any damage to surrounding normal tissue [64]. In a pre-clinical model, water-based ferrofluid 10 nm particles were injected into mice with xenografted human prostate tumors, to evaluate how the infusion flow rate affected nanoparticle distribution and tumor temperature. High-resolution microCT imaging was used to assess nanoparticle distribution in the tumour tissue. A non-uniform distribution of these nanoparticles was observed in the tumor xenograft. After hyperthermia, an increase of nanoparticles was observed in the in tumors, suggesting a re-distribution in the tumors during the heating process [78].

Magnetic iron oxide nanoparticles (~15 nm in diameter) were administered in a mouse xenograft model of a human head and neck squamous cell carcinoma cell line (Tu212). Pathological analysis showed destruction of epithelial tumor cells associated with the hyperthermia treatment [79].

3.4. Quantum Dots

General Presentation

The semiconductor nanoparticle and rare-earth nanoparticle families are large and heterogeneous. Among them, some commercial solutions based on light-emitting colloidal nanocrystals are available, such as Quantum dots® (Life Technologies) or Lumidots® (Sigma-Aldricht).

Quantum dots®-Lumidots® have a broad excitation spectrum, and a narrow range of emission wavelengths, which depends on the core size and composition. They also have a high photobleaching threshold, enabling longterm monitoring, and a flexible surface enabling various types of conjugation. For *in vitro* and *in vivo* applications, cadmium selenide cores are coated with a layer of ZnS. These nanoparticles have light absorbance, bright fluorescence, narrow symmetric emission bands, and high photo-stability [80]. For their optical properties, using the quantum confinement effect, Quantum dots®-Lumidots®, made of the same material, emit light of different colors in relation to the size of the nanoparticles.

Fluorescent light energy (color) is inversely proportional to nanoparticle size. Quantum dots®-Lumidots® have been used for *in vitro* imaging of pre-labeled cells (tracking cells). For specific cell targeting, antibodies, streptavidin [81], peptides [82] nucleic acid aptamers [83] or small-molecule ligands can be conjugated to Quantum dots®-Lumidots®.

The chemical nature of Quantum dots®-Lumidots® implies that they have potential toxicity for *in vivo* applications. *In vitro* studies have shown that Cadmium Selenium nanocrystals are highly toxic on cell cultures when UV-excited because under UV illumination the highly toxic cadmium ions are released. However, quantum dots with a stable polymer coating do not show any toxicity in absence of UV irradiation [84,85]. In addition, only little is known about the excretion process of quantum dots from living organisms [86]. Since the core of Quatum Dots/Lumidots contains cadmium and zinc, injection of these nanoparticles in humans is prohibited. Cadmium is an inorganic toxicant of great environmental and occupational concern, classified as a human carcinogen in 1993. Moreover, the association of cadmium with zinc increases the carcinogenic potential of these materials [87]. The penetration of quantum dot particles through human skin has been studied in *ex vivo* models and the Quantum dot particles were found in deep layers by non-invasive multiphoton and confocal laser scanning microscopy [88].

Here, we will not review the *in vivo* distribution of Quantum dots/Lumidots in preclinical models since these nanoparticles cannot be used in translational research because of their potential toxicity in human.

3.5. Carbon Nanoparticles

3.5.1. General Presentation

Carbon nanotubes (CNT) can be either single-walled (SWNTs) or multi-walled carbon nanotubes (MWNTs). They are made from one-atom-thick sheets of carbon. These sheets are rolled at specific ("chiral") angles, and the combination of the rolling angle and the radius determines the nanotube properties.

Most single-walled carbon nanotubes (SWCNT) have a diameter close to 1 nanometer, with a tube length that can be many millions of times greater. The structure of a SWNT can be conceptualized by wrapping a one-atom-thick layer of graphite called a graphene onto a seamless cylinder defined by a pair of indices, n and m, which denote the number of unit vectors along two directions. They can be metallic or semiconducting depending on their structure. Multi-walled nanotubes consist of multiple rolled layers (concentric tubes) of graphite.

The needle-like fiber shape of CNTs is similar to asbestos fibers. Widespread use of carbon nanotubes can lead to pleural mesothelioma, a cancer of the lining of the lungs, or peritoneal mesothelioma, a cancer of the lining of the abdomen [89]. Exposure of the mesothelial lining of the abdomen of mice to long multiwalled carbon nanotubes leads to asbestos-like, length-dependent, inflammatory reaction with granuloma reaction.

Results of experimental studies on rodents collectively show that regardless of the process by which CNTs were synthesized and the types and amounts of metals they contained, CNTs were capable of producing inflamma-

tion, epithelioid granulomas (microscopic nodules), fibrosis, and biochemical/toxicological changes in the lungs. Comparative toxicity studies in which mice were given equal weights of test materials showed that SWCNTs were more toxic than quartz. As a control, ultrafine carbon black particles were shown to produce minimal lung responses in mice [90].

3.5.2. *In Vivo* Biodistribution

After SWNTs injection in mice, a large percentage can be sequestered in the liver, depending on the surface change [91,92]. These nanoparticles can be taken up in organs such as the spleen, lymph node, or bone marrow, which are part of the reticuloendothelial system and contain numerous macrophages [93]. To escape macrophage uptake, MWCNTs have to be coated with ammonium and diethylentriaminepentaacetic chelator functional groups [94]. After intravenous injection, SWCNT functionalised with ammonium do not show any liver uptake and are rapidly excreted in the urine. In contrast, intra-peritoneal injection of SWCNT functionalized with hydroxyl leads to accumulation in the liver and kidneys, with urine excretion in 18 days [95]. To prolong circulation time, SWNTs can also be modified using PEG. PEG-5400-modified SWCNTs have a circulation time (t1/2 = 2 h) that is much longer than PEG-2000-modified counterpart SWCNT (t1/2 = 0.5 h) [92]. Other polymers can be used to functionalize SWCNTs. When PEG is grafted to branched polymers (poly(maleicanhydride-alt-1-octadecene)-PEG methyl ethers (PMHC18-mPEG), the blood circulation time of functionalised SWCNTs was prolonged and their half-time evaluated at 22.1 h [96]. Both SWCNT and MWCNT can aggregate *in vivo*, but SWCNT agglomerates remain the same size and can translocate from the injection site, whereas MWCNT agglomerates grow larger and are not able to translocate [97]. After functionalization of PEGylated SWCNTs with arginine-glycine-aspartic acid (RGD) peptide, the accumulation in integrin-positive U87MG tumors was significantly improved from 3% to 4% to 10% to 15% of the total injected dose (ID)/g, as a result of specific RGD-integrin avb3 recognition [92].

Numerous biomedical applications of SWCNTs and MWCNTs have been demonstrated over the years. Owing to their ability to absorb light at a wide range of wavelengths spanning the ultraviolet, visible near infrared (NIR) and micro-wave spectral ranges, CNTs are natural contrast agents for photoacoustic (PA) and photothermal (PT) techniques. The first demonstration of CNTs as PA contrast agents was performed by Zharov *et al*. in 2007 by *in vitro* and *in vivo* detection of circulating CNTs alone, and by circulating Staphylococcus aureus and Escherichia coli labelled with CNTs in the blood flow [98]. In 2008, de la Zerda *et al*. demonstrated the first *in vivo* PA imaging of CNTs by molecularly targeting SWCNTs to tumor neovasculature using arginine-glycine-aspartic acid (RGD) peptides in living mice [99]. In these two studies, chemical modifications of CNTs included coating with organic optical dyes [100], gold and folates, and antibodies for molecular targeting of circulating tumor cells (CTCs), or endothelial lymphatic LYVE1 receptors. [101,102], or tumor cells [103]. CNTs were also used as contrast agents for sentinel lymph node (SLN) imaging [21,102,104,105]. SWCNTs can also be excited by microwaves (3 GHz frequency) generating a thermoacoustic signal [105].

Recent cytometry explorations based on PT and PA Raman spectroscopy were able to detect nonlinear effects in CNTs *in vivo* [106]. The PT and PA effects in CNTs can be further used for nanophotothermolysis as demonstrated in 2003. Pulsed PT nanotherapy, with laser induced nano- and microbubbles around overheated gold nanoparticles, led to tumor cell death [107,108]. When compared with continuous wave laser for phototherapy for tumors [12,109] laser in pulsed mode is able to precisely kill individual cancer cells with a spatial accuracy of a few micrometers without harmful effects to the surrounding normal cells [108,110].

4. Conclusions

The *in vivo* biodistribution pattern of inorganic nanoparticles depends on particle size and surface engineering. Surface PEGylation reduces the uptake rate by macrophages and prolongs the circulation half-life.

Similarly, nanoparticle accumulation in tumors is also dependent on the size and surface engineering of inorganic nanoparticles. Some of them are devoid of toxicity and could be further developed in clinics for imaging and/or therapeutic purposes.

5. Acknowledgements

This work was supported by Agence Nationale de la Recherche (ANR) grant 2010-INTB-1003-02. Ms Angela Swayne reviewed the English language.

REFERENCES

[1] B. Chen, W. Wu and X. Wang, "Magnetic Iron Oxide Nanoparticles for Tumor-Targeted Therapy," *Current Cancer Drug Targets*, Vol. 11, No. 2, 2011, pp. 184-189. doi:10.2174/156800911794328475

[2] C. S. Paulo, R. Pires das Neves and L. S. Ferreira, "Nanoparticles for Intracellular-Targeted Drug Delivery," *Nanotechnology*, Vol. 22, No. 49, 2011, Article ID 494002. doi:10.1088/0957-4484/22/49/494002

[3] V. P. Torchilin, "Passive and Active Drug Targeting: Drug Delivery to Tumors as an Example," *Drug Delivery*, Vol. 197, 2010, pp. 3-53.

doi:10.1007/978-3-642-00477-3_1

[4] A. Agarwal, X. Shao, J. R. Rajian, H. Zhang, D. L. Chamberland, N. A. Kotov and X. Wang, "Dual-Mode Imaging with Radiolabeled Gold Nanorods," *Journal of Biomedical Optics*, Vol. 16, No. 5, 2011, Article ID 051307. doi:10.1117/1.3580277

[5] D. A. Shah, S. J. Kwon, S. S. Bale, A. Banerjee, J. S. Dordick and R. S. Kane, "Regulation of Stem Cell Signaling by Nanoparticle-Mediated Intracellular Protein Delivery," *Biomaterials*, Vol. 32, No. 12, 2011, pp. 3210-3219. doi:10.1016/j.biomaterials.2010.11.077

[6] M. A. Hahn, A. K. Singh, P. Sharma, S. C. Brown and B. M. Moudgil, "Nanoparticles as Contrast Agents for *In-Vivo* Bioimaging: Current Status and Future Perspectives," *Analytical and Bioanalytical Chemistry*, Vol. 399, No. 1, 2011, pp. 3-27. doi:10.1007/s00216-010-4207-5

[7] A. L. van de Ven, P. Kim, O. Haley, J. R. Fakhoury, G. Adriani, J. Schmulen, P. Moloney, F. Hussain, M. Ferrari, X. Liu, S. H. Yun and P. Decuzzi, "Rapid Tumoritropic Accumulation of Systemically Injected Plateloid Particles and Their Biodistribution," *Journal of Controlled Release*, Vol. 158, No. 1, 2011, pp. 148-155. doi:10.1016/j.jconrel.2011.10.021

[8] H. C. Huang, S. Barua, G. Sharma, S. K. Dey and K. Rege, "Inorganic Nanoparticles for Cancer Imaging and Therapy," *Journal of Controlled Release*, Vol. 155, No. 3, 2011, pp. 344-357. doi:10.1016/j.jconrel.2011.06.004

[9] J. V. Jokerst and S. S. Gambhir, "Molecular Imaging with Theranostic Nanoparticles," *Accounts of Chemical Research*, Vol. 44, No. 10, 2011, pp. 1050-1060. doi:10.1021/ar200106e

[10] S. H. Radwan and H. M. Azzazy, "Gold Nanoparticles for Molecular Diagnostics," *Expert Review of Molecular Diagnostics*, Vol. 9, No. 5, 2009, pp. 511-524. doi:10.1586/erm.09.33

[11] K. S. Park, J. Tae, B. Choi, Y. S. Kim, C. Moon, S. H. Kim, H. S. Lee, J. Kim, J. Park, J. H. Lee, J. E. Lee, J. W. Joh and S. Kim, "Characterization, *in Vitro* Cytotoxicity Assessment, and *in Vivo* Visualization of Multimodal, RITC-Labeled, Silica-Coated Magnetic Nanoparticles for Labeling Human Cord Blood-Derived Mesenchymal Stem Cells," *Nanomedicine*, Vol. 6, No. 2, 2010, pp. 263-276. doi:10.1016/j.nano.2009.07.005

[12] L. R. Hirsch, R. J. Stafford, J. A. Bankson, S. R. Sershen, B. Rivera, R. E. Price, J. D. Hazle, N. J. Halas and J. L. West, "Nanoshell-Mediated Near-Infrared Thermal Therapy of Tumors Under Magnetic Resonance Guidance," *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 100, No. 23, 2003, pp. 13549-13554. doi:10.1073/pnas.2232479100

[13] C. Loo, L. Hirsch, M. H. Lee, E. Chang, J. West, N. Halas and R. Drezek, "Gold Nanoshell Bioconjugates for Molecular Imaging in Living Cells," *Optic Letters*, Vol. 30, No. 9, 2005, pp. 1012-1014. doi:10.1364/OL.30.001012

[14] X. Huang, I. H. El-Sayed, W. Qian and M. A. El-Sayed, "Cancer Cell Imaging and Photothermal Therapy in the Near-Infrared Region by Using Gold Nanorods," *Journal of the American Chemical Society*, Vol. 128, No. 6, 2006, pp. 2115-2120. doi:10.1021/ja057254a

[15] W. Lu, M. P. Melancon, C. Xiong, Q. Huang, A. Elliott, S. Song, R. Zhang, L. G. Flores 2nd, J. G. Gelovani, L. V. Wang, G. Ku, R. J. Stafford and C. Li, "Effects of Photoacoustic Imaging and Photothermal Ablation Therapy Mediated by Targeted Hollow Gold Nanospheres in an Orthotopic Mouse Xenograft Model of Glioma," *Cancer Research*, Vol. 71, No. 19, 2011, pp. 6116-6121. doi:10.1158/0008-5472.CAN-10-4557

[16] S. D. Li and L. Huang, "Pharmacokinetics and Biodistribution of Nanoparticles," *Molecular Pharmaceutics*, Vol. 5, No. 4, 2008, pp. 496-504. doi:10.1021/mp800049w

[17] H. Maeda, J. Wu, T. Sawa, Y. Matsumura and K. Hori, "Tumor Vascular Permeability and the EPR Effect in Macromolecular Therapeutics: A Review," *Journal of Controlled Release*, Vol. 65, No. 1-2, 2000, pp. 271-284. doi:10.1016/S0168-3659(99)00248-5

[18] J. Chen, D. Wang, J. Xi, L. Au, A. Siekkinen, A. Warsen, Z. Y. Li, H. Zhang, Y. Xia and X. Li, "Immuno Gold Nanocages with Tailored Optical Properties for Targeted Photothermal Destruction of Cancer Cells," *Nano Letters*, Vol. 7, No. 5, 2007, pp. 1318-1322. doi:10.1021/nl070345g

[19] K. K. Jain, "Advances in the Field of Nanooncology," *BMC Medicine*, Vol. 8, 2010, p. 83. doi:10.1186/1741-7015-8-83

[20] M. Hu, J. Chen, Z. Y. Li, L. Au, G. V. Hartland, X. Li, M. Marquez and Y. Xia, "Gold Nanostructures: Engineering Their Plasmonic Properties for Biomedical Applications," *Chemical Society Reviews*, Vol. 35, No. 11, 2006, pp. 1084-1094. doi:10.1039/b517615h

[21] E. I. Galanzha, M. S. Kokoska, E. V. Shashkov, J. W. Kim, V. V. Tuchin and V. P. Zharov, "*In Vivo* Fiber-Based Multicolor Photoacoustic Detection and Photothermal Purging of Metastasis in Sentinel Lymph Nodes Targeted by Nanoparticles," *Journal of Biophotonics*, Vol. 2, No. 8-9, 2009, pp. 528-539. doi:10.1002/jbio.200910046

[22] A. S. Thakor, J. Jokerst, C. Zavaleta, T. F. Massoud and S. S. Gambhir, "Gold Nanoparticles: A Revival in Precious Metal Administration to Patients," *Nano Letters*, Vol. 11, No. 10, 2011, pp. 4029-4036. doi:10.1021/nl202559p

[23] L. Tong, Q. Wei, A. Wei and J. X. Cheng, "Gold Nanorods as Contrast Agents for Biological Imaging: Optical Properties, Surface Conjugation and Photothermal Effects," *Photochemistry and Photobiology*, Vol. 85, No. 1, 2009, pp. 21-32. doi:10.1111/j.1751-1097.2008.00507.x

[24] Y. Akiyama, T. Mori, Y. Katayama and T. Niidome, "The Effects of PEG Grafting Level and Injection Dose on Gold Nanorod Biodistribution in the Tumor-Bearing Mice," *Journal of Controlled Release*, Vol. 139, No. 1, 2009, pp. 81-84. doi:10.1016/j.jconrel.2009.06.006

[25] P. Diagaradjane, A. Shetty, J. C. Wang, A. M. Elliott, J. Schwartz, S. Shentu, H. C. Park, A. Deorukhkar, R. J. Stafford, S. H. Cho, J. W. Tunnell, J. D. Hazle and S. Krishnan, "Modulation of *in Vivo* Tumor Radiation Response via Gold Nanoshell-Mediated Vascular-Focused Hyperthermia: Characterizing an Integrated Antihypoxic

and Localized Vascular Disrupting Targeting Strategy," *Nano Letters*, Vol. 8, No. 5, 2008, pp. 1492-1500. doi:10.1021/nl080496z

[26] X. Yang, A. Maurudis, J. Gamelin, A. Aguirre, Q. Zhu and L. V. Wang, "Photoacoustic Tomography of Small Animal Brain with a Curved Array Transducer," *Journal of Biomedical Optics*, Vol. 14, No. 5, 2009, Article ID 054007. doi:10.1117/1.3227035

[27] A. Agrawal, S. Huang, A. Wei Haw Lin, M. H. Lee, J. K. Barton, R. A. Drezek and T. J. Pfefer, "Quantitative Evaluation of Optical Coherence Tomography Signal Enhancement with Gold Nanoshells," *Journal of Biomedical Optics* , Vol. 11, No. 4, 2006, Article ID 041121. doi:10.1117/1.2339071

[28] C. H. Su, H. S. Sheu, C. Y. Lin, C. C. Huang, Y. W. Lo, Y. C. Pu, J. C. Weng, D. B. Shieh, J. H. Chen and C. S. Yeh, "Nanoshell Magnetic Resonance Imaging Contrast Agents," *Journal of the American Chemical Society*, Vol. 129, No. 7, 2007, pp. 2139-2146. doi:10.1021/ja0672066

[29] D. P. O'Neal, L. R. Hirsch, N. J. Halas, J. D. Payne and J. L. West, "Photo-Thermal Tumor Ablation in Mice Using near Infrared-Absorbing Nanoparticles," *Cancer Letters*, Vol. 209, No. 2, 2004, pp. 171-176. doi:10.1016/j.canlet.2004.02.004

[30] G. F. Paciotti, L. Myer, D. Weinreich, D. Goia, N. Pavel, R. E. McLaughlin and L. Tamarkin, "Colloidal Gold: A Novel Nanoparticle Vector for Tumor Directed Drug Delivery," *Drug Delivery*, Vol. 11, No. 3, 2004, pp. 169-183. doi:10.1080/10717540490433895

[31] T. Niidome, M. Yamagata, Y. Okamoto, Y. Akiyama, H. Takahashi, T. Kawano, Y. Katayama and Y. Niidome, "PEG-Modified Gold Nanorods with a Stealth Character for *in Vivo* Applications," *Journal of Controlled Release*, Vol. 114, No. 3, 2006, pp. 343-347. doi:10.1016/j.jconrel.2006.06.017

[32] S. Manohar, C. Ungureanu and T. G. Van Leeuwen, "Gold Nanorods as Molecular Contrast Agents in Photoacoustic Imaging: The Promises and the Caveats," *Contrast Media Mol Imaging*, Vol. 6, No. 5, 2011, pp. 389-400. doi:10.1002/cmmi.454

[33] X. D. Zhang, D. Wu, X. Shen, P. X. Liu, N. Yang, B. Zhao, H. Zhang, Y. M. Sun, L. A. Zhang and F. Y. Fan, "Size-Dependent *in Vivo* Toxicity of PEG-Coated Gold Nanoparticles," *International Journal of Nanomedicine*, Vol. 6, 2011, pp. 2071-2081. doi:10.2147/IJN.S21657

[34] C. Lasagna-Reeves, D. Gonzalez-Romero, M. A. Barria, I. Olmedo, A. Clos, V. M. Sadagopa Ramanujam, A. Urayama, L. Vergara, M. J. Kogan and C. Soto, "Bioaccumulation and Toxicity of Gold Nanoparticles after Repeated Administration in Mice," *Biochemical and Biophysical Research Communications*, Vol. 393, No. 4, 2010, pp. 649-655. doi:10.1016/j.bbrc.2010.02.046

[35] W. H. De Jong, W. I. Hagens, P. Krystek, M. C. Burger, A. J. Sips and R. E. Geertsma, "Particle Size-Dependent Organ Distribution of Gold Nanoparticles after Intravenous Administration," *Biomaterials*, Vol. 29, No. 12, 2008, pp. 1912-1919. doi:10.1016/j.biomaterials.2007.12.037

[36] G. Sonavane, K. Tomoda and K. Makino, "Biodistribution of Colloidal Gold Nanoparticles after Intravenous Administration: Effect of Particle Size," *Colloids Surf B Biointerfaces*, Vol. 66, No. 2, 2008, pp. 274-280. doi:10.1016/j.colsurfb.2008.07.004

[37] J. H. Kim, K. W. Kim, M. H. Kim and Y. S. Yu, "Intravenously Administered Gold Nanoparticles Pass through the Blood-Retinal Barrier Depending on the Particle Size, and Induce No Retinal Toxicity," *Nanotechnology*, Vol. 20, No. 50, 2009, Article ID 505101. doi:10.1088/0957-4484/20/50/505101

[38] K. H. Song, C. Kim, K. Maslov and L. V. Wang, "Noninvasive *in Vivo* Spectroscopic Nanorod-Contrast Photoacoustic Mapping of Sentinel Lymph Nodes," *European Journal of Radiology*, Vol. 70, No. 2, 2009, pp. 227-231. doi:10.1016/j.ejrad.2009.01.045

[39] X. Yang, E. W. Stein, S. Ashkenazi and L. V. Wang, "Nanoparticles for Photoacoustic Imaging," *Wiley Interdisciplinary Reviews*: *Nanomedicine and Nanobiotechnology*, Vol. 1, No. 4, 2009, pp. 360-368. doi:10.1002/wnan.42

[40] X. Wang, G. Ku, M. A. Wegiel, D. J. Bornhop, G. Stoica and L. V. Wang, "Noninvasive Photoacoustic Angiography of Animal Brains *in Vivo* with Near-Infrared Light and an Optical Contrast Agent," *Optic Letters*, Vol. 29, No. 7, 2004, pp. 730-732. doi:10.1364/OL.29.000730

[41] X. Yang, S. E. Skrabalak, Z. Y. Li, Y. Xia and L. V. Wang, "Photoacoustic Tomography of a Rat Cerebral Cortex *in Vivo* with au Nanocages as an Optical Contrast Agent," *Nano Letters*, Vol. 7, No. 12, 2007, pp. 3798-3802. doi:10.1021/nl072349r

[42] H. Cui and X. Yang, "*In Vivo* Imaging and Treatment of Solid Tumor Using Integrated Photoacoustic Imaging and High Intensity Focused Ultrasound System," *Medical Physics*, Vol. 37, No. 9, 2010, pp. 4777-4781. doi:10.1118/1.3480963

[43] J. Park, A. Estrada, K. Sharp, K. Sang, J. A. Schwartz, D. K. Smith, C. Coleman, J. D. Payne, B. A. Korgel, A. K. Dunn and J. W. Tunnell, "Two-Photon-Induced Photoluminescence Imaging of Tumors Using Near-Infrared Excited Gold Nanoshells," *Optics Express*, Vol. 16, No. 3, 2008, pp. 1590-1599. doi:10.1364/OE.16.001590

[44] M. L. Li, J. C. Wang, J. A. Schwartz, K. L. Gill-Sharp, G. Stoica and L. V. Wang, "*In-Vivo* Photoacoustic Microscopy of Nanoshell Extravasation from Solid Tumor Vasculature," *Journal of Biomedical Optics*, Vol. 14, No. 1, 2009, Article ID 010507. doi:10.1117/1.3081556

[45] G. Zhang, Z. Yang, W. Lu, R. Zhang, Q. Huang, M. Tian, L. Li, D. Liang and C. Li, "Influence of Anchoring Ligands and Particle Size on the Colloidal Stability and *in Vivo* Biodistribution of Polyethylene Glycol-Coated Gold Nanoparticles in Tumor-Xenografted Mice," *Biomaterials*, Vol. 30, No. 10, 2009, pp. 1928-1936. doi:10.1016/j.biomaterials.2008.12.038

[46] N. Venkatesan, J. Yoshimitsu, Y. Ito, N. Shibata and K. Takada, "Liquid Filled Nanoparticles as a Drug Delivery Tool for Protein Therapeutics," *Biomaterials*, Vol. 26, No. 34, 2005, pp. 7154-7163. doi:10.1016/j.biomaterials.2005.05.012

[47] J. Lu, Z. Li, J. I. Zink and F. Tamanoi, "*In Vivo* Tumor Suppression Efficacy of Mesoporous Silica Nanoparticles-Based Drug-Delivery System: Enhanced Efficacy by Folate Modification," *Nanomedicine*, Vol. 8, No. 2, 2012, pp. 212-220. doi:10.1016/j.nano.2011.06.002

[48] M. R. Papasani, G. Wang and R. A. Hill, "Gold Nanoparticles: The Importance of Physiological Principles to Devise Strategies for Targeted Drug Delivery," *Nanomedicine*: *Nanotechnology, Biology and Medicine*, 2012, Article in Press. doi:10.1016/j.nano.2012.01.008

[49] J. M. Rosenholm, V. Mamaeva, C. Sahlgren and M. Linden, "Nanoparticles in Targeted Cancer Therapy: Mesoporous Silica Nanoparticles Entering Preclinical Development Stage," *Nanomedicine* (*Lond*), Vol. 7, No. 1, 2012, pp. 111-120. doi:10.2217/nnm.11.166

[50] R. P. Bagwe, L. R. Hilliard and W. Tan, "Surface Modification of Silica Nanoparticles to Reduce Aggregation and Nonspecific Binding," *Langmuir*, Vol. 22, No. 9, 2006, pp. 4357-4362. doi:10.1021/la052797j

[51] P. F. Holmes, E. P. Currie, J. C. Thies, H. C. van der Mei, H. J. Busscher and W. Norde, "Surface-Modified Nanoparticles as a New, Versatile, and Mechanically Robust Nonadhesive Coating: Suppression of Protein Adsorption and Bacterial Adhesion," *Journal of Biomedical Materials Research Part A*, Vol. 91, No. 3, 2009, pp. 824-833. doi:10.1002/jbm.a.32285

[52] J. M. Rosenholm, C. Sahlgren and M. Linden, "Towards Multifunctional, Targeted Drug Delivery Systems Using Mesoporous Silica Nanoparticles—Opportunities & Challenges," *Nanoscale*, Vol. 2, No. 10, 2010, pp. 1870-1883. doi:10.1039/c0nr00156b

[53] J. M. Rosenholm, E. Peuhu, J. E. Eriksson, C. Sahlgren and M. Linden, "Targeted Intracellular Delivery of Hydrophobic Agents Using Mesoporous Hybrid Silica Nanoparticles as Carrier Systems," *Nano Letters*, Vol. 9, No. 9, 2009, pp. 3308-3311. doi:10.1021/nl901589y

[54] J. Kobler, K. Moller and T. Bein, "Colloidal Suspensions of Functionalized Mesoporous Silica Nanoparticles," *ACS Nano*, Vol. 2, No. 4, 2008, pp. 791-799. doi:10.1021/nn700008s

[55] J. L. Vivero-Escoto, Slowing, II, B. G. Trewyn and V. S. Lin, "Mesoporous Silica Nanoparticles for Intracellular Controlled Drug Delivery," *Small*, Vol. 6, No. 18, 2010, pp. 1952-1967. doi:10.1002/smll.200901789

[56] H. Nabeshi, T. Yoshikawa, K. Matsuyama, Y. Nakazato, K. Matsuo, A. Arimori, M. Isobe, S. Tochigi, S. Kondoh, T. Hirai, T. Akase, T. Yamashita, K. Yamashita, T. Yoshida, K. Nagano, Y. Abe, Y. Yoshioka, H. Kamada, T. Imazawa, N. Itoh, S. Nakagawa, T. Mayumi, S. Tsunoda and Y. Tsutsumi, "Systemic Distribution, Nuclear Entry and Cytotoxicity of Amorphous Nanosilica Following Topical Application," *Biomaterials*, Vol. 32, No. 11, 2011, pp. 2713-2724. doi:10.1016/j.biomaterials.2010.12.042

[57] R. Kumar, I. Roy, T. Y. Ohulchanskky, L. A. Vathy, E. J. Bergey, M. Sajjad and P. N. Prasad, "*In Vivo* Biodistribution and Clearance Studies Using Multimodal Organically Modified Silica Nanoparticles," *ACS Nano*, Vol. 4, No. 2, 2010, pp. 699-708. doi:10.1021/nn901146y

[58] X. He, H. Nie, K. Wang, W. Tan, X. Wu and P. Zhang, "*In Vivo* Study of Biodistribution and Urinary Excretion of Surface-Modified Silica Nanoparticles," *Analytical Chemistry*, Vol. 80, No. 24, 2008, pp. 9597-9603. doi:10.1021/ac801882g

[59] H. Nabeshi, T. Yoshikawa, K. Matsuyama, Y. Nakazato, A. Arimori, M. Isobe, S. Tochigi, S. Kondoh, T. Hirai, T. Akase, T. Yamashita, K. Yamashita, T. Yoshida, K. Nagano, Y. Abe, Y. Yoshioka, H. Kamada, T. Imazawa, N. Itoh, S. Tsunoda and Y. Tsutsumi, "Size-Dependent Cytotoxic Effects of Amorphous Silica Nanoparticles on Langerhans Cells," *Pharmazie*, Vol. 65, No. 3, 2010, pp. 199-201.

[60] J. Lu, M. Liong, Z. Li, J. I. Zink and F. Tamanoi, "Biocompatibility, Biodistribution, and Drug-Delivery Efficiency of Mesoporous Silica Nanoparticles for Cancer Therapy in Animals," *Small*, Vol. 6, No. 16, 2010, pp. 1794-1805. doi:10.1002/smll.201000538

[61] T. H. Chung, S. H. Wu, M. Yao, C. W. Lu, Y. S. Lin, Y. Hung, C. Y. Mou, Y. C. Chen and D. M. Huang, "The Effect of Surface Charge on the Uptake and Biological Function of Mesoporous Silica Nanoparticles in 3T3-L1 Cells and Human Mesenchymal Stem Cells," *Biomaterials*, Vol. 28, No. 19, 2007, pp. 2959-2966. doi:10.1016/j.biomaterials.2007.03.006

[62] C. Morelli, P. Maris, D. Sisci, E. Perrotta, E. Brunelli, I. Perrotta, M. L. Panno, A. Tagarelli, C. Versace, M. F. Casula, F. Testa, S. Ando, J. B. Nagy and L. Pasqua, "PEG-Templated Mesoporous Silica Nanoparticles Exclusively Target Cancer Cells," *Nanoscale*, Vol. 3, No. 8, 2011, pp. 3198-3207. doi:10.1039/c1nr10253b

[63] D. Artemov, "Molecular Magnetic Resonance Imaging with Targeted Contrast Agents," *Journal of Cellular Biochemistry*, Vol. 90, No. 3, 2003, pp. 518-524. doi:10.1002/jcb.10660

[64] T. Kobayashi, "Cancer Hyperthermia Using Magnetic Nanoparticles," *Biotechnology Journal*, Vol. 6, No. 11, 2011, pp. 1342-1347. doi:10.1002/biot.201100045

[65] X. H. Peng, X. Qian, H. Mao, A. Y. Wang, Z. G. Chen, S. Nie and D. M. Shin, "Targeted Magnetic Iron Oxide Nanoparticles for Tumor Imaging and Therapy," *International Journal of Nanomedicine*, Vol. 3, No. 3, 2008, pp. 311-321.

[66] C. Alexiou, R. Jurgons, R. J. Schmid, C. Bergemann, J. Henke, W. Erhardt, E. Huenges and F. Parak, "Magnetic Drug Targeting—Biodistribution of the Magnetic Carrier and the Chemotherapeutic Agent Mitoxantrone after Locoregional Cancer Treatment," *Journal of Drug Targeting*, Vol. 11, No. 3, 2003, pp. 139-149. doi:10.3109/1061186031000150791

[67] K. Lind, M. Kresse, N. P. Debus and R. H. Muller, "A Novel Formulation for Superparamagnetic Iron Oxide (SPIO) Particles Enhancing MR Lymphography: Comparison of Physicochemical Properties and the *in Vivo* Behaviour," *Journal of Drug Targeting*, Vol. 10, No. 3, 2002, pp. 221-230. doi:10.1080/10611860290022651

[68] O. Veiseh, C. Sun, J. Gunn, N. Kohler, P. Gabikian, D. Lee, N. Bhattarai, R. Ellenbogen, R. Sze, A. Hallahan, J. Olson and M. Zhang, "Optical and MRI Multifunctional

Nanoprobe for Targeting Gliomas," *Nano Letters*, Vol. 5, No. 6, 2005, pp. 1003-1008. doi:10.1021/nl0502569

[69] M. Meng Lin, H. H. Kim, H. Kim, M. Muhammed and D. Kyung Kim, "Iron Oxide-Based Nanomagnets in Nanomedicine: Fabrication and Applications," *Nano Reviews*, Vol. 1, 2010.

[70] C. E. Neumaier, G. Baio, S. Ferrini, G. Corte and A. Daga, "MR and Iron Magnetic Nanoparticles. Imaging Opportunities in Preclinical and Translational Research," *Tumori*, Vol. 94, No. 2, 2008, pp. 226-233.

[71] V. I. Shubayev, T. R. Pisanic, 2nd and S. Jin, "Magnetic Nanoparticles for Theragnostics," *Advanced Drug Delivery Reviews*, Vol. 61, No. 6, 2009, pp. 467-477. doi:10.1016/j.addr.2009.03.007

[72] A. J. Cole, A. E. David, J. Wang, C. J. Galban and V. C. Yang, "Magnetic Brain Tumor Targeting and Biodistribution of Long-Circulating PEG-Modified, Cross-Linked Starch-Coated Iron Oxide Nanoparticles," *Biomaterials*, Vol. 32, No. 26, 2011, pp. 6291-6301.

[73] T. K. Jain, M. K. Reddy, M. A. Morales, D. L. Leslie-Pelecky and V. Labhasetwar, "Biodistribution, Clearance, and Biocompatibility of Iron Oxide Magnetic Nanoparticles in Rats," *Molecular Pharmaceutics*, Vol. 5, No. 2, 2008, pp. 316-327. doi:10.1021/mp7001285

[74] E. K. Schlachter, H. R. Widmer, A. Bregy, T. Lonnfors-Weitzel, I. Vajtai, N. Corazza, V. J. Bernau, T. Weitzel, P. Mordasini, J. Slotboom, G. Herrmann, S. Bogni, H. Hofmann, M. Frenz and M. Reinert, "Metabolic Pathway and Distribution of Superparamagnetic Iron Oxide Nanoparticles: *In Vivo* Study," *International Journal of Nanomedicine*, Vol. 6, 2011, pp. 1793-800.

[75] J. Feng, H. Liu, K. K. Bhakoo, L. Lu and Z. Chen, "A Metabonomic Analysis of Organ Specific Response to USPIO Administration," *Biomaterials*, Vol. 32, No. 27, 2011, pp. 6558-6569. doi:10.1016/j.biomaterials.2011.05.035

[76] B. R. Smith, J. Heverhagen, M. Knopp, P. Schmalbrock, J. Shapiro, M. Shiomi, N. I. Moldovan, M. Ferrari and S. C. Lee, "Localization to Atherosclerotic Plaque and Biodistribution of Biochemically Derivatized Superparamagnetic Iron Oxide Nanoparticles (SPIONs) Contrast Particles for Magnetic Resonance Imaging (MRI)," *Biomedical Microdevices*, Vol. 9, No. 5, 2007, pp. 719-727. doi:10.1007/s10544-007-9081-3

[77] M. Kettering, H. Richter, F. Wiekhorst, S. Bremer-Streck, L. Trahms, W. A. Kaiser and I. Hilger, "Minimal-Invasive Magnetic Heating of Tumors Does Not Alter Intra-Tumoral Nanoparticle Accumulation, Allowing for Repeated Therapy Sessions: An *in Vivo* Study in Mice," *Nanotechnology*, Vol. 22, No. 50, 2011, Article ID 505102. doi:10.1088/0957-4484/22/50/505102

[78] A. Attaluri, R. Ma, Y. Qiu, W. Li and L. Zhu, "Nanoparticle Distribution and Temperature Elevations in Prostatic Tumours in Mice During Magnetic Nanoparticle Hyperthermia," *International Journal of Hyperthermia*, Vol. 27, No. 5, 2011, pp. 491-502. doi:10.3109/02656736.2011.584856

[79] Q. Zhao, L. Wang, R. Cheng, L. Mao, R. D. Arnold, E. W. Howerth, Z. G. Chen and S. Platt, "Magnetic Nanoparticle-Based Hyperthermia for Head & Neck Cancer in Mouse Models," *Theranostics*, Vol. 2, 2012, pp. 113-121. doi:10.7150/thno.3854

[80] G. P. Drummen, "Quantum Dots-From Synthesis to Applications in Biomedicine and Life Sciences," *International Journal of Molecular Science*, Vol. 11, No. 1, 2010, pp. 154-163. doi:10.3390/ijms11010154

[81] M. Howarth, W. Liu, S. Puthenveetil, Y. Zheng, L. F. Marshall, M. M. Schmidt, K. D. Wittrup, M. G. Bawendi and A. Y. Ting, "Monovalent, Reduced-Size Quantum Dots for Imaging Receptors on Living Cells," *Nature Methods*, Vol. 5, No. 5, 2008, pp. 397-399. doi:10.1038/nmeth.1206

[82] M. E. Akerman, W. C. Chan, P. Laakkonen, S. N. Bhatia and E. Ruoslahti, "Nanocrystal Targeting *in Vivo*," *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 99, No. 20, 2002, pp. 12617-12621. doi:10.1073/pnas.152463399

[83] S. Dwarakanath, J. G. Bruno, A. Shastry, T. Phillips, A. A. John, A. Kumar and L. D. Stephenson, "Quantum Dot-Antibody and Aptamer Conjugates Shift Fluorescence upon Binding Bacteria," *Biochemical and Biophysical Research Communications*, Vol. 325, No. 3, 2004, pp. 739-743. doi:10.1016/j.bbrc.2004.10.099

[84] B. Ballou, B. C. Lagerholm, L. A. Ernst, M. P. Bruchez and A. S. Waggoner, "Noninvasive Imaging of Quantum Dots in Mice," *Bioconjugate Chemistry*, Vol. 15, No. 1, 2004, pp. 79-86. doi:10.1021/bc034153y

[85] J. L. Pelley, A. S. Daar and M. A. Saner, "State of Academic Knowledge on Toxicity and Biological Fate of Quantum Dots," *Toxicological Science*, Vol. 112, No. 2, 2009, pp. 276-296. doi:10.1093/toxsci/kfp188

[86] H. S. Choi, W. Liu, P. Misra, E. Tanaka, J. P. Zimmer, B. Itty Ipe, M. G. Bawendi and J. V. Frangioni, "Renal Clearance of Quantum Dots," *Nature Biotechnology*, Vol. 25, No. 10, 2007, pp. 1165-1170. doi:10.1038/nbt1340

[87] M. P. Waalkes, "Cadmium Carcinogenesis in Review," *Journal of Inorganic Biochemistry*, Vol. 79, No. 1-4, 2000, pp. 241-244. doi:10.1016/S0162-0134(00)00009-X

[88] T. Gratieri, U. F. Schaefer, L. Jing, M. Gao, K. H. Kostka, R. F. Lopez and M. Schneider, "Penetration of Quantum Dot Particles through Human Skin," *Journal of Biomedical Nanotechnology*, Vol. 6, No. 5, 2010, pp. 586-595. doi:10.1166/jbn.2010.1155

[89] C. A. Poland, R. Duffin, I. Kinloch, A. Maynard, W. A. Wallace, A. Seaton, V. Stone, S. Brown, W. Macnee and K. Donaldson, "Carbon Nanotubes Introduced into the Abdominal Cavity of Mice Show Asbestos-Like Pathogenicity in a Pilot Study," *Nature Nanotechnology*, Vol. 3, No. 7, 2008, pp. 423-428. doi:10.1038/nnano.2008.111

[90] C. W. Lam, J. T. James, R. McCluskey, S. Arepalli and R. L. Hunter, "A Review of Carbon Nanotube Toxicity and Assessment of Potential Occupational and Environmental Health Risks," *Critical Review in Toxicology*, Vol. 36, No. 3, 2006, pp. 189-217. doi:10.1080/10408440600570233

[91] R. S. Yang, L. W. Chang, J. P. Wu, M. H. Tsai, H. J. Wang, Y. C. Kuo, T. K. Yeh, C. S. Yang and P. Lin,

"Persistent Tissue Kinetics and Redistribution of Nanoparticles, Quantum Dot 705, in Mice: ICP-MS Quantitative Assessment," *Environmenatal Health Perspectives*, Vol. 115, No. 9, 2007, pp. 1339-1343. doi:10.1289/ehp.10290

[92] Z. Liu, W. Cai, L. He, N. Nakayama, K. Chen, X. Sun, X. Chen and H. Dai, "*In Vivo* Biodistribution and Highly Efficient Tumour Targeting of Carbon Nanotubes in Mice," *Nature Nanotechnology*, Vol. 2, No. 1, 2007, pp. 47-52. doi:10.1038/nnano.2006.170

[93] T. M. Saba, "Physiology and Physiopathology of the Reticuloendothelial System," *Archives of International Medicne*, Vol. 126, No. 6, 1970, pp. 1031-152. doi:10.1001/archinte.1970.00310120093014

[94] R. Singh, D. Pantarotto, L. Lacerda, G. Pastorin, C. Klumpp, M. Prato, A. Bianco and K. Kostarelos, "Tissue Biodistribution and Blood Clearance Rates of Intravenously Administered Carbon Nanotube Radiotracers," *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 103, No. 9, 2006, pp. 3357-3362. doi:10.1073/pnas.0509009103

[95] H. Wang, J. Wang, X. Deng, H. Sun, Z. Shi, Z. Gu, Y. Liu and Y. Zhao, "Biodistribution of Carbon Single-Wall Carbon Nanotubes in Mice," *Journal of Nanoscience and Nanotechnology*, Vol. 4, No. 8, 2004, pp. 1019-1024. doi:10.1166/jnn.2004.146

[96] G. Prencipe, S. M. Tabakman, K. Welsher, Z. Liu, A. P. Goodwin, L. Zhang, J. Henry and H. Dai, "PEG Branched Polymer for Functionalization of Nanomaterials with Ultralong Blood Circulation," *Journal of the American Chemical Society*, Vol. 131, No. 13, 2009, pp. 4783-4787. doi:10.1021/ja809086q

[97] A. Fraczek, E. Menaszek, C. Paluszkiewicz and M. Blazewicz, "Comparative *in Vivo* Biocompatibility Study of Single- and Multi-Wall Carbon Nanotubes," *Acta Biomaterialia*, Vol. 4, No. 6, 2008, pp. 1593-1602. doi:10.1016/j.actbio.2008.05.018

[98] V. P. Zharov, E. I. Galanzha, E. V. Shashkov, J. W. Kim, N. G. Khlebtsov and V. V. Tuchin, "Photoacoustic Flow Cytometry: Principle and Application for Real-Time Detection of Circulating Single Nanoparticles, Pathogens, and Contrast Dyes *in Vivo*," *Journal of Biomedical Optics*, Vol. 12, No. 5, 2007, Article ID 051503. doi:10.1117/1.2793746

[99] A. De la Zerda, C. Zavaleta, S. Keren, S. Vaithilingam, S. Bodapati, Z. Liu, J. Levi, B. R. Smith, T. J. Ma, O. Oralkan, Z. Cheng, X. Chen, H. Dai, B. T. Khuri-Yakub and S. S. Gambhir, "Carbon Nanotubes as Photoacoustic Molecular Imaging Agents in Living Mice," *Nature Nanotechnology*, Vol. 3, No. 9, 2008, pp. 557-562. doi:10.1038/nnano.2008.231

[100] A. de la Zerda, Z. Liu, S. Bodapati, R. Teed, S. Vaithilingam, B. T. Khuri-Yakub, X. Chen, H. Dai and S. S. Gambhir, "Ultrahigh Sensitivity Carbon Nanotube Agents for Photoacoustic Molecular Imaging in Living Mice," *Nano Letters*, Vol. 10, No. 6, 2010, pp. 2168-2172. doi:10.1021/nl100890d

[101] E. I. Galanzha, E. V. Shashkov, T. Kelly, J. W. Kim, L. Yang and V. P. Zharov, "*In Vivo* Magnetic Enrichment and Multiplex Photoacoustic Detection of Circulating Tumour Cells," *Nature Nanotechnology*, Vol. 4, No. 12, 2009, pp. 855-860. doi:10.1038/nnano.2009.333

[102] J. W. Kim, E. I. Galanzha, E. V. Shashkov, H. M. Moon and V. P. Zharov, "Golden Carbon Nanotubes as Multimodal Photoacoustic and Photothermal High-Contrast Molecular Agents," *Nature Nanotechnology*, Vol. 4, No. 10, 2009, pp. 688-694. doi:10.1038/nnano.2009.231

[103] L. Xiang, Y. Yuan, D. Xing, Z. Ou, S. Yang and F. Zhou, "Photoacoustic Molecular Imaging with Antibody-Functionalized Single-Walled Carbon Nanotubes for Early Diagnosis of Tumor," *Journal of Biomedical Optics*, Vol. 14, No. 2, 2009, Article ID 021008. doi:10.1117/1.3078809

[104] E. I. Galanzha, E. V. Shashkov, V. V. Tuchin and V. P. Zharov, "*In Vivo* Multispectral, Multiparameter, Photoacoustic Lymph Flow Cytometry with Natural Cell Focusing, Label-Free Detection and Multicolor Nanoparticle Probes," *Cytometry A*, Vol. 73, No. 10, 2008, pp. 884-894. doi:10.1002/cyto.a.20587

[105] M. Pramanik, M. Swierczewska, D. Green, B. Sitharaman and L. V. Wang, "Single-Walled Carbon Nanotubes as a Multimodal-Thermoacoustic and Photoacoustic-Contrast Agent," *Journal of Biomedical Optics*, Vol. 14, No. 3, 2009, Article ID 034018. doi:10.1117/1.3147407

[106] E. V. Shashkov, E. I. Galanzha and V. P. Zharov, "Photothermal and Photoacoustic Raman Cytometry *in Vitro* and *in Vivo*," *Optics Express*, Vol. 18, No. 7, 2010, pp. 6929-6944. doi:10.1364/OE.18.006929

[107] V. P. Zharov, E. N. Galitovskaya, C. Johnson and T. Kelly, "Synergistic Enhancement of Selective Nanophotothermolysis with Gold Nanoclusters: Potential for Cancer Therapy," *Lasers in Surgery and Medicine*, Vol. 37, No. 3, 2005, pp. 219-226. doi:10.1002/lsm.20223

[108] V. P. Zharov, J. W. Kim, D. T. Curiel and M. Everts, "Self-Assembling Nanoclusters in Living Systems: Application for Integrated Photothermal Nanodiagnostics and Nanotherapy," *Nanomedicine*, Vol. 1, No. 4, 2005, pp. 326-345. doi:10.1016/j.nano.2005.10.006

[109] N. W. Kam, M. O'Connell, J. A. Wisdom and H. Dai, "Carbon Nanotubes as Multifunctional Biological Transporters and Near-Infrared Agents for Selective Cancer Cell Destruction," *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 102, No. 33, 2005, pp. 11600-11605. doi:10.1073/pnas.0502680102

[110] V. Pustovalov, L. Astafyeva and B. Jean, "Computer Modeling of the Optical Properties and Heating of Spherical Gold and Silica-Gold Nanoparticles for Laser Combined Imaging and Photothermal Treatment," *Nanotechnology*, Vol. 20, No. 22, 2009, Article ID 225105. doi:10.1088/0957-4484/20/22/225105

