Review article

Journal of Applied Toxicology

Received: 18 February 2017, Revised: 21 March 2017, Accepted: 21 March 2017 Published online in Wiley Online Library: 6 June 2017

(wileyonlinelibrary.com) DOI 10.1002/jat.3476

Potential applications and human biosafety of nanomaterials used in nanomedicine

Hong Su[a†], Yafei Wang[a†], Yuanliang Gu[a†], Linda Bowman[b], Jinshun Zhao[a,b*] and Min Ding[b*]

ABSTRACT: With the rapid development of nanotechnology, potential applications of nanomaterials in medicine have been widely researched in recent years. Nanomaterials themselves can be used as image agents or therapeutic drugs, and for drug and gene delivery, biological devices, nanoelectronic biosensors or molecular nanotechnology. As the composition, morphology, chemical properties, implant sites as well as potential applications become more and more complex, human biosafety of nanomaterials for clinical use has become a major concern. If nanoparticles accumulate in the human body or interact with the body molecules or chemical components, health risks may also occur. Accordingly, the unique chemical and physical properties, potential applications in medical fields, as well as human biosafety in clinical trials are reviewed in this study. Finally, this article tries to give some suggestions for future work in nanomedicine research. Copyright © 2017 John Wiley & Sons, Ltd.

Keywords: nanoparticles (NPs); nanomaterials; physical and chemical properties; potential applications; nanomedicine products; clinical trials; human biosafety

Introduction

Nanotechnology is considered one of three major technologies of the twenty-first century (Pautler and Brenner, 2010; Reese, 2013), which is a technology of nanometer scale used to control material structure. The concept of nanotechnology was first mentioned in 1959 by Richard Feynman (2011) a renowned physicist in his talk "There's Plenty of Room at the Bottom," in which he described the possibility of synthesis of nanomaterials via direct manipulation of atoms. After more than half a century of development, nanotechnology has become a mature technology. Now humans are constantly benefiting from nanotechnology. Unique properties make nanomaterials display new features as well as distinct and excellent performance (Macwan *et al.,* 2011). In addition, nanomaterials have a broad application prospect in the medical field (Fan and Alexeeff, 2010; Fu, 2014; Kunzmann *et al.,* 2011; Zhao and Castranova, 2011).

Nanomaterials have been investigated in the medical field for drugs (Cao *et al.,* 2014; Ranganathan *et al.,* 2012; Tan *et al.,* 2011; Zhao *et al.,* 2013) and genes carriers (Banizs *et al.,* 2014; Eroglu *et al.,* 2013; Liu *et al.,* 2014), cancer therapy (Frank *et al.,* 2014; Jabir *et al.,* 2012; Marques *et al.,* 2014; Wang *et al.,* 2013), gene therapy (Rodriguez-Gascon *et al.,* 2014), antibacterial agents (Amrol, 2007; Seil and Webster, 2012; Taylor and Webster, 2011), antiviral agents (du Toit *et al.,* 2010; Mahajan *et al.,* 2012; Parboosing *et al.,* 2012), tissue engineering (Mazaheri *et al.,* 2015; Partha and Conyers, 2009; Tautzenberger *et al.,* 2012; Tonelli *et al.,* 2012), medical diagnosis (Chen *et al.,* 2013; Kang *et al.,* 2015; Madani *et al.,* 2013), medical imaging (Cao *et al.,* 2014; Peng *et al.,* 2008; Sheng *et al.,* 2014; Yi *et al.,* 2014), etc., which bring many benefits to humans. At the same time, studies on the biosafety of nanomaterials have been soaring in recent years (Vega-Villa *et al.,* 2008). As Schütz *et al.* (2013) pointed out: "Care must be taken to ensure biocompatibility of the carrier or therapeutic nanomaterials and to ensure that their intrinsic toxicity does not overtake their benefits." Owing to the unique characteristics, such as small size, surface area, quantum size and so on, nanomaterials could trigger a special biological effect and bring potential adverse influence to human health. Accordingly, the unique physical and chemical properties, potential applications in medical fields and human biosafety of nanomaterials in clinical trials are reviewed in this paper. To optimize the development of nanomedicine, strategies for future work in nanomedicine research are also discussed.

Unique physical and chemical properties of nanomaterials

Nanometer material is made of a nanostructural unit of all different types of material. In principle, nanomaterial is material of which a single unit is sized (in at least one dimension) between 1 and 100 nm (Shi *et al.,* 2013) and it often owns unique physical and chemical properties. The electronic, optical and chemical properties of each individual component may be very different

*Correspondence to: Jinshun Zhao, MD, PhD, Department of Preventative Medicine, Zhejiang Provincial Key Laboratory of Pathological and Physiological Technology, School of Medicine, Ningbo University, 818 Fenghua Road, Ningbo, Zhejiang Province 315211, People's Republic of China.
E-mail: zhaojinshun@nbu.edu.cn

Min Ding, MD, PhD, Toxicology and Molecular Biology Branch, Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Morgantown, WV, 26505, USA.
E-mail: mid5@cdc.gov

†These authors contributed equally to this work.

[a]Department of Preventative Medicine, Zhejiang Provincial Key Laboratory of Pathological and Physiological Technology, School of Medicine, Ningbo University, 818 Fenghua Road, Ningbo, Zhejiang Province 315211, People's Republic of China

[b]Toxicology and Molecular Biology Branch, Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Morgantown, WV, 26505, USA

from others in their bulk state. At the nanoscale, materials may behave very differently compared to their larger scales. In the field of nanomedicine, the common acceptance extends this definition to particles or nanotherapeutics with dimensions up to 1000 nm (Schütz *et al.,* 2013). Schütz *et al.* (2013) classified medical nanoparticles (NPs) as three main categories: soft; hard solid; and other. Soft particles normally consist of polymers, proteins or lipids, including polymer NPs, micelles, vesicles, liposomes, core–shell systems, gels, polymeric drugs or polymer–drug conjugates. Hard solid-core NPs include metal and ceramic NPs such as oxides, semiconductors or carbon nanotubes, as well as nanocrystal drug formulations. Solid-core NPs with a polymer coating were also considered as solid NPs. Dimensions of solid-core NPs are well defined and less dependent on environmental parameters. However, the size and shape of soft nanoparticulate systems can largely depend on environmental factors, such as temperature, pH, ionic strength or medium characteristics. Other particles, the third category, such as antibody–drug conjugates, albumin particles, can also enable nanotype interactions due to their nanosize and construction.

Physiochemical properties of NPs and the corresponding bulk substitute may be quite different. For example, zinc oxide NPs have been found to have superior ultraviolet blocking properties compared to its bulk substitute. This is one of the reasons why it is often used in the preparation of sunscreen lotions. The unique physical and chemical properties of nanomaterials can be classified as the following aspects (Amanchi Bala *et al.,* 2015).

Small size

While most microstructure materials have similar properties as the corresponding bulk materials, the properties of materials with nanometer dimensions are significantly different from those of bulk materials (Cao *et al.,* 2013). The nanometer size of the nanomaterials renders them: (1) large fraction of surface atoms; (2) high surface energy; (3) spatial confinement; and (4) reduced imperfections, which normally do not exist in the corresponding bulk material.

At the nanometer scale, properties become size-dependent, which include: (1) chemical properties – reactivity, catalysis; (2) thermal properties – melting temperature; (3) mechanical properties – adhesion, capillary forces; (4) optical properties – absorption and scattering of light; (5) electrical properties – tunneling current; and (6) magnetic properties – superparamagnetic effect.

Surface area

As the particle size gets smaller for nanometer particles, the ratio of the surface atomic number to the total number of atoms increases sharply (Jacobs *et al.,* 1997). The surface-to-volume ratio (and consequently the fraction of the surface atoms with respect to the bulk ones) also increases (Issa *et al.,* 2013). The large surface-to-volume ratio of the NPs is the key factor to the novel physical, chemical and mechanical properties compared to those of the corresponding bulk state. The surface area to volume ratio in NPs has a significant effect on the particle properties. As particle size decreases, a greater portion of the atoms are found at the surface compared to those inside, which results in NPs having a much greater surface area per unit volume compared with larger particles. It leads to NPs being more chemically reactive meaning that a given mass of material in nanoparticulate form will be much more reactive than the same mass of material made up of larger particles (Buzea *et al.,* 2007).

Quantum size

One of the most direct effects of reducing the size of materials to the nanometer range is the appearance of quantum effects due to the confinement of the movement of electrons. This leads to discrete energy levels depending on the size of the structure. When the size of a particle is smaller than the de Broglie wavelength, electrons and holes are spatially confined and electric dipoles are formed, and discrete electronic energy levels would be formed in all materials. Once particles get so small, the quasi-continuous assumption for the Fermi–Dirac probability distribution is no longer valid and the energy levels must be considered discrete for the electrons because different mechanisms take precedence, resulting in a different phenomenon such that the oxidation, reduction and catalytic properties change (Ekimov *et al.,* 1993). Early in 1996, Volokitin *et al.* (1996) had already confirmed that quantum size strongly influences the thermodynamic properties of metallic NPs. Following this line, artificial structures with properties different from those of the corresponding bulk materials can be created.

Chemical reaction properties

Owing to a large number of active atoms on the surface, NPs have specific surface adsorption, dispersion, aggregation, rheological properties and viscosity of colloidal suspension properties (Spampinato *et al.,* 2015). Normally, the NPs are chemically more active than those of the corresponding bulk material are (Luo *et al.,* 2006). As Li and Zhu (2006) reported the high chemical reactivity of silver (Ag) NPs with hydrochloric acid and characterized by X-ray powder diffraction gives direct evidence of the reaction, which has been proven impossible for the bulk Ag.

Catalytic properties

NPs are small in size but large in surface area, which is excellent support for active catalytic activity (Zhang and Fu, 2013). Normally, NP catalytic activities are quite different from the traditional catalysts. Metal NPs dispersed on an oxide support often show a much higher catalytic activity than the single-component NPs (Lee *et al.*, 2010b). Poreless nanometer catalysts can avoid the use of conventional means when the reactants to the pore diffusion, which are not attached to the inert carrier, can go directly into the liquid phase of the reaction system (Trovarelli, 1997).

Optical properties

The optical properties of metal NPs have been of interest in physical chemistry for a long time. NPs often possess unexpected optical properties, as they are small enough to confine their electrons and produce quantum effects. For example, gold NPs appear deep red to black in solution (Mandal, 2012). Depending on the size of the smallest feature, the interaction of light with structured materials can be very different. The coupling between several metallic NPs can induce a field enhancement in the surrounding media, which can increase phenomena such as scattering, absorption, luminescence or Raman scattering (Berginc, 2011).

Other properties

NPs also have higher hardness, higher plasticity, higher specific heat and thermal expansion, higher conductivity, higher

Journal of Applied Toxicology

diffusivity, lower sintering temperature and sintering shrinkage than large ones (Zhang *et al.*, 2013a). Other properties unique among NPs include surface plasmon resonance in some metal particles and superparamagnetism in magnetic materials (Chen *et al.*, 2015d; Estelrich *et al.*, 2015; Hussein-Al-Ali *et al.*, 2014; Skopalik *et al.*, 2014), which makes them desirable as a magnetic-targeting tool for medical applications.

Potential applications of nanomaterials in nanomedicine

In this paper, we use the words "potential applications" meaning at present most of the research evidence for nanomaterials used in nanomedicine is from cell culture or animal experiments. As Marchal *et al.* (2015) pointed out: "The hope raised by promising preclinical studies has often resulted in disappointment when nanomedicines have been applied in patients."

Applications in medical diagnosis

Disease diagnosis is a very important part of clinical medicine, and accurate and high precision of diagnosis is an important requirement for effective treatment. NPs can be smaller than red blood cells. After the NPs have been injected into the bloodstream, it can free flow in the blood vessels and be transported to various parts of the body. In this way, NPs can be used as a means of monitoring and diagnosis of diseases. Studies indicate that nanotechnology has brought a rapid development of diagnostic technology in medical fields, which can be mainly classified into the following aspects.

Imaging diagnosis. NPs are being used to enhance medical imaging. Several approved applications utilizing iron oxide NPs for *in vivo* magnetic resonance imaging (MRI) enhancement were identified, some are under clinical investigation (Etheridge *et al.*, 2013; Wang *et al.*, 2001). Nanoshells possess highly favorable optical and chemical properties for biomedical imaging and therapeutic applications (Loo *et al.*, 2004). A new type optical coherence tomography (OCT) with gold nanoshells can improve the detection of certain diseases (Agrawal *et al.*, 2006; Brezinski *et al.*, 1996; Loo *et al.*, 2004). The axial resolution of OCT is limited to about 1 μm (Fuchs *et al.*, 2016), so the precision is thousands of times higher than the computed tomography and MRI. Unlike X-ray, computed tomography or magnetic resonance (nuclear magnetic resonance spectroscopy), OCT with nanoscale imaging technology may find the disease at an earlier stage without damaging the living cells (Leary *et al.*, 2006). For example, Chao *et al.* (2010) have shown that the technique of OCT using gold nanoshells enables integrated structural and molecular-targeted imaging for cancer markers. Kim *et al.* (2009) showed that a multimodal delivery of antibody-conjugated PEGylated gold NPs in a hamster model could enhance the contrast *in vivo* OCT images of oral dysplasia. Fuchs *et al.* (2016) proposed extreme ultraviolet coherence tomography as a new technique for non-invasive cross-sectional imaging by using nanometer structures. In addition, Au *et al.* (2011) evaluated a near-infrared (NIR) absorbing contrast agent based on polypyrrole NPs for quantitative OCT studies on tissue phantoms and Mie scattering calculations. They suggested that polypyrrole NPs might be a potential OCT contrast agent for cancer imaging.

Laboratory diagnosis. Nanotechnology and its applications in biomedical sciences principally in molecular nanodiagnostics are known as nanomolecular diagnostics (Gorjikhah *et al.*, 2016). Biomedical nanotechnology on laboratory diagnostics is widely investigated, such as the magnetic NPs linked with antibodies to mark special molecules and structures. A lab-on-a-chip is a tool that incorporates numerous laboratory tasks on to a small device, usually only millimeters or centimeters in size (Gorjikhah *et al.*, 2016), where advantage is taken from nanotechnology to enable precise control of the biochemical cellular environment. These tools also offer the possibility of analyzing the composition of single cells (Andersson and van den Berg, 2004). In recent years, biological assays with light color code and nanopore analysis of nucleic acids have been developed. Nanopores have emerged as a new tool for studying the properties of nucleic acids at the single-molecule level (Larkin *et al.*, 2013). According to different diagnostic and testing purposes, tiny probe technology allows the nanoprobe to locate in different parts of the body, or travel with the blood in the bloodstream, which provides the body with a variety of biological feedback information supplied by an *in vitro* recording device. This tiny probe technology has great potential in clinical medicine. In an effort to improve the early diagnostic rate of pancreatic cancer, Liu *et al.* (2016) demonstrated that a nanoprobe Fe_3O_4@SiO_2 modified with an anti-mesothelin antibody could effectively target pancreatic cancer *in vitro* and *in vivo* and might be a promising agent for diagnosis of pancreatic cancer.

Genetic disease diagnosis. Owing to the unique optical properties, nanomaterials can be used in gene diagnosis and base mutation detection (Oh and Lee, 2011). Chin *et al.* (2014a) proposed that nanostructured DNA biosensors could detect base mutations. To speed up and simplify mutation screening in genes, Vanden Bon *et al.* (2014) developed a method based on the change in heat transfer resistance upon thermal denaturation of double-stranded DNA on nanocrystalline diamond to detect the mutation in entire exons of the phenylalanine hydroxylase gene in phenylketonuria patients. To determine whether a fetus had a genetic defect, amniotic fluid technology, an expensive and possibly harmful diagnosis, normally was used. With the application of nanotechnology, now a much more efficient and safe way has been developed. At the early stages of pregnancy, very small amounts of fetal cells in the maternal blood can be used for genetic defect diagnosis (Bianchi and Hanson, 2006). Liu *et al.* (2011) developed a new method to study the electrochemical behavior of dGTP utilizing carbon multi-walled nanotube-modified glassy carbon electrodes for mapping of the pancreatic cancer genetic fingerprint and screening of genetic alterations. Their results indicated that the coupling of random amplified polymorphic DNA and nanoelectrochemical sensors could be successfully applied to the screening of genetic alterations in pancreatic cancer and for mapping of DNA fingerprints. Chin *et al.* (2014b) demonstrated that by using a nanostructured biosensor it is possible to differentiate effectively between a haplotype mutation and normal genes in the MD-2 gene promoter. A barrier layer with a nanohemisphere array of anodic aluminum oxide was used as the substrate for the biosensor. They found a clear distinction between the haplotype mutation samples and the normal target samples, even using samples produced by a five-cycle polymerase chain reaction process.

Tumor early diagnosis. Detecting cancer at an early stage is one of the most important factors associated with the survival rate of

patients. Cancer detection at early stages using NPs have been widely explored in recent years (Dassie *et al.,* 2015). Dassie *et al.* (2015) investigated the use of polysaccharide NPs loaded with [4-(dicyanomethylene)-2-methyl-6-(4-dimethylaminostyryl)-4H–pyran] (DCM), as a potential diagnostic tool in an experiment using a rat model of Barrett's esophageal adenocarcinoma. Their results suggested that polysaccharide NPs loaded with DCM might be useful as a targeted carrier for photoactive and bioactive molecules in esophageal cancer diagnosis. Nanowires and nanocantilever arrays are used for the early detection of precancerous and malignant lesions from biological fluids (Ferrari, 2005). Qian *et al.* (2008) reported that biocompatible and non-toxic NPs such as pegylated gold NPs and surface-enhanced Raman scattering are effective for *in vivo* tumor targeting and detection. NIR fluorescent NPs are also considered promising candidates for use as contrast agents for tumor detection (Ren *et al.,* 2015). Kolitz-Domb *et al.* (2014) demonstrated that bioactive conjugated NIR fluorescent proteinoid-poly (L-lactic acid) hollow NPs can be used for optical detection of colon cancer in a chicken embryo model. Will *et al.* (2006) assessed the diagnostic precision of MRI with Ferumoxtran-10, an ultrasmall superparamagnetic iron oxide NPs, used as a contrast agent for the diagnosis of lymph node metastases. Compared with that of unenhanced MRI and final histological diagnosis, they found that the Ferumoxtran-10-enhanced MRI is sensitive and specific in the detection of lymph node metastases for various tumors, which offers higher diagnostic precision than does unenhanced MRI for detection of lymph node metastases.

Applications as drug carriers

With the mutual infiltration of nanoscience and modern technology, nanomaterials are widely investigated in the medical field for drug delivery (Xing and Zhang, 2004). Drug delivery focuses on maximizing bioavailability both at specific places in the body and over a period of time, which can potentially be achieved through molecular targeting by nanoengineered carriers (Drbohlavova *et al.,* 2013). Compared with traditional drugs, drugs carried by nanocarriers have the following characteristics.

(1) Nanodrug carriers may not only pass through the circulation of the blood into the capillaries, but also pass through the endothelial cell gap into the lesions (Nichols and Bae, 2012). Drugs delivered by the nanodrug carriers can be absorbed by cells in the form of pinocytosis. In this way, bioavailability of the drugs will be largely improved.
(2) Owing to the large surface area, nanosize carriers can be used to embed hydrophobic drugs, thus increasing the solubility of the drug and reducing the side effects of co-solvents normally used in conventional drugs.
(3) Nanodrug carriers modified with targeted tissue specificity, such as folic acid modification for drug-loading NPs and magnetic NPs, help the drugs reach the targeted tissue efficiently, which can not only reduce the administration dosage, but also reduce the side effects of the drugs.
(4) Nanometer drug carriers can prolong the half-life of drug elimination and improve the time and efficacy of the drug concentration in the blood (Wang *et al.,* 2015a), thus reducing the drug administration frequency.
(5) Nanometer drug carriers can pass through the biological barriers of the human body, such as the blood–brain barrier, blood–eye barrier, as well as other cell biological membrane barriers, allowing drugs to reach the lesions, and increasing efficacy (Lim and Helpern, 2002; Patravale *et al.,* 2004; Stowe *et al.,* 2012).
(6) Compared with drug administration alone, drugs delivered by nanocarriers can achieve targeted drug therapy with high bioavailability (Pandey *et al.,* 2005) and reduce the side effects of drugs accompanying decreases in drug dosage (Bishwajitsutradhar and Amin, 2014) and treatment cost (Master and Sen Gupta, 2012).

Applications in medical instruments

Instead of macro-level conventional instruments, nanomedical instruments can be designed at micro-levels for disease diagnosis or treatment. Dramatically, nanotechnology may bring us new instruments to examine tissue in unprecedented detail. Sensors smaller than a cell may give us an insight and exquisitely precise look at ongoing functions of the human body (Merkle, 1996).

Nanoprobe. A nanosensor probe may be designed according to different diagnostic and monitoring purposes and then transported and localized to different parts of the body through the bloodstream circulation. Feedback of biological information will be possible by using *in vitro* recording devices (Li *et al.,* 2014; Panchapakesan *et al.,* 2011). Won *et al.* (2005) reported a technology named magnetism-based interaction capture, which can be used to identify molecular targets based on induced movement of superparamagnetic NPs inside living cells. They developed a transducible fusogenic peptide to mediate the intracellular uptake of superparamagnetic NPs (coated with a small molecule of interest). Nanoprobes captured the small molecule's labeled target protein and are translocated in a direction specified by the magnetic field. In this way, magnetism-based interaction capture might be useful for screening identified protein targets of a drug or monitoring signal-dependent modification and multiple interactions of proteins. Chen *et al.* (2015b) designed a photoacoustic nanoprobe for pH detection. They found that this nanoprobe is safe and easy to operate with depth-independent accuracy for real-time *in vivo* pH imaging of entire tumors. Liu *et al.* (2015b) developed an NIR fluorescence nanoprobe by coating CuInS2/ZnS quantum dots with an amphiphilic bioconjugate and investigated the feasibility of the constructed NIR fluorescence probe *in vivo* imaging. A decorated nanoprobe was found to be highly selective for targeted integrin αvβ3-overexpressed tumor cell imaging. A preclinical evaluation of a nanoprobe in rhesus monkeys concluded that the nanoprobe of urokinase plasminogen activator receptor-targeted magnetic iron oxide NPs has the potential to be used as receptor-targeted MRI contrasts as well as theranostic agents for the detection and treatment of human cancers (Chen *et al.,* 2015c).

Nanorobot. In recent years, a new science named nanorobotics is emerging. It is an interdisciplinary science of nanomaterial science and robotics technology with biological knowledge (Toumey, 2013). As early as 2007, Popov *et al.* (2007) proposed a set of nanoelectromechanical systems based on the relative motion of carbon wall nanotubes for use in medical nanorobots, which includes electromechanical nanothermometers, jet nanoengines and nanosyringes. A molecular medicine robot is the organic combination of both a nanomechanical and a biological system (Jacob *et al.,* 2011). The nanorobot could carry gene sequences to the targeted cells acting as a miniature doctor in biomedical engineering to solve the problems that were difficult

for the traditional human doctor to treat. The biggest advantage is that the nanorobot can be injected directly into human blood vessels, and perform health examination and treat certain diseases. It can also be used to repair human organs, such as cosmetic surgery, remove harmful DNA from genes or install normal DNA into the genes for therapeutic purposes. In the treatment of atherosclerosis, it also can be used for the removal of blood clots, clean the wound, dispel parasites, remove kidney stones and artificial insemination (Biswas and Sen, 2016; Hariharan and Manohar, 2010; Kaewkamnerdpong *et al.,* 2015). Of course, nanorobot technology is now still in the early development stage and more studies are needed for transformation to clinical application.

Handheld disease diagnosis instrument. This is a mini-instrument that can diagnose disease, which can be put in a pocket. A team of investigators from the Massachusetts General Hospital and Harvard Medical School have developed a fast, portable molecular imaging device in combination with magnetic NPs and a smartphone (Haun *et al.,* 2011). The device is named a hand-held nuclear magnetic resonance spectroscopy instrument. They found that the device trumps traditional pathological methods, both in terms of speed and diagnostic accuracy by using fine needle biopsies taken from human patients with cancer. These nanodiagnostic technologies are potentially applicable to global health applications, as they are supposed to be inexpensive, portable and easy-to-use for the detection of diseases (Lee *et al*., 2010a).

Nanosensor. The unique chemical and physical properties of NPs make them extremely suitable for designing new and improved sensing devices, particularly electrochemical sensors and biosensors (Luo *et al.,* 2006). Nanosensors include nanobiological and chemical sensors, nanometer gas sensitive sensors and other types of nanometer sensors (flow, pressure and temperature, etc.) (Feng and Yong, 2012). The development of nanotechnology, not only provides good sensitive material for nanosensors, such as NPs, nanotubes, nanowires, nanofilm, but also provides an innovative idea for many manufacturers of sensors. Compared with traditional sensors, the size of the nanosensors is reduced, but the precision is improved greatly (Li *et al.,* 2011). In the field of medicine, nanosensors have great potentials to measure *in vivo* cell temperature, volume, concentration, displacement, speed, weight, electrical and magnetic forces, or pressure. Nanosensors may also be useful in distinguishing between normal and cancerous cells at the molecular level for the early diagnosis of cancer. Zhou *et al.* (2011) developed a label-free biosensor based on Ag NPs array for clinical detection of serum p53 in patients with head and neck squamous cell carcinoma. The nanosensor consists of a triangular Ag NPs array with single particle dimension of 120 nm in-plane width and 45 nm out-of-plane height. They concluded that this kind of nanobiosensor might provide a promising platform with attractive advantages for the serological diagnosis or molecular diagnosis of tumors in the future.

Applications in tissue engineering

Nanobone. The "nanobone" material could potentially replace traditional bone material because "Nanobone implants" may have a better capacity to interact with living tissue, allowing the body to repair itself much faster (Boos *et al.,* 2016). Injectable and forming nanobone materials are non-toxic with good restoring and histocompatibility. These bone materials can promote bone tissue growth and recovery function (Abshagen *et al.,* 2009). Harms *et al.* (2012) tested the osteogenic capacity of nanocrystalline bone cement in a weight-bearing defect at the ovine tibial metaphysis and found that nanobone is a highly potent bone substitute material with osteoconductive properties in a loaded large animal defect model, supporting the potential use of nanobone in humans. Dau *et al.* (2016) evaluated bone formation in monocortical mandibular critical size defects after augmentation with two synthetic nanostructured (NanoBone®, Ostim®) and one xenogenous hydroxyapatite bone substitute (Bio-Oss®) in an *in vivo* animal study with mini-pigs. They found no significant difference in the biological hard tissue response between NanoBone® and Bio-Oss®. The water-soluble Ostim® initially induced an increased amount of new bone but was highly compressed indicating a negative effect in less stable augmentations of the jaw.

Nanoscale red blood cell substitutes. Lack of perfusion of oxygen to the tissue is one of the most fundamental and fast acting conditions, which can be harmful to the human body. Advances in nanotechnology have suggested a possible treatment for this condition in the form of microelectromechanical red blood cell analogs called "respirocytes." Primary applications of "respirocytes" include: transfusable blood substitution; partial treatment for anemia, perinatal/neonatal and lung disorders; prevention of asphyxia; and artificial breathing. Scientists tried using a biodegradable polymer as shell material together with hemoglobin (Hb) to form a nanoscale red blood cell substitute to enhance tissue oxygenation (Zhao *et al.,* 2008). The results of *in vitro* and *in vivo* studies indicated that Hb-loaded particles did not cause significant changes in total platelet count and suggest that the Hb-loaded NPs may be useful as a potential candidate in substitution for red blood cells (Zhao *et al.,* 2008). Chang *et al.* (2003) studied a novel nanodimension artificial red blood cell substitute based on ultrathin polyethylene-glycol–polylactide (PEG–PLA) membrane nanocapsules (80–150 nm diameter) containing Hb and enzymes. They found the best PEG–PLA Hb nanocapsules were prepared using a combination of the following four factors: polymerized Hb; higher molecular weight PLA; higher concentrations of PEG–PLA; and crosslinking of the newly formed PEG–PLA Hb nanocapsules.

Ghanaati *et al.* (2013) assessed the *de novo* bone formation capacity of a nanocrystalline hydroxyapatite bone substitute 3 and 6 months after its insertion into the human sinus cavity. They found that bone tissue formation started from the bone–biomaterial interface into the most cranial parts of the augmented region, but there was no statistically significant difference in new bone formation during this time.

New tissue engineering nanomaterials. Regulation of cellular behavior by nanotechnology is one of many examples demonstrating the significant applications of nanoengineering in biomedicine for the repair or regeneration of tissues and organs (Kim *et al.,* 2014). Tonelli *et al.* (2012) reported that carbon nanotubes could be used to produce scaffolds for tissue engineering by interacting with extracellular matrix proteins. Zhang *et al*. (2013b) have shown that silk fibroin/levorotatory polylactic acid, a new tissue engineering nanomaterial, has good biocompatibility and is a safe implant material. Other nanometer materials have been applied in the field of organ transplantation including the NPs coated on the surface to prevent the rejection of artificial organs in human organ transplants. Wen *et al.* (2015) tested a new method to generate covalent bonds between

collagen and cellulose to improve the immobilization of collagen on bacterial cellulose by using a facile dialdehyde bacterial cellulose/collagen peptide nanocomposite. Cell tests indicated that this nanocomposite was bioactive and suitable for cell adhesion and attachment, indicating it might be a promising material for tissue engineering and regeneration. Gandhimathi *et al.* (2014) evaluated the biocomposite nanofibers for the controlled release of biomolecules for skin tissue regeneration. Their results suggest that the accessibility of human dermal fibroblasts cultured on poly(L-lactic acid)-co-poly-(ε-caprolactone)/silk fibroin/vitamin E/curcumin nanofibrous scaffolds is a potential scaffold for skin tissue regeneration. Dyondi *et al.* (2013) tested gellan xanthan gels along with chitosan NPs of basic fibroblast growth factor, and bone morphogenetic protein 7 in a dual growth factor delivery system to promote the differentiation of human fetal osteoblasts. Their results suggested that encapsulation and stabilization of growth factors within NPs and gels are promising for bone regeneration. In addition, they also found that gellan xanthan gels have antibacterial effects against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Staphylococcus epidermidis*, the common pathogens in implant failure. Yao *et al.* (2013) investigated the nanostructured polyurethane and poly-lactic-co-glycolic acid scaffolds in an *in vivo* study, and found that nanostructured polyurethane and poly-lactic-co-glycolic acid composites could significantly increase bladder tissue repair.

Applications for therapeutic drugs

Antibacterial nanodrugs. Antibacterial efficiency of nanosize Ag particles are usually associated with the total surface area of the NPs, and are much higher than traditional fine Ag particles (Rai *et al.,* 2009). Nanosize Ag particles (10–100 nm) can easily enter pathogens and combine with the bacteria protein enzyme thiol quickly, and then kill the bacteria. Metal-based NPs have a broad antibacterial effect on a range of gram-negative and -positive bacteria and antibiotic-resistant bacteria strains (Ge *et al.,* 2014). Although Ag NPs alone are strong bactericidal agents (Murugan *et al.,* 2014), they are also cytotoxic (Ge *et al.,* 2014). Embedding them in a polymer matrix may reduce their cytotoxicity (Liu *et al.,* 2010). Juan *et al.* (2010) tried to deposit Ag NPs on a titanium surface to obtain antibacterial properties. The diameter of these NPs ranged from 10 to several hundred nanometers. Two species of bacteria, *S. aureus* and *Escherichia coli*, were used to test the antibacterial effect of the titanium Ag NP treated surface. After a 24 h incubation, 94% of *S. aureus* and over 95% of *E. coli* had been killed on the titanium Ag NP surface, suggesting that Ag NP-modified titanium is a promising material with an antibacterial property. In another study, they reported that Ag NP-modified titanium had uncompromised cytocompatibility (Liao *et al.,* 2010). Shameli *et al.* (2010) evaluated the antibacterial activities of the Ag/poly (lactic acid) nanocomposite films against gram-negative bacteria (*E. coli* and *Vibrio parahaemolyticus*) and gram-positive bacteria (*S. aureus*) by a diffusion method using Mueller–Hinton agar. Their results indicated that Ag/poly (lactic acid) nanocomposite films possessed a strong antibacterial activity with an increase in the percentage of Ag NPs in the PLA (Shameli *et al.,* 2010). Nagy *et al.* (2011) evaluated the antibacterial properties of Ag NPs embedded within a zeolite membrane (Ag NP ZM). Their results indicated that Ag NP ZM provide a novel matrix for gradual release of Ag(+). It has been suggested that the antibacterial mechanism of Ag NP ZM is related to the exhaustion of antioxidant capacity.

Many other metal or metal compound NPs also show antibacterial activity, such as ZnO NPs (Mehmood *et al.,* 2015), TiO_2 nanotubes (Liu *et al.,* 2015a), gold NPs (Mocan *et al.,* 2014), gold-supported cerium oxide NPs (Babu *et al.,* 2014), etc. It is worthy to note that most of the research is *in vitro* experiments; therefore, more *in vivo* as well as clinical experiments are needed for elucidating the antibacterial activity of different NPs.

Antiviral nanodrugs. Heavy metal such as Ag, copper, lead or mercury can inactivate enzymes by reacting with thiols in proteins. It has been reported that Ag NPs have higher antiviral activity than Ag ions, due to species differences as they release Ag^0 (atomic) and Ag^+ (ionic) clusters, whereas Ag salts release Ag^+ only (Ge *et al.,* 2014). Research evidence shows that Ag NPs possess antiviral activity against HIV-1 (Lara *et al.,* 2010; Sun *et al.,* 2005), hepatitis B virus (Lu *et al.,* 2008), respiratory syncytial virus (Taylor *et al.,* 2005) and human influenza virus (Xiang *et al.,* 2013). However, the heavy metal ions in the unstable solution limit its wide application. Influenza is one of the fatal diseases and Miao *et al.* (2010) derived a study suggesting that Ag NPs have obvious inhibitory effects on the influenza virus H3N2. To analyze the anti-HIV activities and cytotoxicity of Ag NPs, the research suggests that Ag NPs can inhibit HIV-1 and HIV-2 replication *in vitro* by its virucide activity and no acute toxicity is found on the mice treated by nasal dropping of Ag NPs (Wang *et al.,* 2009).

Antitumor nanodrugs. Tumors are one of the main causes of death in the world, the ideal antitumor drugs can be targeted and can cross gaps in the tumor tissue wall, then once embedded in the tumor tissue can destroy tumor cells, and have little or no effect on normal cells. The specific surface-modified gold NPs can selectively kill tumor cells, but have no significant effect on normal cells. These findings support the concept that tagging tumor necrosis factor (TNF)-gold NPs with tumor vasculature homing peptides have improved antitumor activity, likely because of an active targeting mechanism (Curnis *et al.,* 2015; England *et al.,* 2013). Under pathological and physiological conditions, depending on the difference in biological effects and cell sensitivity, nanomaterials can be designed artificially as organelles to target antitumor nanodrug carriers for targeting tumor treatment (Guo *et al.*, 2015a; Spadavecchia *et al.,* 2016).

Analgesic and anti-inflammatory nanodrugs. Analgesic and anti-inflammatory drugs are drugs with analgesic, as well as, most have anti-inflammatory and anti-rheumatoid effects. Owing to the special anti-inflammatory effect, they are also known as non-steroidal anti-inflammatory drugs (NSAIDs). Because the traditional NSAIDs have adverse reactions to the stomach and kidney, more effective and safer NSAIDs need to be developed. One of the current research trends is the development of nanopreparations of NSAIDs, which can enhance the efficacy and reduce the side effects of the drug. Studies have shown that the anti-inflammatory effect of nanopreparations of aspirin is two times greater than ordinary aspirin at the same dose (Rajesh *et al.,* 2004). Research evidence shows that the use of nanoliposomes as the carriers for diclofenac sodium can improve the analgesic and anti-inflammatory effects (Fan *et al.,* 2007).

Other nanomaterial drugs. Nanomaterials have many other applications in the field of nanomedicine, such as NPs encapsulating hormone drugs (Ayano *et al.,* 2012; Hariri *et al.,* 2015; Ishihara *et al.*, 2009a,b; Santander-Ortega *et al.,* 2009; Silva

et al., 2015; Swaminathan *et al.,* 2013), nanometer polypeptide and protein drugs (Peppas and Kavimandan, 2006), nanotraditional Chinese medicine and so on. Nanotraditional Chinese medicine refers to bioactive ingredients, bioactive parts, medicinal materials or complex prescriptions, being approximately 100 nm in size, which are processed by nanotechnology (Huang *et al.,* 2015).

Applications in nanogene medicine

Gene therapy by means of nanotechnology can be simply defined as nanogene medicine. Exogenous genes and their construction are mostly DNA molecules of nanometer size. Technology using nanocarriers to deliver these exogenous genes into the receptor cells belongs to nanogene medicine (Wu *et al.,* 2016). Biodegradable and biocompatible polymeric nanocarriers due to unique properties such as excellent biocompatibility, prolonged gene circulation time, prevented gene degradation, passive targeting by using the enhanced permeability and retention effect, and possibly modulating polymer structure to obtain desirable therapeutic efficacy, are considered as the most promising gene delivery vehicles (Mokhtarzadeh *et al.,* 2016). For example, lipid NPs, called liposome protamine/DNA lipoplex (LPD), are used for ocular gene delivery (Wang *et al.,* 2015b). Recently, there have been promising results achieved with LPD NPs to deliver functional genes and microRNA to treat retinal diseases (Rajala *et al.,* 2014; Takahashi *et al.,* 2015). Some advantages of these peptide-modified LPD NPs might be: (1) liposome NPs are able to deliver large molecular cargo; (2) optimization of peptide-modified LPD NPs allows multiple mutant genes to be simultaneously co-delivered to one vector; and (3) peptide-modified LPD NP formulations are more biocompatible and safe (Wang *et al.,* 2015b). Polyak *et al.* (2016) tried using systemic delivery of siRNA by another novel nanocarrier, aminated poly(α) glutamate, for the treatment of solid tumors in an ovarian adenocarcinoma-bearing mice model. They found that it is an efficacious and safe anticancer siRNA delivery vehicle following systemic administration. An anticancer siRNA, siPlk1-polyplex, delivered by this nanocarrier, inhibited tumor growth by 73% and 87% compared with siCtrl-polyplex or saline-treated mice, respectively, leading to prolonged overall survival.

In summary, although certain progress has been achieved in nanogene medicine, it is still in the early developmental stage.

Applications in gene therapy of cancer

As stated above, gene therapy has emerged as an alternative for the treatment of diseases (Mastorakos *et al.,* 2015). One of the advantages of nanotechnology is the feasibility to construct therapeutic particles that carry multiple therapeutics with a defined structure and stoichiometry. Accordingly, the field of RNA nanotechnology is emerging (Chen *et al*., 2015a; Guo, 2010; Roh, 2012; Shukla *et al.,* 2011). However, controlled assembly of stable RNA NPs with multiple functionalities, which retain their original role, is challenging due to refolding after fusion. Researchers at MIT and Harvard Medical School have developed a new kind of NP delivery systems by using siRNA delivery to tumor cells, which can eliminate tumors by destroying the mRNA (Dahlman *et al.,* 2014). The outside of this kind of NP has a layer of membrane and inside is a mixture of siRNA and protein. After entering into the tumor cells, NPs with a protein and siRNA mixture can destroy the targeted mRNA. Ren *et al.* (2012) found that most of the tumors can be eliminated in ovarian tumor-bearing mice by using RNAi (RNA interfere) NP treatment. Scientists at the University of Kentucky have developed thermodynamically stable X-shaped RNA NPs for carrying therapeutic RNA motifs by self-assembly of re-engineered small RNA fragments, which is considered useful for cancer treatments at the RNA and DNA level (Haque *et al.,* 2012).

Applications in nanovaccines

Nanovaccine is a novel approach for vaccinations (Zaman *et al.,* 2013). It consists of nano-sized particles of a biodegradable polymer, which encapsulates an antigen of a pathogen, or the active ingredient. Nanovaccines are more efficient than conventional vaccines in that they induce both a humoral and a cell-mediated immune response. Most nanovaccines are non-invasive, delivered by oral or nasal routes, thus allowing no-pain delivery with minimal damage. In addition, nanovaccines can control the delivery of the associated antigens to a specific location and for prolonged times (Cordeiro *et al.,* 2015). No-pain delivery might be the biggest advantage of nanovaccines over conventional vaccines, which are usually multi-injection and multi-dose delivery systems (Nandedkar, 2009). The disadvantages of nanovaccines may include difficulty in reproducibility of formulation during manufacturing (Sharma *et al.,* 2009) and potentially toxicity induced by a prolonged clearance time of the NPs in the body. Therefore, evaluation of the safety of nanovaccines should be equally essential as the study of their efficacy (Nandedkar, 2009). Nanomaterials can be used for nanovaccines including natural nanocarriers (i.e. as bacterial spores, virus-like particles, exosomes and bacteriophages) and synthetic nanocarriers (i.e. proteosomes, liposomes, virosomes, SuperFluids and nanobeads) (Gill, 2013). Nanomaterials are frequently evaluated as the nanocarriers for nanovaccines, which are in the experimental stage at present, including liposomes (Ghaffar *et al.,* 2014; Hu *et al.,* 2014; Marasini *et al.,* 2016), polysaccharide (Cordeiro *et al.,* 2015; Vicente *et al.,* 2014), polyanhydride (Carrillo-Conde *et al.,* 2011; Chavez-Santoscoy *et al.,* 2012), polymeric (Guo *et al*., 2015b; Vicente *et al.,* 2013), chitosan (Danesh-Bahreini *et al.,* 2011; Doavi *et al.,* 2016; Hunsawong *et al.,* 2015) and silica NPs (Mahony *et al.,* 2014; Mody *et al.,* 2015), etc.

Nanovaccines stimulate the human body's immune system curing infections, preventing infections and diseases from spreading (Zaman *et al.,* 2013) and possibly promising chronic disease treatments, such as cancers (Nandedkar, 2009; Paulis *et al.,* 2013), HIV (Vela Ramirez *et al.,* 2014), influenza (Petukhova *et al.,* 2013) and others.

Applications as radiosensitizers in radiation therapy

Along with the rapid development of nanotechnology, the potential value of NPs as novel radiosensitizers in radiation therapy has been investigated in recent years (Su *et al.,* 2014). Radiation therapy is one of the most commonly used non-surgical interventions in tumor treatment but is often hindered by low efficacy (Bergs *et al.,* 2015). Research evidence shows that NPs can enhance the efficacy of the radiation therapy by acting as both a therapeutic and a carrier for other therapeutics. NPs, particularly high atomic number metal NPs such as gold, can either sensitize cancer cells to ionizing radiation via their physicochemical properties, or encapsulate radiation sensitizing agents, thereby protecting them from degradation (Bergs *et al.,* 2015; Kwatra

et al., 2013). At present, the most commonly investigated nanoparticulate radiosensitizers in preclinical models include gold (Botchway *et al.,* 2015; Dorsey *et al.,* 2013; Hainfeld *et al.,* 2008; Her *et al.,* 2015; Jain *et al.,* 2012; Lee *et al.,* 2014; Yamada *et al.,* 2015), Ag (Swanner *et al.,* 2015; Wu *et al.,* 2015; Yamada *et al.,* 2015) and iron oxide (Bhana *et al.,* 2015; Klein *et al.,* 2014; Sun *et al.,* 2016; Zhao *et al.,* 2012) NPs.

In conclusion, due to the potential for a better therapeutic index with radiation therapy, NPs are being widely investigated for cancer therapy.

Human biosafety of nanomaterials used in clinical trials

Nanomaterials are being widely developed for medical and pharmaceutical purposes with the rapid development of nanotechnology. Despite the many proposed advantages of nanomaterials, increasing concerns have been expressed on human biosafety (Zhao and Castranova, 2011). First, as stated above, NPs possess different physicochemical properties compared to their bulk analogues due to extremely small size and large surface area, which makes the particles more reactive and catalytic. Second, in the field of nanomedicine, exogenous NPs may be delivered into the human body without passing through the normal gastrointestinal absorption process after intravenous or interstitial injections. Third, in the human body, NPs have the potential to interact with biological molecules or to accumulate in human tissues or organs. Therefore, human biosafety evaluation is the key point for the safe use of nanomaterial products in clinical practice. However, it is worthy to note that, until now, most published data related to nanomaterial products in nanomedicine are still staying at *in vitro* cell culture or *in vivo* animal experiment stages. Human clinical trials should be the last but the most important step for clinical translation of nanomaterial products. However, the transition probability might be low because clinical translation in nanomedicine is a long, arduous, resource intensive process (Satalkar *et al.,* 2016). In addition, human biosafety uncertainty raises a variety of ethical concerns in clinical trials for nanomedicine products (Resnik and Tinkle, 2007).

Under conditions of occupational and environmental exposures, inhalation of NPs is normally the primary route of entry into the human body. However, intravenous and interstitial injections of nanoparticulate carriers represent the specific exposure routes in nanomedicine. In a previous article entitled as "Toxicology of nanomaterials used in nanomedicine" (Zhao and Castranova, 2011), we reviewed biosafety evaluation data related with animal studies of NPs. The research data on the acute and chronic toxicity, genotoxicity, carcinogenicity, as well as reproductive and developmental toxicity of NPs in animal studies are expanding but are not yet complete. Animal experiments suggest that some of the NPs may potentially exhibit adverse health effects on the human body. For example, TiO_2 and carbon black NPs are classified as possibly carcinogenic to humans by WHO/International Agency for Research on Cancer based solely on carcinogenicity data in experimental animals (Baan, 2007). Limited results are reported for the carcinogenicity of multiwalled carbon nanotubes in animal experiments after intraperitoneal injections (Sakamoto *et al.,* 2009), but no data are available for lung inhalation exposure. Research reports related to the genotoxicity of NPs are still limited (Zhao and Castranova, 2011). In addition, no data are available to confirm carcinogenicity among other NPs (Tsuda *et al.,* 2009). However, Sargent *et al.* (2014) have shown that inhalation of multiwalled carbon nanotubes can cause tumorigenesis in mice. Whether human exposure to NPs designed for medical use induces cancer also remains unclear (Zhao and Castranova, 2011). All these animal experimental results do have certain implications for human biosafety evaluation of nanomaterials designed for nanomedicine. However, animal experiments generally employ high dose and short exposure time, which is quite different from human administration routes in clinical practice. In addition, due to the profound differences in anatomy, physiology and genetics between humans and animals, results from animal experiments cannot directly be extrapolated to humans and in most instances animals have been poor predictors for how humans will respond to drugs.

Accordingly, in this paper, we mainly focus on human clinical trials and try to provide a general perspective on the current landscape related to human biosafety of nanomedicine products used or will be used in clinical practice. We try to answer: During the clinical trials, are there any adverse health effects or toxicities after human exposure?

In 2013, Etheridge *et al.* (2013) did a detailed search of the literatures with the keywords of "clinical trial and nanomedicine" through websites such as PubMed, Google and Google Scholar, etc. They identified a total of 247 applications and products, which were approved or in various stages of a clinical study, and all of the actively targeted products are aimed at diagnosing or treating various forms of cancer. In 2015, Marchal *et al.* (2015) identified 145 clinical studies for the terms "nanoparticles" and "cancer" through the US National Institute of Health database, which was much lower than the number of 45 139 clinical studies at the same time in oncology registered by this website. This reflects a big gap between NP design, quoting more than 9000 publications in PubMed, and clinical translation. Of course, a large number of companies developing NPs published the detailed clinical trial information on approved systems or nanoparticulate systems on their websites instead of open journals, which might be a reason for such a low number in clinical studies for nanomedicine products (Schütz *et al.,* 2013). In phase III failures of clinical trials reported between 2007 and 2010, Thomson Reuters Life Science Consulting found that 21% of the failures were due to safety issues (Arrowsmith, 2011). However, most of these clinical trial failures also cannot be found in open scientific literature.

Nanoconstructions are used for anticancer drug delivery, including liposomes, spherical structures ranging from 100 to 400 nm in size, and polymer-based chemical entities (less than 100 nm). Specific structures such as albumin-bound NPs or antibody–drug conjugates are also used. After extensive reviewing on clinical trial articles of nanomedicine in public journals, we found that most of them focused mainly on reporting the findings of the therapeutic effectiveness of the drugs (Wagner *et al.,* 2006), but not on evaluating the human biosafety of the nanomaterials. Although all the materials assigned for clinical trials have demonstrated biocompatibility using current standards, it remains unclear whether persistence in the human body will produce acute or chronic adverse effects (Etheridge *et al.,* 2013).

Unique properties of nanomaterials make them available to be used as effective antitumor agents or as a compound of combined therapy for improving the therapeutic effectiveness of existing antitumor drugs. However, despite considerable amounts of described nanotechnology-based formulations, only a limited

Journal of Applied Toxicology

number of them have been introduced into clinical trials (Piktel *et al.,* 2016).

NP albumin-bound paclitaxel (nab-paclitaxel) (Abraxane®) is a colloidal suspension of 130 nm particles homogenized in human serum albumin bound to paclitaxel (Al-Hajeili *et al.,* 2014), which has been approved for the treatment of metastatic breast cancer, based on a phase III clinical trial in 460 patients. A significant difference was reported in the overall survival in patients receiving nab-paclitaxel vs. solvent-based paclitaxel. Subsequently, a number of clinical trials have examined different schedules, doses, and combinations in an effort to optimize nab-paclitaxel-based therapy for metastatic and early stage breast cancer (Martin, 2015). No significant adverse effects related to human serum albumin nanocarriers were reported in these clinical trials. However, in a pivotal comparative randomized phase III study for the treatment of breast cancer, Yamamoto *et al.* (2011) found that the nab-paclitaxel-treated group showed a higher incidence of sensory neuropathy than the solvent-based paclitaxel group. Nab-paclitaxel is a novel formulation of paclitaxel that does not require solvents such as polyoxyethylated castor oil and ethanol. Use of these solvents has been associated with a toxic response, including hypersensitivity reactions and prolonged sensory neuropathy, as well as a negative impact in relation to the therapeutic index of paclitaxel (Yamamoto *et al.,* 2011). The reason for the phenomenon, why the human serum albumin nanocarrier group showed a higher incidence of sensory neuropathy than the solvent-based paclitaxel group was not reasonably explained in this clinical trial. The authors only mentioned that these adverse side effects rapidly resolved after interruption of treatment and dose reduction. Animal studies indicate that alterations in coagulation, renal, cardiovascular and pulmonary functions were mainly involved as potentially causing adverse effects following high-dose administration of human serum albumin (Gales and Erstad, 1993). It remains unclear if there is any relationship between a higher incidence of sensory neuropathy and ***human serum albumin nanocarriers.

In a clinical study, 66 patients (59 with recurrent glioblastoma) received neuronavigationally controlled intratumoral instillation of an aqueous dispersion of iron oxide (magnetite) NPs (Nano-Cancer® therapy) and subsequent heating of the particles in an alternating magnetic field. Treatment was combined with fractionated stereotactic radiotherapy. The side effects of this therapeutic approach were moderate, and no serious complications were observed. They concluded that thermotherapy using magnetic NPs in conjunction with a reduced radiation dose is safe and effective (Maier-Hauff *et al.,* 2011). Another clinical trial of 14 patients with glioblastoma multiforme received three-dimensional image guided intratumoral injection of aminosilane-coated iron oxide NPs (core diameter: 15 nm) dispersed in water, with an iron concentration of 112 mg ml^{-1}. Patients received 4–10 (median: 6) thermotherapy treatments following instillation of 0.1–0.7 ml (median: 0.2) of magnetic fluid per ml tumor volume and single fractions (2 Gy) of a radiotherapy series of 16–70 Gy (median: 30). They concluded that thermotherapy using magnetic NPs was tolerated well by all patients with minor or no side effects (Maier-Hauff *et al.,* 2007).

In a prospective phase I clinical trial, the treatment-related morbidity and quality of life following intraprostatic injection of a NP dispersion and thermotherapy using superparamagnetic NPs were investigated in 10 patients with biopsy-proven locally recurrent prostate cancer. Results showed that NP deposits were detectable in the prostates 1 year after thermal therapy. At a median follow-up of 17.5 months (3–24), no systemic toxicity was observed (Johannsen *et al.,* 2007). This clinical trial indirectly reminds us that therapeutic or carrier NPs may stay in the human body for a long time (more than 1 year).

Maeda *et al.* (2016) developed a tumor-targeted nanoprobe, *N*-(2-hydroxypropyl)methacrylamide copolymer-conjugated pirarubicin (P-THP). They found this tumor-targeted nanoprobe exhibited prolonged blood circulation time, thereby resulting in good tumor-selective accumulation. In a clinical pilot study of a patient with stage IV prostate cancer with multiple metastases in the lung and bone, P-THP (50–75 mg administered once every 2–3 weeks) was shown to clear the metastatic nodules in the lung almost completely after three treatments. In this study, the patient retained an excellent quality of life during the treatment without any apparent adverse side effects.

With the rapid development of nanotechnology, Ag nanoproducts have broadened their application as an antibacterial, antiviral and anti-inflammatory therapy (Munger *et al.,* 2014). In an *in vivo* human time–exposure study, Munger *et al.* (2014) conducted a prospective, controlled, parallel design systematic study with two oral doses (10 and 32 ppm, 5–10 nm and 25–40 nm, respectively) of a commercial Ag NP solution over a 3–14 day monitored exposure in 60 healthy volunteers (between 18 and 80 years of age). They found that this colloidal elemental Ag particulate formulation produces detectable Ag ions in human serum, but does not demonstrate any clinically significant changes in metabolic, hematologic, urine, physical findings, sputum morphology or imaging. Their results asserted that the Ag detected in the serum of the human subjects is absorbed in the upper gastrointestinal tract into the blood stream in an ionic form, but not in an intact Ag metallic particle form. Therefore, the fate of nanomaterials through different administration routes, for example, orally, or intravenous or interstitial injections, are entirely different. If there is any toxicity of Ag nanoproducts through intravenous injections into the human body it is still unknown.

Oleanolic acid and ursolic acid are considered relatively non-toxic, and have been used in cosmetics and health products through oral administration (Liu, 1995). Liu (1995) evaluated the single- and multiple-dose pharmacokinetics (PK) as well as the safety of ursolic acid nanoliposomes (UANL) in healthy volunteers and in patients with advanced solid tumors. Twenty-four healthy volunteers in the single dose PK study were divided into three different groups, which received 37, 74 and 98 mg m^{-2} of UANL via a 4 h intravenous infusion, respectively. Eight patients in the multiple dose PK study were administered 74 mg m^{-2} of UANL daily for 14 days. No drug accumulation was observed with repeated doses of UANL. Nausea, diarrhea and abdominal distention were the common adverse effects that were observed. Most UANL-associated adverse effects were either grade 1 or 2. Only one patient developed grade 3 adverse effects in the form of elevated aspartate aminotransferase and alanine aminotransferase levels with diarrhea at the same time after receiving 74 mg m^{-2} of UANL (Liu, 1995). The size (nm) of the UANLs was not clearly stated in this paper.

A novel hard solid nanoparticulate formulation, CYT-6091, constructed by simultaneously binding recombinant human TNF alpha (rhTNF) and thiolated PEG to the surface of 27 nm colloidal gold NPs was tested in a phase I dose escalation clinical trial in 29 patients with advanced stage cancer (Libutti *et al.,* 2010). This formulation was injected intravenously (50–600 μg m^{-2}), and one cycle of treatment consisted of two treatments administered 14 days apart. Biopsies (punch, core or excisional) were obtained

from 20 subjects from both tumor and adjacent healthy tissue 24 h post-administration of CYT-6091. Adverse events were graded according to the National Cancer Institute–Common Terminology Criteria for Adverse Events (version 3.0). Results showed that the half-life for rhTNF and gold were quite similar (182 and 217 min, respectively), suggesting that the CYT-6091 nanoconstruction remains intact in the circulation. Electron micrographs of the patient biopsies, taken 24 h after administration, indicated that the gold NPs travel to tumors having fenestrations of 200–400 nm in size. Gold NPs were also found in healthy liver tissue, but were not detected in healthy skin or breast tissue. The most frequent grade 3 and 4 adverse events were lymphopenia (26 of 29 patients), hypoalbuminemia (five of 29), hypokalemia (five of 29), hypophosphatemia (five of 29), hyperbilirubinemia (five of 29) and increased aspartate aminotransferase (five of 29). In addition, redistribution in circulating lymphocytes and neutrophils that was dose dependent was observed. All other adverse events were not considered as dose-limiting toxicities. Depending on the results, it is difficult to distinguish which adverse events were induced by gold NPs, rhTNF, or both of them. However, from this clinical trial we can get the following conclusions: First, a certain dose of hard solid gold NPs can be administrated through the intravenous injection route into the human body without severe acute adverse effects. Second, the human body can tolerate nanoparticulate formulation of gold NPs in sizes of 200–400 nm. Third, gold NPs will be distributed to not only tumor tissue, but also normal tissues or organs. Therefore, the chronic potential adverse effects should be further evaluated even if this therapeutic formulation is approved for clinical use.

In conclusion:

(1) Most nanomedicine products approved for clinical use are from soft NPs. Moreover, a large proportion of the soft NPs are composed of liposomal and polymer-conjugated formulations (see Table 1) (Ko, 2016; Nishiyama *et al.,* 2016; Zatsepin *et al.,* 2016). These therapeutic or carrier NPs are normally believed to be less toxic than hard solid NPs except for human essential element solid particles, such as iron oxide NPs. More than two-thirds of the nanoparticulate systems presently approved for therapeutic clinical use in humans are soft particles and the majority are liposomes NPs (Schütz *et al.,* 2013). That might be the reason why the soft particles have received significantly more attention in recent research for developing therapeutics for human therapy. It is worth noticing that after checking through PubMed, one can find, as early as in 1976, scientists had already reported the role of liposomes in drug delivery systems for cancer chemotherapy (Juliano, 1976). Superparamagnetic iron oxide NPs (iron is an essential element for the human body), gold and Ag NPs are the principal hard solid nanoparticulate types currently used in clinics (Junghanns and Müller, 2008), but both of them are predominantly used for diagnostic imaging for MRI or radiosensitizers.

(2) Most reports of clinical trials for nanomedicine products mainly pay attention to the therapeutic effectiveness of drugs, not the biosafety or adverse effects of the NPs on the human body.

(3) Most clinical trials did not provide detailed information on the dynamic size of the therapeutic or carrier NPs, although the size and shape of these soft nanoparticulate systems depend largely on environmental factors, such as temperature, pH, ionic strength or medium characteristics. As Schütz *et al.* (2013) concluded the publicly available databases of approved products and clinical trials do not generally mention the nanoparticulate characteristics of the therapeutics under development. Although soft NPs are relatively low toxicity, large counterparts may accumulate in vital organs and cause toxic problems.

(4) Clinical trials for nanomedicine products are well organized in countries or organizations such as US Food and Drug Administration and National Institutes of Health, Swiss Agency for Therapeutic Products and the European Union. China has not yet set a unified organization to schedule the clinical trials for nanomedicine products although research articles published by Chinese scientists in nanomedical fields have been soaring in recent years.

Strategies for research in nanomedicine

In summary, unique chemical and physical properties, potential applications and human biosafety in clinical trials of NPs are reviewed in this paper. The unique chemical and physical properties of NPs include small size, surface area, quantum size, chemical reaction properties, catalytic properties, optical properties and other properties. The potential applications of NPs in medicine mainly include medical diagnosis, drug carriers, medical instruments, tissue engineering, therapeutic drugs, nanogene medicine, gene therapy of cancer, nanovaccines and radiosensitizers. Nanotechnology allows a quicker, more accurate and more credible diagnosis process. Drug carrier NPs can be easily delivered and absorbed, greatly increase the time of the drug half-life and reduce the drug dosage. In the gene therapy of cancer, more accurate targeting can avoid drugs causing too much damage to normal tissue/cells. In tissue engineering, nanotechnology may also play an irreplaceable role. Liposomal and polymer-conjugated formulations are the NPs frequently used for nanomedicine products at present. Human biosafety information from open published clinical trials is limited. Most clinical trial reports for nanomedicine products focused mainly on the therapeutic effectiveness of drugs without paying enough attention to the human biosafety or adverse effects.

To help optimize development of nanomedicine, we summarized the following strategies as suggestions for future work in nanomedicine research.

(1) Unique chemical and physical properties of NPs are a double-edged sword. While developing NPs for medical use, it should first be determined if these unique properties potentially cause acute or chronic adverse effects to humans. NPs themselves potentially may be toxic to the liver, kidneys, lungs, heart, vascular system and the immune system (Schütz *et al.,* 2013). Some NPs may not be suitable for human use particularly through intravenous or interstitial injections even if certain chemical modifications have been applied, except for non-invasive medical devices or drugs for external use only. Therefore, before any clinical application, all nanomedicine products designed for invasive administration should provide complete safety data.

(2) Searching through PubMed, one may find thousands of various publications published every year to investigate the potential medical application of different NPs. However, these studies mainly employ *in vitro* cell culture or *in vivo* animal experiments, transitioning from lab to bedside for medical use still has a long way to go. In addition, we found that so

Table 1. Nanomedicine products approved for clinical use

Category	Drug name	Active ingredient	Manufacturer	Indications	Reference
Soft NPs Liposome	Abelcet®	Amphotericin B	Sigma-Tau Pharmaceuticals	Fungal infections	Ventola, 2012b
	Doxil®(USA)/Caelyx® (EU)	Doxorubicin	Centocor Ortho Biotech, J&J/Schering Plough, Janssen Biotech	HIV-related Kaposi's sarcoma, metastatic breast cancer, metastatic ovarian cancer	Sainz *et al.*, 2015; Tinkle *et al.*, 2014
	DaunoXome®	Daunorubicin citrate	NeXstar Pharmaceuticals/Gilead Sciences Ltd/Galen Ltd	HIV-related Kaposi's sarcoma	Tinkle *et al.*, 2014; Wang *et al.*, 2012
	Ambisome®	Amphotericin B	Astellas Pharma USA/Gilead Ltd	Fungal and protozoal infections	Barratt and Bretagne, 2006
	Depocyt®	Cytarabine	Pacira Pharms/Sigma-Tau Pharmaceuticals	Malignant lymphomatous meningitis	Svenson, 2012
	Myocet®	Doxorubicin	Sopherion Therapeutics/ Cephalon	Combination therapy with cyclophosphamide in metastatic breast cancer	Heidel and Davis, 2011; Wang *et al.*, 2012
	Visudyne®	Verteporfin	Novartis	Macular degeneration, central serous retinopathy	Schütz *et al.*, 2013
	Marqibo®	Vincristine sulfate	Inex(liposometech)/Enzon/ Thalon therapeutics	Philadelphia chromosome-negative lymphoblastic leukemia	Rodriguez and Pytlik, 2009; Zamboni, 2008
	Depodur®	Morphine sulfate	ERK Therapeutics	Postsurgical analgesia	Howell, 2001
	Mepact®/L-MTP-PE	Mifamurtide	IDM Pharma	Osteosarcoma	Howell, 2001
	L-Annamycin	Annamycin	Callisto	Acute lymphocytic leukemia, acute myeloid leukemia	Wetzler *et al.*, 2013
	SLIT Cisplatin	Cisplatin	Transave	Progressive osteogenic sarcoma metastatic to the lung	Chou *et al.*, 2007
	Sarcodoxome	Doxorubicin	GP-Pharm	Soft tissue sarcoma	Patravale *et al.*, 2012
	AeroLEF	Fentanyl	Delex Therapeutics	Postoperative analgesic	Clark *et al.*, 2008
	OSI-211	Lurtotecan	OSI Pharmaceuticals	Ovarian cancer	Seiden *et al.*, 2004
	Onco TCS	Vincristine	Inex, Enzon	Non-Hodgkin's lymphoma	Gopalakrishna and Ceballos-Coronel, 2013
	Amphotec	Amphotericin B	Alkopharma	Invasive aspergillosis	Ventola, 2012a
	LEP-ETU	Paclitaxel	Neopharma	Ovarian/breast/lung cancers	Gopalakrishna and Ceballos-Coronel, 2013
	Atragen	*Trans*-retinoic acid	Aronex Pharmaceuticals	Acute promyelocytic leukemia	Patatanian and Thompson, 2008

(*Continues*)

Table 1. *(Continued)*

Category	Drug name	Active ingredient	Manufacturer	Indications	Reference
Polymer-protein conjugate	Adagen®/Pegademase bovine	Bovine adenosine deaminase	Sigma-Tau Pharmaceuticals/Enzon	Severe combined immunodeficiency disease associated with ADA deficiency	Vicent *et al.*, 2009
	Oncaspar®/PEG-L-asparaginase	Asparaginase	Enzon	Acute lymphoblastic leukemia	Pasut and Veronese, 2009
	PEGINTRON®	IFN-α2b	Schering Plough	Chronic hepatitis C	Pasut and Veronese, 2009; Vicent *et al.*, 2009
	Neulasta®	PEGfilgrastim (granulocyte-colony stimulating factor)	Amgen	Neutropenia associated with cancer chemotherapy	Pasut and Veronese, 2009
	PEGASYS®	IFN-α2a	Hoffmann-laRoche	Hepatitis C	Vicent *et al.*, 2009
	Somavert®	PEGvisomant (protein) (HGH antagonist)	Pfizer	Acromegaly	Vicent *et al.*, 2009
	Mircera®	Methoxy polyethylene glycol-epoetin β	Hoffman-LaRoche	Anemia associated with chronic kidney disease	Curran and McCormack, 2008
	Cimzia®/Certolizumab Pegol,CDP870	Humanized anti-TNF-α, Certolizumab pegol	Nektar/UCB S.A	Rheumatoid arthritis and Crohn's disease	Mease, 2011
	Krystexxa®/Pegloticase, Puricase®	Uricase (urate oxidase)	Savient Pharmaceuticals	Chronic gout	Duncan, 2006
	Ontak	Interleukin-2 diphtheria toxin fusion protein	Eisai	Cutaneous T-cell lymphoma	Ventola, 2012a
Polymer-polymeric drug	Copaxone®/Glatiramer Acetate, Copolymer 1	Glatiramer acetate (polymer), Glu-Ala –Tyr copolymer	Teva	Multiple sclerosis	Karussis *et al.*, 2010
	Renagel®	Sevelamer hydrochloride	Genzyme	End-stage renal disease	Duncan, 2006
	Welchol®	Colesevelam Hydrochloride	Daiichi Sankyo Co., Ltd	Hyperlipidemia, type 2 diabetes	Goldfine *et al.*, 2010
Polymer-aptamer conjugate	Macugen®	siRNA anti-VEGF inhibitor (PEG)	Eyetech Inc.	Age-related macular degeneration	Vicent *et al.*, 2009
	Mircera	Methoxy PEG-epoetin beta	Hoffman La Roche	Symptomatic anemia associated with CKD	Jelkmann, 2013
Polymeric	ProLindac	HPMA copolymer–DACH platinate	Access Pharmaceuticals	Ovarian cancers	Duncan, 2011; Zhang *et al.*, 2008
	Basulin	L-Leucine, L-glutamate copolymer, and insulin	Flamel Technologies	Type I diabetes	Zhang *et al.*, 2008
	Hepacid	PEG–arginine deaminase	Phoenix	Hepatocellular carcinoma	Zhang *et al.*, 2008
	Prothecan	PEG–camptothecin	Enzon	Various cancers	Duncan, 2003
	NKTR-118	PEG–naloxol	Nektar		Eldon *et al.*, 2007

(*Continues*)

Table 1. *(Continued)*

Category	Drug name	Active ingredient	Manufacturer	Indications	Reference
				Opioid-induced constipation	
	Puricase	PEG–uricase	Phoenix	Hyperuricemia from gout	Sherman *et al.*, 2008
	SP1049C	Pluronic block-copolymer doxorubicin	Supratek Pharma	Esophageal carcinoma	Kabanov *et al.*, 2002
	IT-101	Polycyclodextrin camptothecin	Insert Therapeutics	Metastatic solid tumors	Zhang *et al.*, 2008
	CT-2106	Polyglutamate camptothecin	Cell Therapeutics	Colorectal and ovarian cancers	Takimoto *et al.*, 2004
	Xyotax	Polyglutamate paclitaxel	Cell Therapeutics	NSCLC, ovarian cancer	Vicent and Duncan, 2006
	Transdrug	Poly(iso-hexyl-cyanoacrylate) doxorubicin	BioAlliance Pharma	Hepatocellular carcinoma	Zhang *et al.*, 2008
Micelle	Taxol®	Paclitaxel	Bristol Myers Squibb	Ovarian/breast/lung/pancreatic cancers	Heidel and Davis, 2011
	Taxotere	Docetaxel	Sanofi-Aventis	Metastatic breast cancer	Heidel and Davis, 2011
	Estrasorb™	Estradiol hemihydrate	Novavax/Graceway	Hormone replacement therapy	Simon & Group, 2006
	Genexol-PM®	Paclitaxel	Samyang Co.	Ovarian/breast/lung/pancreatic cancers	Heidel and Davis, 2011; Wang *et al.*, 2012
	EndoTAG-1	Paclitaxel	Medigene/SynCore Biotechnology	Breast cancer/pancreatic cancer	von Roemeling *et al.*, 2016
Virosome	Inflexal®V	Vaccine	Berna Biotech/Crucell	Influenza	Herzog *et al.*, 2009
	Epaxal®/HAVpur®	Vaccine	Berna Biotech, Crucell	Hepatitis A	Bovier, 2008
	Influvac®plus	Vaccine	Solvay Pharma Abbott	Influenza	Herzog *et al.*, 2009
Nanoemulsion	Bepanthen® spray mousse	Dexpanthenol	Bayer	Improve wound healing	Schütz *et al.*, 2013
Hard solid NPs					
SPION/iron oxide	Endorem®/Feridex IV®, AMI-25	Iron oxide nanoparticles	Guerbet/Amag Pharmaceuticals	MRI diagnostic	Chu, 1995
	GastorMARK™/ LumiremTM, AMI-121	Superparamagnetic iron oxide	Advanced magnetics/ Amag Pharmaceuticals/ Guerbet	MRI diagnostic	Chu, 1995
	Resovist®/Cliavist®, Ferucarbotran, SHU 555 A, BAY86	Superparamagnetic iron oxide	Bayer Schering Pharma AG Bayer Healthcare Pharmaceuticals	MRI diagnostic	Wang *et al.*, 2001
	Feraheme®	Ferumoxytol	Amag Pharmaceuticals/ Takeda	Anemia	Singh *et al.*, 2008
	Ferrlecit®	Sodium ferric gluconate complex	Sanofi-Aventis	Iron deficiency anemia	Schütz *et al.*, 2013

(*Continues*)

Journal of Applied Toxicology

Table 1. *(Continued)*

Category	Drug name	Active ingredient	Manufacturer	Indications	Reference
	Venofer®	Iron oxide + sucrose	Fresenius/Luitpold	Iron deficiency anemia	Duncan, 2006
Nanocrystal	Rapamune®	Sirolimus	Wyeth Pharms/Pfizer	Organ transplant rejection, lymphangio-leiomyomatosis	Junghanns and Müller, 2008
	Emend®	Aprepitant	Merck and Co.	Postoperative nausea and vomiting, chemotherapy-induced nausea and vomiting	Hesketh *et al.*, 2003
	TriCor®	Fenofibrate	Abbott Laboratories	Hypercholesterolemia, hypertriglyceridemia	Junghanns and Müller, 2008
	Megace®ES	Megestrol acetate	Par Pharmaceutical Companies	Breast, endometrial, and prostate cancers	Junghanns and Müller, 2008
	Triglide®	Fenofibrate	SkyePharma/First Horizon/ Sciele Pharma	Hypercholesterolemia, hypertriglyceridemia	Junghanns and Müller, 2008
	Paliperidone palmitate	Nanocrystalline paliperidone palmitate	Elan, Johnson &Johnson	Schizophrenia	Chen *et al.*, 2011
	Panzem NCD	Nanocrystalline 2-methoxyestradiol	Elan, EntreMed	Various cancers	Shim, 2011
Gold	Verigene® platform	Gold NPs	Nanosphere	Disease detection and diagnostic	Baptista, 2014
	AuroShell	Gold nanoshells	Nanospectra Biosciences, Inc	AuroLase therapy (cancer)	Gopalakrishna and Ceballos-Coronel, 2013
Others					
	Abraxane®/ABI-007,nab-Paclitaxel, NSC-736631	Paclitaxel	Abraxis Bioscience/ Celgene Europe	Metastatic breast cancer	Wang *et al.*, 2012
	Nanocoll®	NPs(HSA) +99mTc	GE Healthcare	Radio diagnostic	Gommans *et al.*, 2009
	Mylotarg	Gentuzumab-ozogamicin	Wyeth-Ayerst	Acute myeloid leukemia	Gopalakrishna and Ceballos-Coronel, 2013
	Alimta	Pemetrexed	Lilly	Non-squamous NSCLC, malignant pleural mesothelioma	Ventola, 2012a
	Eligard	Leuprolide acetate and PLGH polymer formulation	Sanof	Advanced prostate cancer	Ventola, 2012a
	BioVant	Calcium phosphate nanoparticle vaccine adjuvant	BioSante	Vaccine adjuvant	Gil *et al.*, 2010
	NB-001		NanoBio	Herpes labialis	Lipuma *et al.*, 2008

(*Continues*)

Journal of AppliedToxicology

Table 1. *(Continued)*

Category	Drug name	Active ingredient	Manufacturer	Indications	Reference
		Nanoemulsion-based therapy			
	NB-002	Nanoemulsion-based therapy	NanoBio	Onychomycosis	Lipuma *et al.*, 2008
	AI-850	Paclitaxel nanoparticles in porous, hydrophilic matrix	Acusphere	Solid tumors	Ledet and Mandal, 2012
	VivaGel	Poly-L-lysine dendrimer	Starpharma	Antimicrobial protection from genital herpes and HIV infection	Rupp *et al.*, 2007
	Propofol IDD-D	Propofol IDD-D	SkyePharma	Anesthetic	Kilpatrick and Tilbrook, 2006

ADA, adenosine deaminase; CKD, chronic kidney disease; DACH, diamino-cyclohexane; Glu, glutamate; HGH, human growth hormone; HIV, human immunodeficiency virus; HPM, hydroxypropyl methacrylate; HSA, human serum albumin; IDD-D, insoluble drug delivery-MicroDroplet; MRI, magnetic resonance imaging; NPs, nanoparticles; NSCLC, non-small cell lung cancer; PEG, polyethylene glycol; PLGH, placental growth hormone; SPION, superparamagnetic iron oxide nanoparticle; TNF-α, tumor necrosis factor-alpha; VEGF, vascular endothelial growth factor.

many scientists or researchers involved in nanomedicine research are from pure material science fields without any medical backgrounds. These situations inevitably form a gap between labs and clinical use of NPs. A research team consisting of multidisciplinary sciences such as chemical, material, toxicological, biological, animal, basic and clinical medical sciences should be the best way to develop the nanomedicine products. Accordingly, research teams consisting of multidisciplinary scientists, particularly medical physicians, is vital and should get priority when applying for funding for clinical use of NPs.

(3) Through reviewing, we found that there are two major obstacles for the development of nanomedicine. First, there exists a big gap between research and clinical transition. For clinical medicine, transitioning from lab to bedside use is the final goal. As stated above, a bridge between lab and bedside should be built through multidisciplinary scientists' cooperation. Second, human biosafety evaluation of clinical trials is very important for future healthy and rapid development of new nanomedicine products. Therefore, clinical trial results on nanomedicine products should be encouraged to publish in open journals, including both successful and unsuccessful cases. In this way, unsuccessful clinical trials due to human adverse effects may help scientists avoid making similar mistakes in the development of new nanomedicine products.

(4) Most clinical trials for nanomedicine products used as drug carriers focused mainly on the findings in the therapeutic effectiveness of drugs, but not on human biosafety of NPs. In future clinical trials, it will be optimal to determine if the side effects and/or adverse effects are induced by drugs themselves or by the NPs when used as drug carriers. In addition, due to the amount of time it takes for clearance or accumulation in tissue or organs, chronic adverse effects of nanomedicine products, particularly those used for invasive administration, should be further evaluated after clinical use. Epidemiology investigation is also necessary for human biosafety evaluation after several years of application in clinical practice.

(5) At present, human biosafety evaluation for nanomedicine products is still at the preliminary stage. Owing to a lack of standardized evaluation methods, publication results related with preclinical and clinical tests from different labs or hospitals are difficult to compare. A unified standardized test battery for human biosafety evaluation of nanomedicine products in clinical trials should be determined, which may include all indexes of blood chemistry, immune, nervous, reproductive, developmental, carcinogenic, teratogenic and mutagenic toxicities.

(6) While investigating nanomedicine products for human use, we should be prepared for accidental overdose, misuse or accumulation of NPs in human tissue and organs. This means how to neutralize or treat the toxicities of overdose, and how to promote unnecessary NP excretions from the body should also be investigated at the same time. In addition, emergency medicine and emergency medical measures should be explored before any nanomedicine product is permitted for clinical application.

(7) Although soft NPs are normally believed to be less toxic than hard solid NPs, the potential adverse effects after a long time of accumulation in human tissues or organs should not be ignored. In addition, some soft NPs such as liposomes

themselves may be digested or metabolized in the human body. Therefore, several important questions should be answered while evaluating the clinical use of medical nanoproducts in clinical trials. These questions include biodistribution, dynamic change in particle size, elimination half-life and accumulation in human tissues and organs particularly in the non-targeted tissues and organs.

(8) How to prevent contamination of bacteria or other microorganisms in nanomedicine products particularly for those administered intravenously has not been well demonstrated in the most recent of clinical trials. As we know, conventional disinfection methods may not be suitable for NP disinfection. Therefore, standardized disinfection methods for different kinds of nanomedicine products should be explored further.

(9) Last, but not necessarily the least important, preventing nanomedicine product pollution during clinical trials or clinical applications should also be considered. Health risks coming from occupational exposures of researchers and lab workers, healthcare workers and family members during clinical use of nanomedicine products have not previously been studied. Potential risks to the environment have also not been investigated. A more precautionary approach to oversight from such exposures or pollutions seems advisable (Ramachandran *et al.,* 2012; Resnik, 2012).

Funding

This work was partly supported by the National Nature Science Foundation of China (grant no 81273111), the Scientific Projects of Zhejiang Province (2015C33148 and 2015C37117), Ningbo Scientific Innovation Team for Environmental Hazardous Factor Control and Prevention (2016C51001) and KC Wong Magna Fund in Ningbo University.

Conflict of interest

The authors did not report any conflict of interest.

Authors' contributions

HS and JZ were involved in writing the manuscript, BL and MD helped to organize and proof read the final manuscript. All authors read and approved the final manuscript.

Disclaimer

The findings and conclusions of this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention or any other agency.

References

Abshagen K, Schrodi I, Gerber T, Vollmar B. 2009. In vivo analysis of biocompatibility and vascularization of the synthetic bone grafting substitute NanoBone. *J. Biomed. Mater. Res. A* **91**: 557–566.

Agrawal A, Huang S, Lin A, Lee M, Barton J, Drezek R, Pfefer TJ. 2006. Quantitative evaluation of optical coherence tomography signal enhancement with gold nanoshells. *J. Biomed. Opt.* **11**: 215–219.

Al-Hajeili M, Azmi AS, Choi M. 2014. Nab-paclitaxel: potential for the treatment of advanced pancreatic cancer. *Onco. Targets Ther.* **7**: 187–192.

Amanchi Bala SS, Amanchi Vamsi PK, Amanchi A. 2015. Novel process of preparing nano metal and the products thereof. US20150024204[P].

Amrol D. 2007. Single-dose azithromycin microsphere formulation: a novel delivery system for antibiotics. *Int. J. Nanomedicine* **2**: 9–12.

Andersson H, van den Berg A. 2004. Microtechnologies and nanotechnologies for single-cell analysis. *Curr. Opin. Biotechnol.* **15**: 44–49.

Arrowsmith J. 2011. Trial watch: phase III and submission failures: 2007-2010. *Nat. Rev. Drug Discov.* **10**: 87.

Au KM, Lu Z, Matcher SJ, Armes SP. 2011. Polypyrrole nanoparticles: a potential optical coherence tomography contrast agent for cancer imaging. *Adv. Mater.* **23**: 5792–5795.

Ayano E, Karaki M, Ishihara T, Kanazawa H, Okano T. 2012. Poly (N-isopropylacrylamide)-PLA and PLA blend nanoparticles for temperature-controllable drug release and intracellular uptake. *Colloids Surf. B Biointerfaces* **99**: 67–73.

Baan RA. 2007. Carcinogenic hazards from inhaled carbon black, titanium dioxide, and talc not containing asbestos or asbestiform fibers: recent evaluations by an IARC Monographs Working Group. *Inhal. Toxicol.* **19**(Suppl. 1): 213–228.

Babu KS, Anandkumar M, Tsai TY, Kao TH, Inbaraj BS, Chen BH. 2014. Cytotoxicity and antibacterial activity of gold-supported cerium oxide nanoparticles. *Int. J. Nanomedicine* **9**: 5515–5531.

Banizs AB, Huang T, Dryden K, Berr SS, Stone JR, Nakamoto RK, Shi W, He J. 2014. In vitro evaluation of endothelial exosomes as carriers for small interfering ribonucleic acid delivery. *Int. J. Nanomedicine* **9**: 4223–4230.

Baptista PV. 2014. Nanodiagnostics: leaving the research lab to enter the clinics? *Diagnosis* **1**: 305–309.

Barratt G, Bretagne S. 2006. Optimizing efficacy of amphotericin B through nanomodification. *Int. J. Nanomedicine* **1**: 417–432.

Berginc G. 2011. Optical properties of nanostructured materials: a review. *J. Nanophoton.* **5**: 052502.

Bergs JW, Wacker MG, Hehlgans S, Piiper A, Multhoff G, Rodel C, Rodel F. 2015. The role of recent nanotechnology in enhancing the efficacy of radiation therapy. *Biochim. Biophys. Acta* **1856**: 130–143.

Bhana S, Lin G, Wang L, Starring H, Mishra SR, Liu G, Huang X. 2015. Near-infrared-absorbing gold nanopopcorns with iron oxide cluster core for magnetically amplified photothermal and photodynamic cancer therapy. *ACS Appl. Mater. Interfaces* **7**: 11637–11647.

Bianchi DW, Hanson J. 2006. Sharpening the tools: a summary of a National Institutes of Health workshop on new technologies for detection of fetal cells in maternal blood for early prenatal diagnosis. *J. Matern. Fetal Neonatal Med.* **19**: 199–207.

Bishwajitsutradhar K, Amin ML. 2014. Nanotechnology in cancer drug delivery and selective targeting. *ISRN Nanotechnol.* **2014**: 1–12.

Biswas O, Sen A. 2016. Nanorobot the Expected Ever Reliable Future Asset in Diagnosis, Treatment and Therapy[C]: 3rd International Conference on Foundations and Frontiers in Computer, Communication and Electrical Engineering.

Boos AM, Weigand A, Brodbeck R, Beier JP, Arkudas A, Horch RE. 2016. The potential role of telocytes in tissue engineering and regenerative medicine. *Semin. Cell Dev. Biol.* **55**: 70–78.

Botchway SW, Coulter JA, Currell FJ. 2015. Imaging intracellular and systemic in vivo gold nanoparticles to enhance radiotherapy. *Br. J. Radiol.* **88**: 20150170.

Bovier PA. 2008. Epaxal: a virosomal vaccine to prevent hepatitis A infection. *Expert Rev. Vaccines* **7**: 1141–1150.

Brezinski ME, Tearney GJ, Bouma BE, Izatt JA, Hee MR, Swanson EA, Southern JF, Fujimoto JG. 1996. Optical coherence tomography for optical biopsy. Properties and demonstration of vascular pathology. *Circulation* **93**: 1206–1213.

Buzea C, Pacheco II, Robbie K. 2007. Nanomaterials and nanoparticles: sources and toxicity. *Biointerphases* **2**: MR17–MR71.

Cao LS, Lu QF, Wang LC, Li J, Song J, Wang DJ. 2013. Microwave-induced small size effect of $(Ba, Sr)_3MgSi_2O_8$:0.06Eu^{2+}, 0.1Mn^{2+}, phosphor for 660nm-featured bio-lighting. *Ceram. Int.* **39**: 7717–7720.

Cao Z, Zhu W, Wang W, Zhang C, Xu M, Liu J, Feng ST, Jiang Q, Xie X. 2014. Stable cerasomes for simultaneous drug delivery and magnetic resonance imaging. *Int. J. Nanomedicine* **9**: 5103–5116.

Carrillo-Conde B, Song EH, Chavez-Santoscoy A, Phanse Y, Ramer-Tait AE, Pohl NL, Wannemuehler MJ, Bellaire BH, Narasimhan B. 2011. Mannose-functionalized "pathogen-like" polyanhydride nanoparticles target C-type lectin receptors on dendritic cells. *Mol. Pharm.* **8**: 1877–1886.

Chang TM, Powanda D, Yu WP. 2003. Analysis of polyethylene-glycol-polylactide nano-dimension artificial red blood cells in maintaining systemic hemoglobin levels and prevention of methemoglobin formation. *Artif. Cells Blood Substit. Immobil. Biotechnol.* **31**: 231–247.

Chao Z, Tsung-Han T, Adler DC, Hsiang-Chieh L, Cohen DW, Amy M, Yihong W, Connolly JL, Fujimoto JG. 2010. Photothermal optical coherence tomography in ex vivo human breast tissues using gold nanoshells. *Opt. Lett.* **35**: 700–702.

Chavez-Santoscoy AV, Roychoudhury R, Pohl NL, Wannemuehler MJ, Narasimhan B, Ramer-Tait AE. 2012. Tailoring the immune response by targeting C-type lectin receptors on alveolar macrophages using "pathogen-like" amphiphilic polyanhydride nanoparticles. *Biomaterials* **33**: 4762–4772.

Chen B, Pan R, Askhatova D, Chen P. 2015a. Effective small interfering RNA delivery in vitro via a new stearylated cationic peptide. *Int. J. Nanomedicine* **10**: 3303–3314.

Chen H, Khemtong C, Yang X, Chang X, Gao J. 2011. Nanonization strategies for poorly water-soluble drugs. *Drug Discov. Today* **16**: 354–360.

Chen J, Shao R, Zhang XD, Chen C. 2013. Applications of nanotechnology for melanoma treatment, diagnosis, and theranostics. *Int. J. Nanomedicine* **8**: 2677–2688.

Chen Q, Liu X, Chen J, Zeng J, Cheng Z, Liu Z. 2015b. A self-assembled albumin-based nanoprobe for in vivo ratiometric photoacoustic pH imaging. *Adv. Mater.* **27**: 6820–6827.

Chen Y, Gong L, Gao N, Liao J, Sun J, Wang Y, Wang L, Zhu P, Fan Q, Wang YA, Zeng W, Mao H, Yang L, Gao F. 2015c. Preclinical evaluation of a urokinase plasminogen activator receptor-targeted nanoprobe in rhesus monkeys. *Int. J. Nanomedicine* **10**: 6689–6698.

Chen YW, Liou GG, Pan HB, Tseng HH, Hung YT, Chou CP. 2015d. Specific detection of CD133-positive tumor cells with iron oxide nanoparticles labeling using noninvasive molecular magnetic resonance imaging. *Int. J. Nanomedicine* **10**: 6997–7018.

Chin YT, Liao EC, Wu CC, Wang GJ, Tsai JJ. 2014a. Detection of haplotype mutations of the MD-2 gene promoter associated with Der p2-induced allergy using a nanostructured biosensor. *Int. J. Nanomedicine* **9**: 1403–1412.

Chin YT, Liao EC, Wu CC, Wang GJ, Tsai JJ. 2014b. Detection of haplotype mutations of the MD-2 gene promoter associated with Der p2-induced allergy using a nanostructured biosensor. *Int. J. Nanomedicine* **9**: 1403–1412.

Chou AJ, Bell MD, Mackinson C, Gupta R, Meyers PA, Gorlick R. 2007. Phase Ib/IIa study of sustained release lipid inhalation targeting cisplatin by inhalation in the treatment of patients with relapsed/progressive osteosarcoma metastatic to the lung. Asco Meeting Abstracts 25. (18 Suppl).

Chu WJ. 1995. Surface properties of superparamagnetic iron oxide MR contrast agents: Ferumoxides, ferumoxtran, ferumoxsil. *Magn. Reson. Imaging* **13**: 675–691.

Clark A, Rossiter-Rooney M, Valle-Leutri F. 2008. Aerosolized liposome-encapsulated fentanyl (AeroLEF) via pulmonary administration allows patients with moderate to severe post-surgical acute pain to self-titrate to effective analgesia. *J. Pain* **9**: 42–42.

Cordeiro AS, Alonso MJ, de la Fuente M. 2015. Nanoengineering of vaccines using natural polysaccharides. *Biotechnol. Adv.* **33**: 1279–1293.

Curnis F, Gasparri AM, Sacchi A, Fiocchi M, Corti A. 2015. Anti-tumor activity of TNF-gold nanodrugs tagged with tumor vasculature-homing peptides containing the NGR or isoDGR motives. *Cancer Res.* **75**: 4387–4387 (Abstract 4387).

Curran MP, McCormack PL. 2008. Methoxy polyethylene glycol-epoetin beta: a review of its use in the management of anaemia associated with chronic kidney disease. *Drugs* **68**: 1139–1156.

Dahlman JE, Barnes C, Khan OF, Thiriot A, Jhunjunwala S, Shaw TE, Xing Y, Sager HB, Sahay G, Speciner L, Bader A, Bogorad RL, Yin H, Racie T, Dong Y, Jiang S, Seedorf D, Dave A, Singh Sandhu K, Webber MJ, Novobrantseva T, Ruda VM, Lytton-Jean AK, Levins CG, Kalish B, Mudge DK, Perez M, Abezgauz L, Dutta P, Smith L, Charisse K, Kieran MW, Fitzgerald K, Nahrendorf M, Danino D, Tuder RM, von Andrian UH, Akinc A, Panigrahy D, Schroeder A, Koteliansky V, Langer R, Anderson DG. 2014. In vivo endothelial siRNA delivery using polymeric nanoparticles with low molecular weight. *Nat. Nanotechnol.* **9**: 648–655.

Danesh-Bahreini MA, Shokri J, Samiei A, Kamali-Sarvestani E, Barzegar-Jalali M, Mohammadi-Samani S. 2011. Nanovaccine for leishmaniasis: preparation of chitosan nanoparticles containing Leishmania superoxide dismutase and evaluation of its immunogenicity in BALB/c mice. *Int. J. Nanomedicine* **6**: 835–842.

Dassie E, Arcidiacono D, Wasiak I, Damiano N, Dall'Olmo L, Giacometti C, Facchin S, Cassaro M, Guido E, De Lazzari F, Marin O, Ciach T, Fery-Forgues S, Alberti A, Battaglia G, Realdon S. 2015. Detection of fluorescent organic nanoparticles by confocal laser endomicroscopy in a rat model of Barrett's esophageal adenocarcinoma. *Int. J. Nanomedicine* **10**: 6811–6823.

Dau M, Kammerer PW, Henkel KO, Gerber T, Frerich B, Gundlach KK. 2016. Bone formation in mono cortical mandibular critical size defects after augmentation with two synthetic nanostructured and one xenogenous hydroxyapatite bone substitute – in vivo animal study. *Clin. Oral Implants Res.* **27**: 597–603.

Doavi T, Mousavi SL, Kamali M, Amani J, Fasihi RM. 2016. Chitosan-based intranasal vaccine against *Escherichia coli* O157:H7. *Iran. Biomed. J.* **20**: 97–108.

Dorsey JF, Sun L, Joh DY, Witztum A, Kao GD, Alonso-Basanta M, Avery S, Hahn SM, Al Zaki A, Tsourkas A. 2013. Gold nanoparticles in radiation research: potential applications for imaging and radiosensitization. *Transl. Cancer Res.* **2**: 280–291.

Drbohlavova J, Chomoucka J, Adam V, Ryvolova M, Eckschlager T, Hubalek J, Kizek R. 2013. Nanocarriers for anticancer drugs – new trends in nanomedicine. *Curr. Drug Metab.* **14**: 547–564.

du Toit LC, Pillay V, Choonara YE. 2010. Nano-microbicides: challenges in drug delivery, patient ethics and intellectual property in the war against HIV/AIDS. *Adv. Drug Deliv. Rev.* **62**: 532–546.

Duncan R. 2003. The dawning era of polymer therapeutics. *Nat. Rev. Drug Discov.* **2**: 347–360.

Duncan R. 2006. Polymer conjugates as anticancer nanomedicines. *Nat. Rev. Cancer* **6**: 688–701.

Duncan R. 2011. Polymer therapeutics as nanomedicines: new perspectives. *Curr. Opin. Biotechnol.* **22**: 492–501.

Dyondi D, Webster TJ, Banerjee R. 2013. A nanoparticulate injectable hydrogel as a tissue engineering scaffold for multiple growth factor delivery for bone regeneration. *Int. J. Nanomedicine* **8**: 47–59.

Ekimov AI, Efros AL, Onushchenko AA. 1993. Quantum size effect in semiconductor microcrystals. *Solid State Commun.* **88**: 947–950.

Eldon MA, Song D, Neumann TA, Wolff R, Cheng L, Viegas TX, Bentley MD, Fishburn CS, Kugler AR. 2007. NKTR-118 (Oral PEG-Naloxol), a PEGylated Derivative of Naloxone: Demonstration of Selective Peripheral Opioid Antagonism After Oral Administration in Preclinical Models: American Academy of Pain Management Clinical Meeting; September 27–30, 2007; Las Vegas, NV.

England CG, Priest T, Zhang G, Sun X, Patel DN, McNally LR, van Berkel V, Gobin AM, Frieboes HB. 2013. Enhanced penetration into 3D cell culture using two and three layered gold nanoparticles. *Int. J. Nanomedicine* **8**: 3603–3617.

Eroglu E, Tiwari PM, Waffo AB, Miller ME, Vig K, Dennis VA, Singh SR. 2013. A nonviral pHEMA+chitosan nanosphere-mediated high-efficiency gene delivery system. *Int. J. Nanomedicine* **8**: 1403–1415.

Estelrich J, Sanchez-Martin MJ, Busquets MA. 2015. Nanoparticles in magnetic resonance imaging: from simple to dual contrast agents. *Int. J. Nanomedicine* **10**: 1727–1741.

Etheridge ML, Campbell SA, Erdman AG, Haynes CL, Wolf SM, McCullough J. 2013. The big picture on nanomedicine: the state of investigational and approved nanomedicine products. *Nanomedicine* **9**: 1–14.

Fan AM, Alexeeff G. 2010. Nanotechnology and nanomaterials: toxicology, risk assessment, and regulations. *J. Nanosci. Nanotechnol.* **10**: 8646–8657.

Fan R, Liang QH, Wang J, Tang T, Xiong XG. 2007. Enhancement in the percutaneous permeation effects of diclofenac sodium by nanoliposome carrier: A comparative randomized study with common external preparation. *J. Clin. Rehabil. Tissue Eng. Res.* **11**: 3597–3600.

Feng X, Yong Z. 2012. Highly conductive and stretchable silver nanowire conductors. *Adv. Mater.* **24**: 5117–5122.

Ferrari M. 2005. Cancer nanotechnology: opportunities and challenges. *Nat. Rev. Cancer* **5**: 161–171.

Feynman RP. 2011. There's plenty of room at the bottom. *Resonance* **16**: 890–905.

Frank D, Tyagi C, Tomar L, Choonara YE, du Toit LC, Kumar P, Penny C, Pillay V. 2014. Overview of the role of nanotechnological innovations in the detection and treatment of solid tumors. *Int. J. Nanomedicine* **9**: 589–613.

Fu PP. 2014. Introduction to the special issue: nanomaterials – toxicology and medical applications. *J. Food Drug Anal.* **22**: 1–2.

Fuchs S, Rodel C, Blinne A, Zastrau U, Wunsche M, Hilbert V, Glaser L, Viefhaus J, Frumker E, Corkum P, Forster E, Paulus GG. 2016. Nanometer resolution optical coherence tomography using broad bandwidth XUV and soft x-ray radiation. *Sci. Rep.* **6**: 20658.

Gales BJ, Erstad BL. 1993. Adverse reactions to human serum albumin. *Ann. Pharmacother.* **27**: 87–94.

Gandhimathi C, Venugopal JR, Bhaarathy V, Ramakrishna S, Kumar SD. 2014. Biocomposite nanofibrous strategies for the controlled release of

biomolecules for skin tissue regeneration. *Int. J. Nanomedicine* **9**: 4709–4722.
Ge L, Li Q, Wang M, Ouyang J, Li X, Xing MM. 2014. Nanosilver particles in medical applications: synthesis, performance, and toxicity. *Int. J. Nanomedicine* **9**: 2399–2407.
Ghaffar KA, Giddam AK, Zaman M, Skwarczynski M, Toth I. 2014. Liposomes as nanovaccine delivery systems. *Curr. Top. Med. Chem.* **14**: 1194–1208.
Ghanaati S, Barbeck M, Willershausen I, Thimm B, Stuebinger S, Korzinskas T, Obreja K, Landes C, Kirkpatrick CJ, Sader RA. 2013. Nanocrystalline hydroxyapatite bone substitute leads to sufficient bone tissue formation already after 3 months: histological and histomorphometrical analysis 3 and 6 months following human sinus cavity augmentation. *Clin. Implant. Dent. Relat. Res.* **15**: 883–892.
Gil PR, Hühn D, Mercato LLD, Sasse D, Parak WJ. 2010. Nanopharmacy: Inorganic nanoscale devices as vectors and active compounds. *Pharmacol. Res.* **62**: 115–125.
Gill P. 2013. Nanocarriers, nanovaccines, and nanobacteria as nanobiotechnological concerns in modern vaccines. *Scientia Iranica* **20**: 1003–1013.
Goldfine AB, Fonseca VA, Jones MR, Wang AC, Ford DM, Truitt KE. 2010. Long-term safety and tolerability of colesevelam HCl in subjects with type 2 diabetes. *Horm. Metab. Res.* **42**: 23–30.
Gommans GM, Gommans E, van der Zant FM, Teule GJ, van der Schors TG, de Waard JW. 2009. 99m Tc Nanocoll: A radiopharmaceutical for sentinel node localisation in breast cancer – In vitro and in vivo results. *Appl. Radiat. Isot.* **67**: 1550–1558.
Gopalakrishna P, Ceballos-Coronel ML. 2013. Science and technology of the emerging nanomedicines in cancer therapy: A primer for physicians and pharmacists. *Anal. Chem.* **1**: 238–241.
Gorjikhah F, Davaran S, Salehi R, Bakhtiari M, Hasanzadeh A, Panahi Y, Emamverdy M, Akbarzadeh A. 2016. Improving "lab-on-a-chip" techniques using biomedical nanotechnology: a review. *Artif. Cells Nanomed. Biotechnol.* **44**: 1609–1614.
Guo L, Zhang H, Wang F, Liu P, Wang Y, Xia G, Liu R, Li X, Yin H, Jiang H, Chen B. 2015a. Targeted multidrug-resistance reversal in tumor based on PEG-PLL-PLGA polymer nano drug delivery system. *Int. J. Nanomedicine* **10**: 4535–4547.
Guo P. 2010. The emerging field of RNA nanotechnology. *Nat. Nanotechnol.* **5**: 833–842.
Guo Y, Wang D, Song Q, Wu T, Zhuang X, Bao Y, Kong M, Qi Y, Tan S, Zhang Z. 2015b. Erythrocyte membrane-enveloped polymeric nanoparticles as nanovaccine for induction of antitumor immunity against melanoma. *ACS Nano* **9**: 6918–6933.
Hainfeld JF, Dilmanian FA, Slatkin DN, Smilowitz HM. 2008. Radiotherapy enhancement with gold nanoparticles. *J. Pharm. Pharmacol.* **60**: 977–985.
Haque F, Shu D, Shu Y, Shlyakhtenko LS, Rychahou PG, Evers BM, Guo P. 2012. Ultrastable synergistic tetravalent RNA nanoparticles for targeting to cancers. *Nano Today* **7**: 245–257.
Hariharan R, Manohar J. 2010. Nanorobotics as medicament: (Perfect solution for cancer): International Conference on Emerging Trends in Robotics and Communication Technologies. IEEE, 4–7.
Hariri W, Sudha T, Bharali DJ, Cui H, Mousa SA. 2015. Nano-targeted delivery of toremifene, an estrogen receptor-alpha blocker in prostate cancer. *Pharm. Res.* **32**: 2764–2774.
Harms C, Helms K, Taschner T, Stratos I, Ignatius A, Gerber T, Lenz S, Rammelt S, Vollmar B, Mittlmeier T. 2012. Osteogenic capacity of nanocrystalline bone cement in a weight-bearing defect at the ovine tibial metaphysis. *Int. J. Nanomedicine* **7**: 2883–2889.
Haun JB, Castro CM, Wang R, Peterson VM, Marinelli BS, Lee H, Weissleder R. 2011. Micro-NMR for rapid molecular analysis of human tumor samples. *Sci. Transl. Med.* **3**: 1968–1973.
Heidel JD, Davis ME. 2011. Clinical developments in nanotechnology for cancer therapy. *Pharm. Res.* **28**: 187–199.
Her S, Jaffray DA, Allen C. 2015. Gold nanoparticles for applications in cancer radiotherapy: Mechanisms and recent advancements. *Adv. Drug Deliv. Rev.* **109**: 84–101.
Herzog C, Hartmann K, Künzi V, Kürsteiner O, Mischler R, Lazar H, Glück R. 2009. Eleven years of Inflexal®; V – a virosomal adjuvanted influenza vaccine. *Vaccine* **27**: 4381–4387.
Hesketh PJ, Grunberg SM, Gralla RJ, Warr DG, Roila F, De Wit R, Chawla SP, Carides AD, Ianus J, Elmer ME. 2003. The oral neurokinin-1 antagonist aprepitant for the prevention of chemotherapy-induced nausea and vomiting: a multinational, randomized, double-blind, placebo-controlled trial in patients receiving high-dose cisplatin – the Aprepitant Protocol 052 Study Group. *J Clin Oncol.* **21**: 4112–4119.
Howell SB. 2001. Clinical applications of a novel sustained-release injectable drug delivery system: DepoFoam technology. *Cancer J.* **7**: 219–227.
Hu Y, Zheng H, Huang W, Zhang C. 2014. A novel and efficient nicotine vaccine using nano-lipoplex as a delivery vehicle. *Hum. Vaccin. Immunother.* **10**: 64–72.
Huang Y, Zhao Y, Liu F, Liu S. 2015. Nano traditional Chinese medicine: Current progresses and future challenges. *Curr. Drug Targets* **16**: 1548–1562.
Hunsawong T, Sunintaboon P, Warit S, Thaisomboonsuk B, Jarman RG, Yoon IK, Ubol S, Fernandez S. 2015. Immunogenic properties of a BCG adjuvanted chitosan nanoparticle-based dengue vaccine in human dendritic cells. *PLoS Negl. Trop. Dis.* **9**: e0003958.
Hussein-Al-Ali SH, El Zowalaty ME, Hussein MZ, Ismail M, Dorniani D, Webster TJ. 2014. Novel kojic acid-polymer-based magnetic nanocomposites for medical applications. *Int. J. Nanomedicine* **9**: 351–362.
Ishihara T, Kubota T, Choi T, Higaki M. 2009a. Treatment of experimental arthritis with stealth-type polymeric nanoparticles encapsulating betamethasone phosphate. *J. Pharmacol. Exp. Ther.* **329**: 412–417.
Ishihara T, Kubota T, Choi T, Takahashi M, Ayano E, Kanazawa H, Higaki M. 2009b. Polymeric nanoparticles encapsulating betamethasone phosphate with different release profiles and stealthiness. *Int. J. Pharm.* **375**: 148–154.
Issa B, Obaidat IM, Albiss BA, Haik Y. 2013. Magnetic nanoparticles: surface effects and properties related to biomedicine applications. *Int. J. Mol. Sci.* **14**: 21266–21305.
Jabir NR, Tabrez S, Ashraf GM, Shakil S, Damanhouri GA, Kamal MA. 2012. Nanotechnology-based approaches in anticancer research. *Int. J. Nanomedicine* **7**: 4391–4408.
Jacob T, Hemavathy K, Jacob J, Hingorani A, Marks N, Ascher E. 2011. A nanotechnology-based delivery system: Nanobots. Novel vehicles for molecular medicine. *J. Cardiovasc. Surg.* **52**: 159–167.
Jacobs PW, Wind SJ, Ribeiro FH, Somorjai GA. 1997. Nanometer size platinum particle arrays: catalytic and surface chemical properties. *Surf. Sci.* **372**: L249–L253.
Jain S, Hirst DG, O'Sullivan JM. 2012. Gold nanoparticles as novel agents for cancer therapy. *Br. J. Radiol.* **85**: 101–113.
Jelkmann W. 2013. Physiology and pharmacology of erythropoietin. *Transfus. Med. Hemother.* **40**: 302–309.
Johannsen M, Gneveckow U, Taymoorian K, Thiesen B, Waldofner N, Scholz R, Jung K, Jordan A, Wust P, Loening SA. 2007. Morbidity and quality of life during thermotherapy using magnetic nanoparticles in locally recurrent prostate cancer: results of a prospective phase I trial. *Int. J. Hyperth.* **23**: 315–323.
Juan L, Zhimin Z, Anchun M, Lei L, Jingchao Z. 2010. Deposition of silver nanoparticles on titanium surface for antibacterial effect. *Int. J. Nanomedicine* **5**: 261–267.
Juliano RL. 1976. The role of drug delivery systems in cancer chemotherapy. *Prog. Clin. Biol. Res.* **9**: 21–32.
Junghanns JU, Müller RH. 2008. Nanocrystal technology, drug delivery and clinical applications. *Int. J. Nanomedicine* **3**: 295–309.
Kabanov AV, Batrakova EV, Alakhov VY. 2002. Pluronic®; block copolymers for overcoming drug resistance in cancer. *Adv. Drug Deliv. Rev.* **54**: 759–779.
Kaewkamnerdpong B, Boonrong P, Trihirun S, Achalakul T. 2015. *Modeling Nanorobot Control Using Swarm Intelligence for Blood Vessel Repair: A Rigid-Tube Model*. Springer International Publishing.
Kang BJ, Jeun M, Jang GH, Song SH, Jeong IG, Kim CS, Searson PC, Lee KH. 2015. Diagnosis of prostate cancer via nanotechnological approach. *Int. J. Nanomedicine* **10**: 6555–6569.
Karussis D, Teitelbaum DC, Brenner T. 2010. Long-term treatment of multiple sclerosis with glatiramer acetate: natural history of the subtypes of anti-glatiramer acetate antibodies and their correlation with clinical efficacy. *J. Neuroimmunol.* **220**: 125–130.
Kilpatrick GJ, Tilbrook GS. 2006. Drug development in anaesthesia: industrial perspective. *Curr. Opin. Anaesthesiol.* **19**: 385–389.
Kim CS, Wilder-Smith P, Ahn YC, Liaw LH, Chen Z, Kwon YJ. 2009. Enhanced detection of early-stage oral cancer in vivo by optical coherence tomography using multimodal delivery of gold nanoparticles. *J. Biomed. Opt.* **14**: 034008.
Kim ES, Ahn EH, Dvir T, Kim DH. 2014. Emerging nanotechnology approaches in tissue engineering and regenerative medicine. *Int. J. Nanomedicine* **9**(Suppl. 1): 1–5.

Klein S, Sommer A, Distel LV, Hazemann JL, Kroner W, Neuhuber W, Muller P, Proux O, Kryschi C. 2014. Superparamagnetic iron oxide nanoparticles as novel X-ray enhancer for low-dose radiation therapy. *J. Phys. Chem. B* **118**: 6159–6166.

Ko AH. 2016. Nanomedicine developments in the treatment of metastatic pancreatic cancer: focus on nanoliposomal irinotecan. *Int. J. Nanomedicine* **11**: 1225–1235.

Kolitz-Domb M, Corem-Salkmon E, Grinberg I, Margel S. 2014. Synthesis and characterization of bioactive conjugated near-infrared fluorescent proteinoid-poly(L-lactic acid) hollow nanoparticles for optical detection of colon cancer. *Int. J. Nanomedicine* **9**: 5041–5053.

Kunzmann A, Andersson B, Thurnherr T, Krug H, Scheynius A, Fadeel B. 2011. Toxicology of engineered nanomaterials: focus on biocompatibility, biodistribution and biodegradation. *Biochim. Biophys. Acta* **1810**: 361–373.

Kwatra D, Venugopal A, Anant S. 2013. Nanoparticles in radiation therapy: a summary of various approaches to enhance radiosensitization in cancer. *Transl. Cancer Res.* **2**: 330–342.

Lara HH, Ixtepan-Turrent L, Garza-Trevino EN, Rodriguez-Padilla C. 2010. PVP-coated silver nanoparticles block the transmission of cell-free and cell-associated HIV-1 in human cervical culture. *J. Nanobiotechnol.* **8**: 15.

Larkin J, Carson S, Stoloff DH, Wanunu M. 2013. Nanopore-based analysis of chemically modified DNA and nucleic acid drug targets. *Israel J. Chem.* **53**: 431–441.

Leary SP, Liu CY, Apuzzo ML. 2006. Toward the emergence of nanoneurosurgery: part II – nanomedicine: diagnostics and imaging at the nanoscale level. *Neurosurgery* **58**: 805–823 discussion 805–823.

Ledet G, Mandal TK. 2012. Nanomedicine: Emerging therapeutics for the 21st century. *US Pharm.* **37**(Oncology Suppl.): 7–11.

Lee J, Chatterjee DK, Lee MH, Krishnan S. 2014. Gold nanoparticles in breast cancer treatment: promise and potential pitfalls. *Cancer Lett.* **347**: 46–53.

Lee WG, Kim YG, Chung BG, Demirci U, Khademhosseini A. 2010a. Nano/Microfluidics for diagnosis of infectious diseases in developing countries. *Adv. Drug Deliv. Rev.* **62**: 449–457.

Lee Y, Garcia MA, Frey Huls NA, Sun S. 2010b. Synthetic tuning of the catalytic properties of Au-Fe_3O_4 nanoparticles. *Angew. Chem. Int. Ed. Engl.* **49**: 1271–1274.

Li H, Mu Y, Lu J, Wei W, Wan Y, Liu S. 2014. Target-cell-specific fluorescence silica nanoprobes for imaging and theranostics of cancer cells. *Anal. Chem.* **86**: 3602–3609.

Li L, Zhu YJ. 2006. High chemical reactivity of silver nanoparticles toward hydrochloric acid. *J. Colloid Interface Sci.* **303**: 415–418.

Li M, Li R, Li CM, Wu N. 2011. Electrochemical and optical biosensors based on nanomaterials and nanostructures: a review. *Front. Biosci. (Schol. Ed.)* **3**: 1308–1331.

Liao J, Anchun M, Zhu Z, Quan Y. 2010. Antibacterial titanium plate deposited by silver nanoparticles exhibits cell compatibility. *Int. J. Nanomedicine* **5**: 337–342.

Libutti SK, Paciotti GF, Byrnes AA, Alexander HR, Jr, Gannon WE, Walker M, Seidel GD, Yuldasheva N, Tamarkin L. 2010. Phase I and pharmacokinetic studies of CYT-6091, a novel PEGylated colloidal gold-rhTNF nanomedicine. *Clin. Cancer Res.* **16**: 6139–6149.

Lim KO, Helpern JA. 2002. Neuropsychiatric applications of DTI – a review. *NMR Biomed.* **15**: 587–593.

Lipuma JJ, Rathinavelu S, Foster BK, Keoleian JC, Makidon PE, Kalikin LM, Baker JR. 2008. In Vitro activities of a novel nanoemulsion against Burkholderia and other multidrug-resistant cystic fibrosis-associated bacterial species. *Antimicrob. Agents Chemother.* **53**: 249–255.

Liu F, Le W, Mei T, Wang T, Chen L, Lei Y, Cui S, Chen B, Cui Z, Shao C. 2016. In vitro and in vivo targeting imaging of pancreatic cancer using a Fe_3O_4@SiO_2 nanoprobe modified with anti-mesothelin antibody. *Int. J. Nanomedicine* **11**: 2195–2207.

Liu HL, Dai SA, Fu KY, Hsu SH. 2010. Antibacterial properties of silver nanoparticles in three different sizes and their nanocomposites with a new waterborne polyurethane. *Int. J. Nanomedicine* **5**: 1017–1028.

Liu J. 1995. Pharmacology of oleanolic acid and ursolic acid. *J. Ethnopharmacol.* **49**: 57–68.

Liu L, Liu X, Xu Q, Wu P, Zuo X, Zhang J, Deng H, Wu Z, Ji A. 2014. Self-assembled nanoparticles based on the c(RGDfk) peptide for the delivery of siRNA targeting the VEGFR2 gene for tumor therapy. *Int. J. Nanomedicine* **9**: 3509–3526.

Liu Q, Liu A, Gao F, Weng S, Zhong G, Liu J, Lin X, Lin JH, Chen X. 2011. Coupling technique of random amplified polymorphic DNA and nanoelectrochemical sensor for mapping pancreatic cancer genetic fingerprint. *Int. J. Nanomedicine* **6**: 2933–2939.

Liu W, Su P, Gonzales A, 3rd, Chen S, Wang N, Wang J, Li H, Zhang Z, Webster TJ. 2015a. Optimizing stem cell functions and antibacterial properties of TiO2 nanotubes incorporated with ZnO nanoparticles: experiments and modeling. *Int. J. Nanomedicine* **10**: 1997–2019.

Liu Z, Chen N, Dong C, Li W, Guo W, Wang H, Wang S, Tan J, Tu Y, Chang J. 2015b. Facile construction of near infrared fluorescence nanoprobe with amphiphilic protein-polymer bioconjugate for targeted cell imaging. *ACS Appl. Mater. Interfaces* **7**: 18997–19005.

Loo C, Lin A, Hirsch L, Lee MH, Barton J, Halas N, West J, Drezek R. 2004. Nanoshell-enabled photonics-based imaging and therapy of cancer. *Technol. Cancer Res. Treat.* **3**: 33–40.

Lu L, Sun RW, Chen R, Hui CK, Ho CM, Luk JM, Lau GK, Che CM. 2008. Silver nanoparticles inhibit hepatitis B virus replication. *Antivir. Ther.* **13**: 253–262.

Luo X, Aoife M, Anthony JK, Malcolm RS. 2006. Application of nanoparticles in electrochemical sensors and biosensors. *Electroanalysis* **18**: 319–326.

Macwan DP, Dave PN, Chaturvedi S. 2011. A review on nano-TiO_2 sol–gel type syntheses and its applications. *J. Mater. Sci.* **46**: 3669–3686.

Madani SY, Shabani F, Dwek MV, Seifalian AM. 2013. Conjugation of quantum dots on carbon nanotubes for medical diagnosis and treatment. *Int. J. Nanomedicine* **8**: 941–950.

Maeda H, Fang J, Ulbrich K, Etrych T, Nakamura H. 2016. Missile-type tumor-targeting polymer drug, P-THP, seeks tumors via three different steps based on the EPR effect. *Gan To Kagaku Ryoho* **43**: 549–557.

Mahajan SD, Aalinkeel R, Law WC, Reynolds JL, Nair BB, Sykes DE, Yong KT, Roy I, Prasad PN, Schwartz SA. 2012. Anti-HIV-1 nanotherapeutics: promises and challenges for the future. *Int. J. Nanomedicine* **7**: 5301–5314.

Mahony D, Cavallaro AS, Mody KT, Xiong L, Mahony TJ, Qiao SZ, Mitter N. 2014. In vivo delivery of bovine viral diahorrea virus, E2 protein using hollow mesoporous silica nanoparticles. *Nano* **6**: 6617–6626.

Maier-Hauff K, Rothe R, Scholz R, Gneveckow U, Wust P, Thiesen B, Feussner A, von Deimling A, Waldoefner N, Felix R, Jordan A. 2007. Intracranial thermotherapy using magnetic nanoparticles combined with external beam radiotherapy: results of a feasibility study on patients with glioblastoma multiforme. *J. Neuro-Oncol.* **81**: 53–60.

Maier-Hauff K, Ulrich F, Nestler D, Niehoff H, Wust P, Thiesen B, Orawa H, Budach V, Jordan A. 2011. Efficacy and safety of intratumoral thermotherapy using magnetic iron-oxide nanoparticles combined with external beam radiotherapy on patients with recurrent glioblastoma multiforme. *J. Neuro-Oncol.* **103**: 317–324.

Mandal A. 2012. Properties of Nanoparticles, http://www.news-medical.net/life-sciences/Properties-of-Nanoparticles.aspx.

Marasini N, Giddam AK, Ghaffar KA, Batzloff MR, Good MF, Skwarczynski M, Toth I. 2016. Multilayer engineered nanoliposomes as a novel tool for oral delivery of lipopeptide-based vaccines against group A Streptococcus. *Nanomedicine (Lond.)* **11**: 1223–1236.

Marchal S, El Hor A, Millard M, Gillon V, Bezdetnaya L. 2015. Anticancer drug delivery: an update on clinically applied nanotherapeutics. *Drugs* **75**: 1601–1611.

Marques C, Ferreira JM, Andronescu E, Ficai D, Sonmez M, Ficai A. 2014. Multifunctional materials for bone cancer treatment. *Int. J. Nanomedicine* **9**: 2713–2725.

Martin M. 2015. nab-Paclitaxel dose and schedule in breast cancer. *Breast Cancer Res.* **17**: 81.

Master AM, Sen Gupta A. 2012. EGF receptor-targeted nanocarriers for enhanced cancer treatment. *Nanomedicine (Lond.)* **7**: 1895–1906.

Mastorakos P, da Silva AL, Chisholm J, Song E, Choi WK, Boyle MP, Morales MM, Hanes J, Suk JS. 2015. Highly compacted biodegradable DNA nanoparticles capable of overcoming the mucus barrier for inhaled lung gene therapy. *Proc. Natl. Acad. Sci. U. S. A.* **112**: 8720–8725.

Mazaheri M, Eslahi N, Ordikhani F, Tamjid E, Simchi A. 2015. Nanomedicine applications in orthopedic medicine: state of the art. *Int. J. Nanomedicine* **10**: 6039–6053.

Mease PJ. 2011. Certolizumab pegol in the treatment of rheumatoid arthritis: a comprehensive review of its clinical efficacy and safety. *Rheumatology* **50**: 261–270.

Mehmood S, Rehman MA, Ismail H, Mirza B, Bhatti AS. 2015. Significance of postgrowth processing of ZnO nanostructures on antibacterial activity against gram-positive and gram-negative bacteria. *Int. J. Nanomedicine* **10**: 4521–4533.

Merkle RC. 1996. Nanotechnology and medicine. *Adv. Anti-Aging Med.* **I**: 277–286.

Miao YQ, Xiang DX, Zheng CL. 2010. Experimental study of inhibitory effects of silver nanoparticles on influenza virus H3N2. *Shandong Med J.* **50**: 18–20.

Mocan L, Ilie I, Matea C, Tabaran F, Kalman E, Iancu C, Mocan T. 2014. Surface plasmon resonance-induced photoactivation of gold nanoparticles as bactericidal agents against methicillin-resistant *Staphylococcus aureus*. *Int. J. Nanomedicine* **9**: 1453–1461.

Mody KT, Mahony D, Cavallaro AS, Zhang J, Zhang B, Mahony TJ, Yu C, Mitter N. 2015. Silica vesicle nanovaccine formulations stimulate long-term immune responses to the bovine viral diarrhoea virus E2 protein. *PLoS One* **10** e0143507.

Mokhtarzadeh A, Alibakhshi A, Yaghoobi H, Hashemi M, Hejazi M, Ramezani M. 2016. Recent advances on biocompatible and biodegradable nanoparticles as gene carriers. *Expert. Opin. Biol. Ther.* **16**: 771–785.

Munger MA, Radwanski P, Hadlock GC, Stoddard G, Shaaban A, Falconer J, Grainger DW, Deering-Rice CE. 2014. In vivo human time-exposure study of orally dosed commercial silver nanoparticles. *Nanomedicine* **10**: 1–9.

Murugan K, Senthilkumar B, Senbagam D, Al-Sohaibani S. 2014. Biosynthesis of silver nanoparticles using Acacia leucophloea extract and their antibacterial activity. *Int. J. Nanomedicine* **9**: 2431–2438.

Nagy A, Harrison A, Sabbani S, Munson RS, Jr, Dutta PK, Waldman WJ. 2011. Silver nanoparticles embedded in zeolite membranes: release of silver ions and mechanism of antibacterial action. *Int. J. Nanomedicine* **6**: 1833–1852.

Nandedkar TD. 2009. Nanovaccines: recent developments in vaccination. *J. Biosci.* **34**: 995–1003.

Nichols JW, Bae YH. 2012. Odyssey of a cancer nanoparticle: from injection site to site of action. *Nano Today* **7**: 606–618.

Nishiyama N, Matsumura Y, Kataoka K. 2016. Development of polymeric micelles for targeting intractable cancers. *Cancer Sci.* **107**: 867–874.

Oh JH, Lee JS. 2011. Designed hybridization properties of DNA-gold nanoparticle conjugates for the ultraselective detection of a single-base mutation in the breast cancer gene BRCA1. *Anal. Chem.* **83**: 7364–7370.

Panchapakesan B, Book-Newell B, Sethu P, Rao M, Irudayaraj J. 2011. Gold nanoprobes for theranostics. *Nanomedicine (Lond.)* **6**: 1787–1811.

Pandey R, Ahmad Z, Sharma S, Khuller GK. 2005. Nano-encapsulation of azole antifungals: potential applications to improve oral drug delivery. *Int. J. Pharm.* **301**: 268–276.

Parboosing R, Maguire GE, Govender P, Kruger HG. 2012. Nanotechnology and the treatment of HIV infection. *Viruses* **4**: 488–520.

Partha R, Conyers JL. 2009. Biomedical applications of functionalized fullerene-based nanomaterials. *Int. J. Nanomedicine* **4**: 261–275.

Pasut G, Veronese FM. 2009. PEG conjugates in clinical development or use as anticancer agents: An overview. *Adv. Drug Deliv. Rev.* **61**: 1177–1188.

Patatanian E, Thompson DF. 2008. Retinoic acid syndrome: a review. *J. Clin. Pharm. Ther.* **33**: 331–338.

Patravale V, Dandekar P, Jain R. 2012. 6–Clinical trials industrial aspects. *Nanoparticulate Drug Deliv.* **3**: 191–207.

Patravale VB, Date AA, Kulkarni RM. 2004. Nanosuspensions: a promising drug delivery strategy. *J. Pharm. Pharmacol.* **56**: 827–840.

Paulis LE, Mandal S, Kreutz M, Figdor CG. 2013. Dendritic cell-based nanovaccines for cancer immunotherapy. *Curr. Opin. Immunol.* **25**: 389–395.

Pautler M, Brenner S. 2010. Nanomedicine: promises and challenges for the future of public health. *Int. J. Nanomedicine* **5**: 803–809.

Peng XH, Qian X, Mao H, Wang AY, Chen ZG, Nie S, Shin DM. 2008. Targeted magnetic iron oxide nanoparticles for tumor imaging and therapy. *Int. J. Nanomedicine* **3**: 311–321.

Peppas NA, Kavimandan NJ. 2006. Nanoscale analysis of protein and peptide absorption: insulin absorption using complexation and pH-sensitive hydrogels as delivery vehicles. *Eur. J. Pharm. Sci.* **29**: 183–197.

Petukhova NV, Gasanova TV, Stepanova LA, Rusova OA, Potapchuk MV, Korotkov AV, Skurat EV, Tsybalova LM, Kiselev OI, Ivanov PA, Atabekov JG. 2013. Immunogenicity and protective efficacy of candidate universal influenza A nanovaccines produced in plants by Tobacco mosaic virus-based vectors. *Curr. Pharm. Des.* **19**: 5587–5600.

Piktel E, Niemirowicz K, Watek M, Wollny T, Deptula P, Bucki R. 2016. Recent insights in nanotechnology-based drugs and formulations designed for effective anti-cancer therapy. *J. Nanobiotechnol.* **14**: 39.

Polyak D, Krivitsky A, Scomparin A, Eliyahu S, Kalinski H, Avkin-Nachum S, Satchi-Fainaro R. 2016. Systemic delivery of siRNA by aminated poly(alpha)glutamate for the treatment of solid tumors. *J. Control Release pii* **S0168-3659**: 30411–30414. https://doi.org/10.1016/j.jconrel.2016.06.034. [Epub ahead of print].

Popov AM, Lozovik YE, Fiorito S, Yahia L. 2007. Biocompatibility and applications of carbon nanotubes in medical nanorobots. *Int. J. Nanomedicine* **2**: 361–372.

Qian X, Peng XH, Ansari DO, Yin-Goen Q, Chen GZ, Shin DM, Yang L, Young AN, Wang MD, Nie S. 2008. In vivo tumor targeting and spectroscopic detection with surface-enhanced Raman nanoparticle tags. *Nat. Biotechnol.* **26**: 83–90.

Rai M, Yadav A, Gade A. 2009. Silver nanoparticles as a new generation of antimicrobials. *Biotechnol. Adv.* **27**: 76–83.

Rajala A, Wang Y, Zhu Y, Ranjo-Bishop M, Ma JX, Mao C, Rajala RV. 2014. Nanoparticle-assisted targeted delivery of eye-specific genes to eyes significantly improves the vision of blind mice in vivo. *Nano Lett.* **14**: 5257–5263.

Rajesh K, Ming-Hsiung C, Parmar VS, Samuelson LA, Jayant K, Robert N, Subbiah Y, Watterson AC. 2004. Supramolecular assemblies based on copolymers of PEG600 and functionalized aromatic diesters for drug delivery applications. *J. Am. Chem. Soc.* **126**: 10640–10644.

Ramachandran G, Howard J, Maynard A, Philbert M. 2012. Handling worker and third-party exposures to nanotherapeutics during clinical trials. *J. Law Med. Ethics* **40**: 856–864.

Ranganathan R, Madanmohan S, Kesavan A, Baskar G, Krishnamoorthy YR, Santosham R, Ponraju D, Rayala SK, Venkatraman G. 2012. Nanomedicine: towards development of patient-friendly drug-delivery systems for oncological applications. *Int. J. Nanomedicine* **7**: 1043–1060.

Reese M. 2013. Nanotechnology: using co-regulation to bring regulation of modern technologies into the 21st century. *Health Matrix Clevel.* **23**: 537–572.

Ren W, Yan Y, Zeng L, Shi Z, Gong A, Schaaf P, Wang D, Zhao J, Zou B, Yu H, Chen G, Brown EM, Wu A. 2015. A near infrared light triggered hydrogenated black TiO2 for cancer photothermal therapy. *Adv. Healthc Mater.* **4**: 1526–1536.

Ren Y, Cheung HW, von Maltzhan G, Agrawal A, Cowley GS, Weir BA, Boehm JS, Tamayo P, Karst AM, Liu JF, Hirsch MS, Mesirov JP, Drapkin R, Root DE, Lo J, Fogal V, Ruoslahti E, Hahn WC, Bhatia SN. 2012. Targeted tumor-penetrating siRNA nanocomplexes for credentialing the ovarian cancer oncogene ID4. *Sci. Transl. Med.* **4** 147ra112.

Resnik DB. 2012. Responsible conduct in nanomedicine research: environmental concerns beyond the common rule. *J. Law Med. Ethics* **40**: 848–855.

Resnik DB, Tinkle SS. 2007. Ethical issues in clinical trials involving nanomedicine. *Contemp. Clin. Trials* **28**: 433–441.

Rodriguez-Gascon A, del Pozo-Rodriguez A, Solinis MA. 2014. Development of nucleic acid vaccines: use of self-amplifying RNA in lipid nanoparticles. *Int. J. Nanomedicine* **9**: 1833–1843.

Rodriguez MA, Pytlik RT. 2009. Vincristine sulfate liposomes injection (Marqibo) in heavily pretreated patients with refractory aggressive non-Hodgkin lymphoma: report of the pivotal phase 2 study. *Cancer* **115**: 3475–3482.

Roh C. 2012. A facile inhibitor screening of SARS coronavirus N protein using nanoparticle-based RNA oligonucleotide. *Int. J. Nanomedicine* **7**: 2173–2179.

Rupp R, Rosenthal SL, Stanberry LR. 2007. VivaGel (SPL7013 Gel): a candidate dendrimer – microbicide for the prevention of HIV and HSV infection. *Int. J. Nanomedicine* **2**: 561–566.

Sainz V, Conniot J, Matos AI, Peres C, Zupancic E, Moura L, Silva LC, Florindo HF, Gaspar RS. 2015. Regulatory aspects on nanomedicines. *Biochem. Biophys. Res. Commun.* **468**: 504–510.

Sakamoto Y, Nakae D, Fukumori N, Tayama K, Maekawa A, Imai K, Hirose A, Nishimura T, Ohashi N, Ogata A. 2009. Induction of mesothelioma by a single intrascrotal administration of multi-wall carbon nanotube in intact male Fischer 344 rats. *J. Toxicol. Sci.* **34**: 65–76.

Santander-Ortega MJ, Bastos-Gonzalez D, Ortega-Vinuesa JL, Alonso MJ. 2009. Insulin-loaded PLGA nanoparticles for oral administration: an in vitro physico-chemical characterization. *J. Biomed. Nanotechnol.* **5**: 45–53.

Sargent LM, Porter DW, Staska LM, Hubbs AF, Lowry DT, Battelli L, Siegrist KJ, Kashon ML, Mercer RR, Bauer AK, Chen BT, Salisbury JL, Frazer D, McKinney W, Andrew M, Tsuruoka S, Endo M, Fluharty KL, Castranova V, Reynolds SH. 2014. Promotion of lung adenocarcinoma following inhalation exposure to multi-walled carbon nanotubes. *Part. Fibre Toxicol.* **11**: 3.

Satalkar P, Elger BS, Hunziker P, Shaw D. 2016. Challenges of clinical translation in nanomedicine: A qualitative study. *Nanomedicine* **12**: 893–900.

Schütz CA, Juillerat-Jeanneret L, Mueller H, Lynch I, Riediker M, NanoImpactNet Consortium. 2013. Therapeutic nanoparticles in clinics and under clinical evaluation. *Nanomedicine (Lond.)* **8**: 449–467.

Seiden MV, Muggia F, Astrow A, Matulonis U, Campos S, Roche M, Sivret J, Rusk J, Barrett E. 2004. A phase II study of liposomal lurtotecan (OSI-211)

in patients with topotecan resistant ovarian cancer. *Gynecol. Oncol.* **93**: 229–232.
Seil JT, Webster TJ. 2012. Antimicrobial applications of nanotechnology: methods and literature. *Int. J. Nanomedicine* **7**: 2767–2781.
Shameli K, Ahmad MB, Yunus WM, Ibrahim NA, Rahman RA, Jokar M, Darroudi M. 2010. Silver/poly (lactic acid) nanocomposites: preparation, characterization, and antibacterial activity. *Int. J. Nanomedicine* **5**: 573–579.
Sharma S, Mukkur TK, Benson HA, Chen Y. 2009. Pharmaceutical aspects of intranasal delivery of vaccines using particulate systems. *J. Pharm. Sci.* **98**: 812–843.
Sheng Y, Liao LD, Thakor NV, Tan MC. 2014. Nanoparticles for molecular imaging. *J. Biomed. Nanotechnol.* **10**: 2641–2676.
Sherman MR, Saifer MGP, Perez-Ruiz F. 2008. PEG-uricase in the management of treatment-resistant gout and hyperuricemia. *Adv. Drug Deliv. Rev.* **60**: 59–68.
Shi H, Magaye R, Castranova V, Zhao J. 2013. Titanium dioxide nanoparticles: a review of current toxicological data. *Part. Fibre Toxicol.* **10**: 15.
Shim H. 2011. *Methoxyestradiol*. Springer: Berlin.
Shukla GC, Haque F, Tor Y, Wilhelmsson LM, Toulme JJ, Isambert H, Guo P, Rossi JJ, Tenenbaum SA, Shapiro BA. 2011. A boost for the emerging field of RNA nanotechnology. *ACS Nano* **5**: 3405–3418.
Silva CO, Rijo P, Molpeceres J, Figueiredo IV, Ascensao L, Fernandes AS, Roberto A, Reis CP. 2015. Polymeric nanoparticles modified with fatty acids encapsulating betamethasone for anti-inflammatory treatment. *Int. J. Pharm.* **493**: 271–284.
Simon JA, Group ES. 2006. Estradiol in micellar nanoparticles: the efficacy and safety of a novel transdermal drug-delivery technology in the management of moderate to severe vasomotor symptoms. *Menopause* **13**: 222–231.
Singh A, Patel T, Hertel J, Bernardo M, Kausz A, Brenner L. 2008. Safety of ferumoxytol in patients with anemia and CKD. *Am. J. Kidney Dis.* **52**: 907–915.
Skopalik J, Polakova K, Havrdova M, Justan I, Magro M, Milde D, Knopfova L, Smarda J, Polakova H, Gabrielova E, Vianello F, Michalek J, Zboril R. 2014. Mesenchymal stromal cell labeling by new uncoated superparamagnetic maghemite nanoparticles in comparison with commercial Resovist--an initial in vitro study. *Int. J. Nanomedicine* **9**: 5355–5372.
Spadavecchia J, Movia D, Moore C, Maguire CM, Moustaoui H, Casale S, Volkov Y, Prina-Mello A. 2016. Targeted polyethylene glycol gold nanoparticles for the treatment of pancreatic cancer: from synthesis to proof-of-concept in vitro studies. *Int. J. Nanomedicine* **11**: 791–822.
Spampinato V, Ceccone G, Giordani S. 2015. Surface analysis of zinc-porphyrin functionalized carbon nano-onions. *Biointerphases* **10**: 019006.
Stowe TR, Wilkinson CJ, Iqbal A, Stearns T. 2012. The centriolar satellite proteins Cep72 and Cep290 interact and are required for recruitment of BBS proteins to the cilium. *Mol. Biol. Cell* **23**: 3322–3335.
Su XY, Liu PD, Wu H, Gu N. 2014. Enhancement of radiosensitization by metal-based nanoparticles in cancer radiation therapy. *Cancer Biol. Med.* **11**: 86–91.
Sun L, Joh DY, Al-Zaki A, Stangl M, Murty S, Davis JJ, Baumann BC, Alonso-Basanta M, Kaol GD, Tsourkas A, Dorsey JF. 2016. Theranostic application of mixed gold and superparamagnetic iron oxide nanoparticle micelles in glioblastoma multiforme. *J. Biomed. Nanotechnol.* **12**: 347–356.
Sun RW, Chen R, Chung NP, Ho CM, Lin CL, Che CM. 2005. Silver nanoparticles fabricated in Hepes buffer exhibit cytoprotective activities toward HIV-1 infected cells. *Chem. Commun. (Camb.)* **40**: 5059–5061.
Svenson S. 2012. Clinical translation of nanomedicines. *Curr. Opin. Solid State* **16**: 287–294.
Swaminathan S, Vavia PR, Trotta F, Cavalli R. 2013. Nanosponges encapsulating dexamethasone for ocular delivery: formulation design, physicochemical characterization, safety and corneal permeability assessment. *J. Biomed. Nanotechnol.* **9**: 998–1007.
Swanner J, Mims J, Carroll DL, Akman SA, Furdui CM, Torti SV, Singh RN. 2015. Differential cytotoxic and radiosensitizing effects of silver nanoparticles on triple-negative breast cancer and non-triple-negative breast cells. *Int. J. Nanomedicine* **10**: 3937–3953.
Takahashi Y, Chen Q, Rajala RV, Ma JX. 2015. MicroRNA-184 modulates canonical Wnt signaling through the regulation of frizzled-7 expression in the retina with ischemia-induced neovascularization. *FEBS Lett.* **589**: 1143–1149.
Takimoto C, Syed S, Mcnamara M, Doroshow J, Pezzulli S, Eastham E, Bernareggi A, Dupont J. 2004. 504 Phase I study of CT-2106 (polyglutamate camptothecin) in patients with advanced malignancies. *EJC Suppl.* **2**: 154–154.
Tan W, Li Y, Chen M, Wang Y. 2011. Berberine hydrochloride: anticancer activity and nanoparticulate delivery system. *Int. J. Nanomedicine* **6**: 1773–1777.
Tautzenberger A, Kovtun A, Ignatius A. 2012. Nanoparticles and their potential for application in bone. *Int. J. Nanomedicine* **7**: 4545–4557.
Taylor E, Webster TJ. 2011. Reducing infections through nanotechnology and nanoparticles. *Int. J. Nanomedicine* **6**: 1463–1473.
Taylor PL, Ussher AL, Burrell RE. 2005. Impact of heat on nanocrystalline silver dressings. Part I: Chemical and biological properties. *Biomaterials* **26**: 7221–7229.
Tinkle S, Mcneil SE, Mühlebach S, Bawa R, Borchard G, Barenholz Y, Tamarkin L, Desai N. 2014. Nanomedicines: addressing the scientific and regulatory gap. *Ann. N. Y. Acad. Sci.* **1313**: 35–56.
Tonelli FM, Santos AK, Gomes KN, Lorencon E, Guatimosim S, Ladeira LO, Resende RR. 2012. Carbon nanotube interaction with extracellular matrix proteins producing scaffolds for tissue engineering. *Int. J. Nanomedicine* **7**: 4511–4529.
Toumey C. 2013. Nanobots today. *Nat. Nanotechnol.* **8**: 475–476.
Trovarelli A. 1997. ChemInform Abstract: Catalytic Properties of Ceria and CeO2-Containing Materials. *Cheminform* 28.
Tsuda H, Xu J, Sakai Y, Futakuchi M, Fukamachi K. 2009. Toxicology of engineered nanomaterials – a review of carcinogenic potential. *Asian Pac. J. Cancer Prev.* **10**: 975–980.
Vanden Bon N, van Grinsven B, Murib MS, Yeap WS, Haenen K, De Ceuninck W, Wagner P, Ameloot M, Vermeeren V, Michiels L. 2014. Heat-transfer-based detection of SNPs in the PAH gene of PKU patients. *Int. J. Nanomedicine* **9**: 1629–1640.
Vega-Villa KR, Takemoto JK, Yanez JA, Remsberg CM, Forrest ML, Davies NM. 2008. Clinical toxicities of nanocarrier systems. *Adv. Drug Deliv. Rev.* **60**: 929–938.
Vela Ramirez JE, Roychoudhury R, Habte HH, Cho MW, Pohl NL, Narasimhan B. 2014. Carbohydrate-functionalized nanovaccines preserve HIV-1 antigen stability and activate antigen presenting cells. *J. Biomater. Sci. Polym. Ed.* **25**: 1387–1406.
Ventola CL. 2012a. The nanomedicine revolution. *Pharm. Ther.* **40**: 525.
Ventola CL. 2012b. The nanomedicine revolution: Part 2: Current and future clinical applications. *P. T.* **37**: 582–591.
Vicent MJ, Duncan R. 2006. Polymer conjugates: nanosized medicines for treating cancer. *Trends Biotechnol.* **24**: 39–47.
Vicent MJ, Ringsdorf H, Duncan R. 2009. Polymer therapeutics: Clinical applications and challenges for development. *Adv. Drug Deliv. Rev.* **61**: 1117–1120.
Vicente S, Diaz-Freitas B, Peleteiro M, Sanchez A, Pascual DW, Gonzalez-Fernandez A, Alonso MJ. 2013. A polymer/oil based nanovaccine as a single-dose immunization approach. *PLoS One* **8**: e62500.
Vicente S, Goins BA, Sanchez A, Alonso MJ, Phillips WT. 2014. Biodistribution and lymph node retention of polysaccharide-based immunostimulating nanocapsules. *Vaccine* **32**: 1685–1692.
Volokitin Y, Sinzig J, Dejongh LJ, Schmid G, Vargaftik MN, Moiseev II. 1996. Quantum-size effects in the thermodynamic properties of metallic nanoparticles. *Nature* **384**: 621–623.
von Roemeling C, Jiang W, Chan CK, Weissman IL, Kim BY. 2016. Breaking down the barriers to precision cancer nanomedicine. *Trends Biotechnol.* **35**: 159–171.
Wagner V, Dullaart A, Bock AK, Zweck A. 2006. The emerging nanomedicine landscape. *Nat. Biotechnol.* **24**: 1211–1217.
Wang AZ, Langer R, Farokhzad OC. 2012. Nanoparticle delivery of cancer drugs. *Annu. Rev. Med.* **63**: 185–198.
Wang K, Wu X, Wang J, Huang J. 2013. Cancer stem cell theory: therapeutic implications for nanomedicine. *Int. J. Nanomedicine* **8**: 899–908.
Wang X, Wang S, Zhang Y. 2015a. Advance of the application of nano-controlled release system in ophthalmic drug delivery. *Drug Deliv.* **23**: 2897–2901.
Wang Y, Rajala A, Rajala RV. 2015b. Lipid nanoparticles for ocular gene delivery. *J. Funct. Biomater.* **6**: 379–394.
Wang YH, Huang N, Zeng-Guo YU. 2009. Study on anti-HIV activity of silver nanoparticles in vitro. *Mod. Prevent. Med.* **36**: 4123–4126.
Wang YX, Hussain SM, Krestin GP. 2001. Superparamagnetic iron oxide contrast agents: physicochemical characteristics and applications in MR imaging. *Eur. Radiol.* **11**: 2319–2331.
Wen X, Zheng Y, Wu J, Wang LN, Yuan Z, Peng J, Meng H. 2015. Immobilization of collagen peptide on dialdehyde bacterial cellulose

nanofibers via covalent bonds for tissue engineering and regeneration. *Int. J. Nanomedicine* **10**: 4623–4637.

Wetzler M, Thomas DA, Wang ES, Shepard R, Ford LA, Heffner TL, Parekh S, Andreeff M, O'Brien S, Kantarjian HM. 2013. Phase I/II Trial of nanomolecular liposomal annamycin in adult patients with relapsed/refractory acute lymphoblastic leukemia. *Clin. Lymphoma Myeloma Leuk.* **13**: 430–434.

Will O, Purkayastha S, Chan C, Athanasiou T, Darzi AW, Gedroyc W, Tekkis PP. 2006. Diagnostic precision of nanoparticle-enhanced MRI for lymph-node metastases: a meta-analysis. *Lancet Oncol.* **7**: 52–60.

Won J, Kim M, Yi YW, Kim YH, Jung N, Kim TK. 2005. A magnetic nanoprobe technology for detecting molecular interactions in live cells. *Science* **309**: 121–125.

Wu H, Lin J, Liu P, Huang Z, Zhao P, Jin H, Wang C, Wen L, Gu N. 2015. Is the autophagy a friend or foe in the silver nanoparticles associated radiotherapy for glioma? *Biomaterials* **62**: 47–57.

Wu Z, Zhan S, Fan W, Ding X, Wu X, Zhang W, Fu Y, Huang Y, Huang X, Chen R. 2016. Peptide-mediated tumor targeting by a degradable nano gene delivery vector based on pluronic-modified polyethylenimine. *Nanoscale Res. Lett.* **11**: 122.

Xiang D, Zheng Y, Duan W, Li X, Yin J, Shigdar S, O'Connor ML, Marappan M, Zhao X, Miao Y, Xiang B, Zheng C. 2013. Inhibition of A/Human/Hubei/3/2005 (H3N2) influenza virus infection by silver nanoparticles in vitro and in vivo. *Int. J. Nanomedicine* **8**: 4103–4113.

Xing BL, Zhang DS. 2004. Application of medical nanotechnology in treatment of malignant tumor. *Chin J. New Drugs Clin. Remedies* **23**: 303–307.

Yamada M, Foote M, Prow TW. 2015. Therapeutic gold, silver, and platinum nanoparticles. *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.* **7**: 428–445.

Yamamoto Y, Kawano I, Iwase H. 2011. Nab-paclitaxel for the treatment of breast cancer: efficacy, safety, and approval. *Onco Targets Ther.* **4**: 123–136.

Yao C, Hedrick M, Pareek G, Renzulli J, Haleblian G, Webster TJ. 2013. Nanostructured polyurethane-poly-lactic-co-glycolic acid scaffolds increase bladder tissue regeneration: an in vivo study. *Int. J. Nanomedicine* **8**: 3285–3296.

Yi X, Wang F, Qin W, Yang X, Yuan J. 2014. Near-infrared fluorescent probes in cancer imaging and therapy: an emerging field. *Int. J. Nanomedicine* **9**: 1347–1365.

Zaman M, Good MF, Toth I. 2013. Nanovaccines and their mode of action. *Methods* **60**: 226–231.

Zamboni W. 2008. Concept and clinical evaluation of carrier-mediated anticancer agents. *Oncologist* **13**: 248–260.

Zatsepin TS, Kotelevtsev YV, Koteliansky V. 2016. Lipid nanoparticles for targeted siRNA delivery – going from bench to bedside. *Int. J. Nanomedicine* **11**: 3077–3086.

Zhang F, Fu J. 2013. Research on catalytic properties of palladium catalyst prepared by biological reduction method. *China Petroleum Processing Petrochem. Technol.* **15**: 24–30.

Zhang H, Huang XJ, Liu XY, Zhu QM, Liu Y, Lin XF. 2013a. Research advances in safety of nanometer materials and their risk assessment system. *Chin. J. Process. Eng.* **13**: 893–900.

Zhang K, Jinglei W, Huang C, Mo X. 2013b. Fabrication of silk fibroin/P(LLA-CL) Aligned nanofibrous scaffolds for nerve tissue engineering. *Macromolecular Mater. Eng.* **298**: 565–574.

Zhang L, Gu FX, Chan JM, Wang AZ, Langer RS, Farokhzad OC. 2008. Nanoparticles in medicine: therapeutic applications and developments. *Clin. Pharmacol. Ther.* **83**: 761–769.

Zhao D, Sun X, Tong J, Ma J, Bu X, Xu R, Fan R. 2012. A novel multifunctional nanocomposite C225-conjugated Fe3O4/Ag enhances the sensitivity of nasopharyngeal carcinoma cells to radiotherapy. *Acta Biochim. Biophys. Sin. Shanghai* **44**: 678–684.

Zhao J, Castranova V. 2011. Toxicology of nanomaterials used in nanomedicine. *J. Toxicol. Environ. Health B Crit. Rev.* **14**: 593–632.

Zhao J, Shan X, Sheng Y, Wu F, Yuan Y, Liu C. 2008. Preparation of hemoglobin-loaded nanoparticles and safety evaluation in vitro and in vivo. *Sheng Wu Yi Xue Gong Cheng Xue Za Zhi* **25**: 584–588.

Zhao YZ, Du LN, Lu CT, Jin YG, Ge SP. 2013. Potential and problems in ultrasound-responsive drug delivery systems. *Int. J. Nanomedicine* **8**: 1621–1633.

Zhou W, Ma Y, Yang H, Ding Y, Luo X. 2011. A label-free biosensor based on silver nanoparticles array for clinical detection of serum p53 in head and neck squamous cell carcinoma. *Int. J. Nanomedicine* **6**: 381–386.