Digest Journal of Nanomaterials and Biostructures Vol. 15, No. 3, July - September 2020, p. 649 - 661

MULTIFUNCTIONAL NANOPARTICLE PLATFORMS FOR BIOMEDICAL APPLICATIONS: A REVIEW

F. ABOLABAN*, F. DJOUIDER
Nuclear Engineering Department, Faculty of Engineering, King Abdulaziz University, Po Box 80204, Jeddah, 21589, Saudi Arabia

Many scientific and technological areas have benefited, in the last few decades, considerably from the introduction of nanomaterials especially the biomedical field. Despite the use of a myriad of drugs to diagnose and treat diseases, the dilemma is how to selectively target diseased cells while lessening collateral harmfulness to surrounding healthy tissues. Novel advances in theragnostics showed that nanoparticles (NPs), owing to their exceptional physicochemical properties, are an exceptional integrated platform for this purpose. This review paper first depicts nanoparticles in their general structural aspects and second, in their application in medicine (bioconjugation with drugs, cellular internalization, drug delivery and phagocytosis).

(Received April 23, 2020; accepted July 10, 2020)

Keywords: Nanoparticles, Bioconjugation, Drug delivery, Gene delivery, Imaging, Cancer treatment

1. Introduction

Nanotechnology is the manipulation of particles of materials having dimensions ranging between 1 and 100 nm. Nanomedicine, which is the application of nanotechnology in general healthcare concerns the diagnosis and treatment of diseases especially the cancer [1,2]. In the recent years, several fields in the biological and medical sciences [3], molecular biology [4], imaging [5], targeted drug delivery [6], non-viral gene delivery systems [7] and dental implants [8], have greatly beneficiated from the huge development of nanotechnology. Being a multifactorial and complex condition, cancer stems from genetic and/or environmental factors that may affect the human being throughout his entire age span. Radiotherapy, chemotherapy and surgery in cancer treatment are the most frequent and efficient cancer care modalities [9,10]. In the last few years, nanotechnology became a powerful tool in diagnosis, through enhanced imaging and in treatment through targeted drug and radiation dose deliveries, has led to improved therapeutic outcomes for cancer patients [11,12]. The aim of this review is to discuss various potential clinical applications of nanoparticles platforms in biomedical applications, where their unique chemical, pharmacological, and biological factors must be considered during their conception, preclinical evaluation, and clinical applications.

2. Nano-scale structures

NPs are solid and come in colloidal or clustered in different forms, approximately in the 1-100 nanometer range (up to 10^4 times smaller than human cells) and comparable to the size of many proteins and enzymes. Therefore, their use in biological science would enable probing the cellular structure. There are many types of NP structures, although might be categorized into three major groups: organic (liposomes and polymers), inorganic (gold, silica, titanium dioxide, quantum dots), and carbon-based, [13]. They consist of a core material to encapsulate drugs or contrast agents for imaging and a surface coating to avoid their *in vivo* phagocytosis or ligand conjugation. Examples of nano-scale structures used in diagnostic and therapeutic applications include quantum dots (QDs), nanotubes (NTs), nanocages (NCs) magnetic NPs, liposomes, nanowires magnetic resonance imaging (MRI) contrast agents for intraoperative imaging, and

* Corresponding author: fabolaban@kau.edu.sa

novel NP-based methods for the highly specific detection of DNA and protein. Due to their considerably large surface area-to-volume ratio, NPs can encapsulate significant amounts of drugs and be dispersed easily all over the bloodstream.

2.1. Quantum dots

Photon imaging of deep tissue at cellular level remains a key task in treatment of some diseases. Using visible light is appropriate only for skin and shallow tissues and is not appropriate for deep tissue owing their inherent limitations (weak transmission and scattering) [14]. To overcome this issue, some researchers used the near-infrared optical window (700–1,700 nm window) for visual imaging with the help of quantum dots (QDs) [15,16]. QDs are generally spherical colloidal nanocrystalline semiconductor of 1 to 10 nm in diameter that glow different visible colors under UV radiation [17]. Due to their unique electro-optical properties, high fluorescence efficiency, size-tunable fluorescence and good photo-stability [18], they are widely used in cancer detection [19], drug delivery [20] and as a substitute to the traditional fluorescent dyes in bioimaging and biosensing [21,22]. Kobayashi and his colleagues used simultaneously different QDs with comparable physical sizes but different emission spectra for a noninvasive visualization of different lymphatic flows which may predict the cancer metastasis pathway into the lymph nodes [23].

Most of the QDs semiconductors whose fluorescence bands vary according to their composition, size and shell thickness are mixtures of two or more heavy metal fabricated from the elements belonging to the IIB–VIA or IIIA–VA groups (CdS, CdSe, CdTe, ZnSe, InP and InAs) [24]. However, being heavy metals, their key issue is their potential cytotoxicity towards their main target, the liver [25–27]. The intra cell oxidation of the CdSe matrix releases Cd^{2+} which accumulates in the hepatic cells and upset the cellular antioxidant system [28]. To overcome this issue, surface coating of the QDs with an array of biocompatible and low cytotoxic chemical substances, reduce drastically their toxicity [29,30]. Heterogeneous QDs formed by CdSe core covered with ZnS shell within a polymer shell Fig. 1 and CdTe core covered with CdSe shell are extensively used in drug delivery. Yang and his co-workers [31] showed that quercetin pigment conjugated with ZnS coated CdSe were more effective against drug-resistant *Escherichia coli* and *Bacillus subtilis* than quercetin conjugated with CdSe nanoparticles alone.

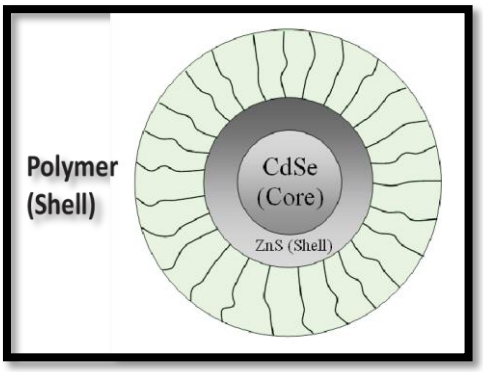

Fig. 1. CdSe-ZnS core-shell quantum dot with the polymer shell [32].

2.2. Liposomes

Liposomes are biocompatible and biodegradable fat globules in hollow spherical structure. These NPs are made of single or multiple bilayer of glycerophospholipid surrounding an aqueous solution core. The double layer consists of two-faced molecules, a hydrophilic phosphate head and a hydrophobic hydrocarbon tail of fatty acids Fig. 2. [31]. Owing to their similarity with plasma membranes, amphiphilic liposomes can easily transfer through the cell membrane and be very effective in targeted drug delivery [33,34]. Wide range of water soluble and poorly soluble drugs can be encapsulated within the core and the lipid bilayer respectively [35].

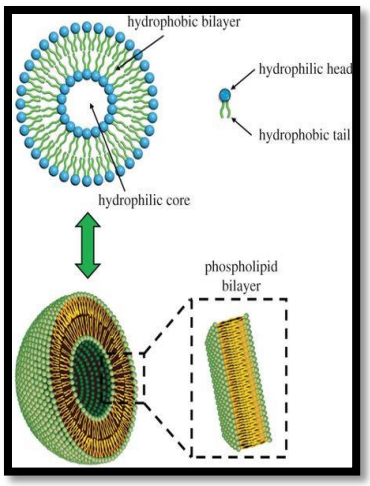

Fig. 2. General scheme of liposomes [36].

To enhance the *in vivo* targeting drug delivery, opsonization of the liposomes must be minimized in order to avoid their phagocytosis by the host immune system [37]. This can be achieved by coating nanoparticles with a corona of biocompatible uncharged hydrophilic polymers e.g., polyethylene glycol (PEG). The PEGylated NPs can then extend their circulation time *in vivo* [38,39]. Allen [40] showed that PEGylation of liposomal drugs increased their lifetime from few hours to 45 hours.

2.3. Polymer-based NPs

Biocompatibility and biodegradability are the main attributes of the FDA approved polymer-based NPs and therefore extensive investigations are being carried out for their preclinical and clinical outcomes [41–43]. These NPs come in capsules (polymeric NPs), amphiphilic micelles (core-shell NPs) or dendrimers (multi-branched NPs). Natural water-soluble polymers such as polysaccharides, chitosan, albumin, and heparin have extensively been used as cargo systems for low water-soluble anticancer drugs in systemic and targeted drug delivery [44–46]. Synthetic polymers such as hydrogel polyacrylamide (PAA), polylactic acid (PLA), poly (L-glutamic) acid (PGLuA), [47–49] and diblock or multiblock copolymer such as N-(2-hydroxypropyl) methacrylamide (HPMA), poly (D,L-lactic-*co*-glycolic) acid (PLGA), and poly(ε-caprolactone) (PCL) [50–52] are usually used, when stabilized with polyethylene glycol (PEG), a**s** delivery cargos for drugs [43], genes [53,54], proteins [55,56] and tumor-imaging photodynamic therapy [57]. In chemotherapy, targeted polymeric NPs are used to deliver drugs to cancer cells with better efficiency while lowering cytotoxicity to surrounding healthy cells [58].

2.4. Metallic (Gold) NPs

Gold NPs are by far the most outstanding members of the metal NP groups. Owing their unique biocompatibility and physico-chemical properties (resistant to oxidation in biological medium), gold NPs can potentially be used in a variety of biomedical applications. They are principally nontoxic [59,60]. However, their cytotoxicity depends greatly on their size of their clusters. Schmid [61] showed very small gold-clusters of 1.4 nm size have strong interaction with DNA grooves, bringing oxidative stress and thereby cause severe cytotoxic effects [62]. The cell surface is negatively charged due mainly to the anionic nature of the phosphate group. The cellular uptake of gold NPs depends greatly on their surface charge (cationic and anionic). For instance, their intrinsic negatively charged surface can be flipped to positively charged by cationic thiol molecule allowing their internalization into cancer cells [63].

2.5. Magnetic NPs

Iron oxide nanoparticles (IONPs) are extensively used in various in vitro and in vivo biomedical field especially as MRI contrast agents for stem cell tracking mainly due to their superparamagnetic properties and higher relaxation values [64,65]. Fundamentally, IONPs come in two basic structures: (i) homogeneous Fe_3O_4 (magnetite) or γ-Fe_2O_3 (maghemite) particles, conjugated with a biocompatible polymer or (ii) a porous biocompatible polymer in which IONPs are incorporated within the pores [66]. The coating of IONPs by hydrophilic biocompatible organic polymers affects their surface charges through electrostatic repulsion [67,68]. This will essentially prevent their aggregation and hence their removal by the immune system [69]. The colloidal stability of the functionalized IONPs also enhances their solubility and intracellular uptake [70], while minimizing their cytotoxicity [71].

2.6. Carbon-based nanoparticles

Among nanoparticles in general, carbon-based NPs (CB-NPs) offer attractive physicochemical characteristics placing them among the most desirable nanomaterials in the biomedical field [72,73]. CB-NPs consist of nano-diamonds (NDs), graphene materials (GPs) and carbon nanotubes (CNTs). Owing to their distinctive light weight, flexibility, structural, electric and optical characteristics, the most used structure in biomedical application is the allotropic CNTs depicted as graphitic single or multi-sheets rolled up as hollow cylinders. They have a wide range of application varying from biosensing and medical diagnosis to cancer treatment, drug and gene delivery [74–77].

3. Nanotechnology in medicine: General considerations

The shape, size and nature of the NPs have a great effect on their *in vivo* pharmacokinetics, cellular endocytosis and the capability to avoid their phagocytosis or removal by the innate immune system [78,79]. Due to their small size that distinguish them from bulky materials and to their exceptional intrinsic chemical and physical properties, NPs can interact with biological systems at the molecular and cellular level [80]. They can easily bind in cell membrane receptors (≈10 nm) and penetrate most cells and interact with DNA (≈1-2 nm), and proteins (≈1–20 nm). Table 1 gives an overview of some NPs structures along with their functionalities.

Table 1. Some Nos structure and their functionalities.

NP	Size (nm)	Shape	Functionality	Ref
Carbon	20-50	Multi wall carbon nanotubes	Drug delivery	[81]
TiO_2	25	Mesoporous nanotubes	Drug delivery Cell imaging	[82]
Superparamagnetic Fe_3O_4	150-250	Spherical coated with polymer	MRI contrast agent	[83]
CdSe/ZnS QD	1-10	Spherical	Drug delivery Gene delivery Cell imaging	[3]
Gold nanoparticle	12-50	Nanorods	Cancer therapy	[42]
Polymer	130	Colloidal suspension	Breast cancer therapy	[84]

4. Bioconjugation of NPs

Typical targeting agents used in nanomedicines include, but not limited to, peptides, aptamers and antibodies [85]. They are usually docked to the NP surface using a poly(ethylene) glycol (PEG) ligand [86,87]. Phagocytosis of the drugs by the innate immune system and their poor solubility in aqueous media, was the rationale of using NPs for efficient drug delivery.

Functionalizing NPs can be designed by conjugating specific targeting ligands on their surface, enabling them to be biodegradable and most importantly to deliver drugs to the impaired cells whilst lessening unwanted side effects elsewhere. Conjugating targeting ligands at the NPs interface can be achieved by either noncovalent weak (van der walls bond) and strong bonds (ionic, hydrogen bonds), or covalent bonding. This will have a great impact on how the drug will be delivered.

5. NP-based targeted drug delivery

Targeted drug delivery is a way of releasing drug to some impaired cells or tissues of the body in a manner that augments its concentration and absorption relative to other healthy parts of the body, thus reducing the required dose and dose frequency [88–90]. The idea of targeting has attracted much of the attention in pharmacology and biomedical research in the last few years [91]. Many researchers conducted challenging investigations in this field when designing new NPs in hopes of offering positive clinical outcomes. The key objectives when designing NPs as a cargo system for carrying the pharmacological active agents is to avoid the clearance by the biological barriers and to reach the impaired cells and delivering the drug at a therapeutically optimum rate with the appropriate dosage, Fig. 3.

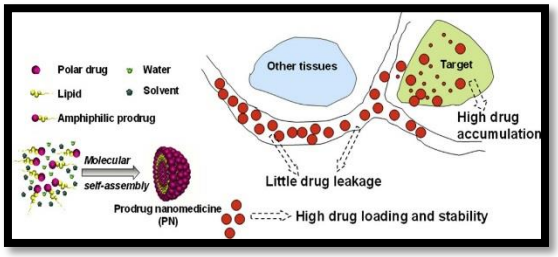

Fig. 3. Nanoparticles based targeted drug delivery [92].

For this purpose, choice of the nature of NPs, their size and surface properties are the main factors to be considered. Smaller NPs have a larger surface area-to-volume ratio; therefore, most of the drug imbedded at the particle surface, lead to a faster drug release. In contrast, larger particles have large cores, which allow more drug to be encapsulated per particle and give slower release [93]. Thus, control of particle size provides a means of tuning drug release rates. Polymeric micelles consisting of hydrophobic and hydrophilic block copolymers received a strong interest owing to their response to pH discrepancy between the tumor cells and its microenvironment to trigger drug release [94]. Yang et al. [95] synthetized micelles formed by the hydrophilic poly (ethylene glycol) with the hydrophobic phenylhydrazone via an acid-labile hydrazone bond. This amphiphilic copolymer displayed a high colloidal stability in vitro and an extended circulation time in vivo due to the presence of the PEG molecule. They encapsulated into the micelle a potent antitumor drug, the hydrophobic paclitaxel, which was released in a smartly pH dependent manner. The delivery was maximum in the slightly acidic medium (6.5-7) of the tumor cells and minimum in the surrounding healthy cell (pH ~ 7.5). Guo and Szoka [96] reported that poly(ethylene glycol)-diortho ester-distearoyl glycerol conjugate (POD) hydrolyses readily at pH 5.5 medium while stays stable in neutral medium. pH-sensitive POD contained liposomes can be suitable for pH triggered drug release systems when targeting slightly acidic bioenvironments such solid cancer.

Systemic drug delivery can be categorized in two large classes: i) the ligand-mediated targeting or active targeting and ii) the enhanced permeation and retention (EPR) or passive targeting. In active targeting, cell surface receptors (plasma membrane-anchored proteins) bind to specified ligands conjugated to NPs. Drugs with low water solubility may be subject to biological clearance before reaching their designated target. The use of hydrophilic NP-based carriers in vivo

such as the non-toxic and biocompatible hydrogel polyacrylamide (PAA) has greatly improved drug delivery [97]. Passive targeting is based on drug buildup in the vicinity of the fast-growing tumor which is surrounded by an abnormally dense and tortuous hyperpermeable vasculature and lymphatic vessels [98]. The extravasation of drugs from the systemic circulation to tumor interstitial space occurs by enhanced permeation and retention (EPR) effect Fig. 4.

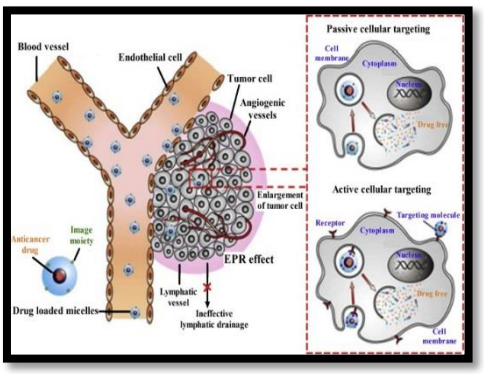

Fig. 4. Schematic diagram of drug targeted via passive and active delivery [99].

6. DNA delivery

Gene therapy describes the treatment of human genetic conditions, both inherited and acquired, by transfection of functional copies of the faulty genes into the impaired cells. Preclinical and clinical studies have shown that cationic polymers when conjugated with genetic material, via complexation with the anionic phosphate group of the DNA strands, are very efficient in non-viral gene transfection [100], although not as effective as the viral counterpart.

Yang and his group showed polymeric NPs *Poly* [2-(*N*,*N*-diethylaminomethyl)-1,3-butadiene] (DMAEMA), obtained by anionic polymerization of the corresponding monomer, was a very efficient gene delivery vector for plasmid DNA [101]. Another attractive approach in non-viral gene delivery is the use of the poly(amidoamine) dendrimers PAMAM-NPs where DNA can bind *in vitro* to the primary amine group present on the dendritic polymer to form a polyplex [102,103]. However, their excessive toxicity and low *in vivo* efficacy restrict their applications. Wood et al. [104] and Qi et al. [105] showed that conjugation of DMAEMA-NPs with polyethylene glycol (DMAEMA-PEG) decreased drastically their cytotoxicity and improve their gene delivery capability in cancer cell-targeting peptides and intramuscular gene expression respectively. Newland and colleagues [106] reported that 8% PEG in the DMAEMA-PEG/DNA polyplex showed higher transfection efficiency with 8% less cytotoxicity compared to the poly (amido amine) dendrimer gene carrier used by Huang et al. [107]. The short half-life (< 2 min) of the glial cell derived neurotrophic growth factor (GDNF), encoded by the *GDNF* gene and fostering the survival and differentiation of dopaminergic neurons [108], hampers its use in the Parkinson disease sufferers who then requires repetitive injections. However, liposome NPs conjugated with PEG and 2-(dimethylamino) ethyl methacrylate (DMAEMA) complexed with DNA showed a prolonged time circulation in the blood system allowing them to be used for brain targeted non-viral transgene expression in the mammalian neurons [109,110].

7. Designing multifunctional NPs

Better understanding of the surface physico-chemistry of NPs in the complex biological microenvironment would enable the design of more efficient and ‘human-friendly’ nanoparticle platforms especially when trying to integrate multifunctional modalities into one single NP. By designing their size, shape and surface coating, NPs performances can be customized to meet their

potential unique biomedical benefit, though various challenges and shortcomings still hamper their multifunctional applications [111,112].

7.1. Surface modification of NPs to avoid immunogenicity

There are a few biological barriers to hinder the effective drug delivery to the impaired site, *i.e* kidneys, liver, innate immune system [113,114]. Macrophages cell (white blood cells including lymphocytes, neutrophils, dendritic cells, monocytes) and other phagocytic cells are part of the mononuclear phagocytic system (MPS), having the role of engulfing and digesting bacteria, cellular fragments, and any exogenous particles, within a matter of few seconds [115]. Rapid clearance of NPs from the blood stream by the MPS is one of the major obstacles to ensuring that NPs can achieve the required accumulation in the target tissue by EPR extravasation.

Depending on their surface properties, NPs can promptly bind to several tens of types of opsonins, plasma proteins present in the systemic circulation, resulting in a protein corona around them. This hydrogen bonding and electrostatic interaction mediated opsonization makes NPs more prone to phagocytosis by the MPS [116,117]. One of the usual ways to avoid phagocytosis of NPs is their coating with the biocompatible and uncharged hydrophilic polymers especially the polyethylene glycol (PEG) which forms a hydrated steric shield, hindering opsonins from adsorbing onto the NP surface [118]. By delaying the phagocytosis mediated clearance of NPs by the MPS, PEGylation may well prolong their residence time in the systemic circulation and contribute to their buildup in the intratumor cells via EPR effect. This potentially improves the NP mediated theragnostic targeting [119,120]. Yang and colleagues showed that 100 nm in size polystyrene nanoparticles PSNPs conjugated with PEG exhibited around 20 times less *in vitro* internalization by human monocytic THP-1 cells than bare PSNPs [121]. Zhang and co-workers [122] also studied the pharmacokinetics of salvianolic acid B (Sal B), a therapeutic drug for cardiovascular related pathologies, loaded in PEGylated phosphatidylcholine-cholesterol liposomes (PC-NPs) when injected into rats. Encapsulation of Sal B in PEGylated liposomes extend its circulation lifetime 3-fold compared to its encapsulation in non-PEGylated liposomes. Although the PEGylation remained the main strategy in NP coating to decrease immunogenicity, a new concept of surface tailoring has been developed in the last decade to inhibit NPs clearance. Rodriguez et al. [123] coated 160-nm polystyrene nanobeads with membrane glycoprotein CD47 a "marker of self" ligand peptide expressed on all cell membranes in humans and mammals. CD47 impedes opsonization-driven clearance of self by signaling through the phagocyte receptor CD172a. After intravenous injection of synthesized CD47 coated nanobeads into mice, they showed that the ratio [nanobead with CD47/nanobead without CD47] increased exponentially with time.

Owing their long circulation lifetime in the body, 80 -100 days before their clearance, red blood cells (RBCs), became in the last decade a novel approach for "stealth" coating of NPs to expand their residence time in the blood circulation. Hu and his colleagues [124] reported a 64% reduction in *in vitro* phagocytosis of poly(lactic-*co*-glycolic acid) NPs coated with RBC membrane. They also reported a clearance half-life of 39.6 hours, longer than the 15.8 hours obtained for the PEGylated poly(lactic-*co*-glycolic acid) NPs. The works done by Piao et al. and Su et al. on different nanostructures supported these findings [125,126]. Membranes of other cell types have been used for NPs coating. Parodi *et al.* [127] coated porous silica nanoparticles (PSNP) with murine J774 macrophages and human THP-1 phagocytic cells membrane along with their cellular self-recognition sialic acid and *N*-acetylglucosamine glycans receptors serving to reduce binding to similar immune cells. They reported a 75 and 50% decrease in PNSP uptake by J774 and THP-1 cells respectively.

7.2. Surface modification of NPs for optimum cellular uptake

Cellular internalization into target cells is an essential step for the specific functionality of NPs [128], while their extracellular localization leads to much less effect [129–131]. Yet the internalization of these bioactive cargos in cells is habitually hindered, for instance, by lipid bilayer of plasma membrane [132]. To overcome this obstacle, bioconjugation of NPs with some cell-penetrating ligands such as peptides have been used to mediate their cell uptake [133].

7.2.1. Effects of surface charge on cellular internalization

One important parameter impacting cellular uptake of NPs is their surface charge. Plasma membrane is a highly selective permeable barrier. Positively charged NPs can promptly be up taken by the cell via electrostatic interaction with the negatively charged hydrophilic portion of lipid bilayer of the cell membrane [134]. High endocytosis activity of cationic polymer coating IONP was detected through tight-fitting vesicles on the negatively charged plasma membrane [128]. However neutral NP coating such as biocompatible dextran polysaccharide shows a lower in vitro cellular internalization for stem cell labelling [135,136]. He et al. [137] showed a good positive correlation between the increase in the macrophage uptake by the phagocytic cells and the surface charge of the negatively charged carboxymethyl chitosan coated NPs. The same positive correlation was observed for the positively charged chitosan hydrochloride coated NPs.

7.2.2. Effects of shape and size of NPs on cellular internalization

The biological behavior of nanomaterials inside the human body is determined mainly by two key properties namely the efficient interaction with targeted tissues [138] and their capacity to escaping their scavenging by the phagocytes, macrophages and other cells of the reticuloendothelial system [139,140]. By studying the binding of Herceptin coated on gold nanoparticles (Her-GNPs) with the overexpressed ErbB2 receptors in plasma membrane of breast cancer cells, Jiang et al. [141] showed that the internalization of Her-GNPs was highly size dependent. Although all nanoparticles within the 2–100 nm size range were internalized, NPs within 25-50 nm size range were most efficiently up taken. On the other hand, Champion et al. showed that the NP spatial curvature or its orientation with respect to the plasma membrane are important in cell internalization. Non-spherical NPs are better internalized, *in vitro*, when approaching microphage cells along their "narrower" side than their "longer" side [142]. Furthermore, particle geometry impacts greatly cellular internalization. Chithrani and Chan [143] reported that HeLa cells have greater tendency, 375–500% more, to internalize spherical GNPs than rod-shaped NPs of similar dimensions. Other workers showed that PEGylated rod-shaped gold NPs were less prone to in vitro phagocytosis by murine macrophage-like cell compared to spherical PEGylated NPs [144].

8. Toxicity and non-degradability of NPs

Owing to their ultra-small size, and once up taken by the human body, NPs can cross the several biological barriers and may ultimately accumulate in the most sensitive organs and upset the cell microenvironment [145,146]. The organs where NPs accumulates more are liver, spleen, brain, lungs and gastrointestinal tract [147,148]. Following the uptake of NPs, the innate immune system will normally clear these "invaders". It is generally admitted that toxicity of a NP is inversely proportional to its size [149,150]. NPs induced cytotoxicity includes oxidative stress, genotoxicity, disruption of signaling pathways, interruption of the mitosis and meiosis and cell death [151]. Several physicochemical processes step behind the mechanisms of the complexity of the NPs cytotoxicity. For instance, metal oxide NPs can induce reactive oxygen species (ROS), highly oxidizing or reducing free radicals ($O_2^{\bullet -}$, $HO_2^{\bullet}$, $OH^{\bullet}$) leading to the oxidative stress resulting in a mitochondrial membrane damage and citric cycle dysfunction and ultimately to cell apoptosis [152–154].

NPs can also disrupt the tightly regulated Ca^{2+} homeostasis in the central nervous system with severe consequences on neuron to neuron synaptic transmission contributing to the memory loss and cognitive decline found in Alzheimer disease sufferers [155,156]. Tang and colleagues [157] studied the in vitro effect of 10 nm size and above CdSe quantum dots on hippocampal neurons and reported cell death after 24 h incubation, attributed to an increase in Ca^{2+} intra cellular concentration. In another study Cao et al., [158] also reported the effects of 38 nm size PbS QDs injected in rat brain. They found an increase in the basal cytosolic Ca^{2+} concentration in hippocampal neurons. Membrane disruption may be the cause of this influx across the L-type calcium channel as suggested by Tang et al. [159].

Cell cycle progresses through successive phases controlled by several antagonist genes coding growth factors and inhibitors. An exposure to NPs results in DNA injuries (double-strand

and single-strand breakages) leading to an arrest of the cell cycle which most frequently happens in the G_2/M phase [160]. Hanagata and colleagues reported the downregulation of up to 90 cell-cycle genes upon in vitro exposure to copper oxide NPs of lung epithelial A549 cells [161]. Setyawati et al. [162] investigated the effect of familiar nanosized food-additives SiO_2, TiO_2, and ZnO on the gastrointestinal intestinal cell lines: DLD-1, SW480, and NCM 460. They observed different levels of NP types induced toxicity on these three cell lines with ZnO NPs inducing greater cytotoxicity. They also found that NPs activate the downregulation of the checkpoint kinase 1 (Chk1) by p53, leading to cell-cycle arrest at the G2/M checkpoint, which in turn might lead to cell death.

Surface charge of the NPs affects also greatly their cytotoxicity. By measuring inhibition of the A549 adenocarcinomic human pulmonary cell proliferation, Wingett et al. [163] found that positively charged zinc oxide NPs induce more cytotoxic effects than their counterions of a similar shape and size. They also reported, upon exposure of these cells to positively charged ZnO-NPs, an abnormal increase in the lactate dehydrogenase (LDH-A) expression, a molecule that nurtures solid tumor progression [164].

9. Nanoparticles in cancer detection and treatment

9.1. NPs in Cancer detection

Recent progress in nanoparticle technology has led to a major forward leap in the field of radiation oncology. NPs can provide a minimally invasive and more efficient practice for the diagnosis or prognosis of some cancers. Due to their significantly large surface area-to-volume ratio, nanoparticle can bind to, and identify numerous cancer-related biomarkers [165,166]. Owing to due to their high quantum yields and tunable emission maxima, QDs are commonly used for the tracking of cancer biomarkers at early stage which might reduce cancer death rate [167]. Fan et al. used fluorescent 3–6 nm shell CdSe QD microarray conjugated with microRNAs (miRNAs) as noninvasive diagnostic biomarkers for lung cancer [168]. Wu et al. [169] used Multicolor QDs as detection elements biomarkers for cytokeratin 19 marker in lung cancer.

9.2. NPs in Radiosensitization

The idea of radiosensitization was introduced in the early eighties, when high atomic number materials were proposed as dose enhancers for cancer radiotherapy [170,171]. The dose enhancement results from the large cross section of photoelectric interaction of low energy incident photons with high-Z elements. This attenuation results in an increase of secondary electron production, and Auger electron cascade in sub-MeV energy range which then leads to an increase in the local dose deposition [172].

Advanced technologies in stereotactic radiotherapy that for instance allow better targeting of the tumor have considerably improved the likelihood curative intent of patients. However, challenges persist. On one hand, many aggressive cancers are quite resistant to radiotherapy where further improvement in curative efficiency of radiotherapy must be achieved in these less radio-responsive tumors. On the other hand, despite its 5-year survival rate of 53% [173], compared to the 40% of the radiotherapy alone [174], chemoradiotherapy suffers a serious setback due to its induced acute toxicity such as hearing loss, bone necrosis, endocrine dysfunction to mention but a few [175].

High Z-NP based radiosensitization is an efficient means for enhancing the therapeutic outcome of chemotherapy. Inert NPs used in intratumoral injection or in implants have been explored for local radiosensitization in external beam radiotherapy. Based on simulation [176,177] and experimental test [178] results have shown that dose is locally delivered to the tumor cells sparing the surrounding healthy tissue. Yet this technology is still in the clinical trials and is showing an impressive potential in cancer cure.

10. Conclusion

The application of nanomaterials in biomedical field is limitless. As explored in this review, nanotechnology made it possible the manufacturing of devices on the cellular and even biomolecules scale, making an exclusive approach to imaging, drug and gene delivery. There is still room for more groundbreaking and exciting applications of nanotechnology where NP can potentially assist in understanding the cellular signaling pathways bringing a deeper knowledge of the complex cell.

References

[1] S. Tinkle et al., Ann. N. Y. Acad. Sci. **1313**, 35 (2014).
[2] A. Wicki, D. Witzigmann, V. Balasubramanian, J. Huwyler, J. Control. release **200**, 138 (2015).
[3] C. T. Matea et al., Int. J. Nanomedicine **12**, 5421 (2017).
[4] E. C. Wang, A. Z. Wang, Integr. Biol. **6**, 9 (2014).
[5] X. Han, K. Xu, O. Taratula, K. Farsad, Nanoscale **11**, 799 (2019).
[6] Y. H. Bae, K. Park, J. Control. release **153**, 198 (2011).
[7] S. M. Dizaj, S. Jafari, A. Y. A. Khosroushahi, Nanoscale Res. Lett. **9**, 252 (2014).
[8] F. Parnia, J. Yazdani, V. Javaherzadeh, S. M. Dizaj, J. Pharm. Pharm. Sci. **20**, 148 (2017).
[9] M. H. Barcellos-Hoff, C. Park, E. G. Wright, Nat. Rev. Cancer **5**, 867 (2005).
[10] G. Delaney, S. Jacob, C. Featherstone, M. Barton, Cancer Interdiscip. Int. J. Am. Cancer Soc. **104**, 1129 (2005).
[11] T. Islam, M. G. Harisinghani, Cancer Biomarkers **5**, 61 (2009).
[12] H. Maeda, H. Nakamura, J. Fang, Adv. Drug Deliv. Rev. **65**, 71 (2013).
[13] J. Jeevanandam, A. Barhoum, Y. S. Chan, A. Dufresne, M. K. Danquah, Beilstein J. Nanotechnol. **9**, 1050 (2018).
[14] H. Zhang et al., J. Biomed. Opt. **21**, 126006 (2016).
[15] A. M. Smith, H. Duan, A. M. Mohs, S. Nie, Adv. Drug Deliv. Rev. **60**, 1226 (2008).
[16] R. Weissleder, Nat. Biotechnol. **19**, 316 (2001).
[17] A. J. Sutherland, Curr. Opin. Solid State Mater. Sci. **6**, 365 (2002).
[18] C. B. Murray, C. R. Kagan, M. G. Bawendi, Annu. Rev. Mater. Sci. **30**, 545 (2000).
[19] M. Fang, M. Chen, L. Liu, Y. Li, Y. J. Biomed. Nanotechnol. **13**, 1 (2017).
[20] S. Pleskova, E. Mikheeva, E. Gornostaeva, Cellular and Molecular Toxicology of Nanoparticles, 323 (Springer, 2018).
[21] W. C. W. Chan et al., Curr. Opin. Biotechnol. **13**, 40 (2002).
[22] I. V. Martynenko et al., AJ. Mater. Chem. B **5**, 6701 (2017).
[23] H. Kobayashi et al., Nano Lett. **7**, 1711 (2007).
[24] F. A. Esteve-Turrillas, A. Abad-Fuentes, Biosens. Bioelectron. **41**, 12 (2013).
[25] A. M. Derfus, W. C. W. Chan, S. N. Bhatia, Nano Lett. **4**, 11 (2004).
[26] L. Qu, Z. A. Peng, X. Peng, Nano Lett. **1**, 333 (2001).
[27] Y. Wang, M. Tang, Sci. Total Environ. **625**, 940 (2018).
[28] L. E. Rikans, T. Yamano, J. Biochem. Mol. Toxicol. **14**, 110 (2000).
[29] Y. Yang et al., Nanotoxicology **8**, 107 (2014).
[30] X. Yang et al., J. Inorg. Biochem. **167**, 36 (2017).
[31] V. P. Torchilin, Nat. Rev. Drug Discov. **4**, 145 (2005).
[32] N. V. Long, C. M. Thi, M. Nogami, Curr. Phys. Chem **4**, 173 (2014).
[33] P. U. Atukorale et al., Bioconjug. Chem. **29**, 1131 (2018).
[34] H.-I. Chang, M.-K. Yeh, Int. J. Nanomedicine **7**, 49 (2012).
[35] A. D. Bangham, Chem. Phys. Lipids **64**, 275 (1993).
[36] M. Marciello et al., Interface Focus **6**, 20160055 (2016).
[37] E. Liliemark et al., Leuk. Lymphoma **18**, 113 (1995).
[38] J. S. Suk, Q. Xu, N.. Kim, J. Hanes, L. M. Ensign, Adv. Drug Deliv. Rev. **99**, 28 (2016).
[39] Y. Su, Z. Xie, G. B. Kim, C. Dong, J. Yang, ACS Biomater. Sci. Eng. **1**, 201 (2015).

[40] T. M. Allen, Drugs **54**, 8 (1997).
[41] K. S. Soppimath, T. M. Aminabhavi, A. R. Kulkarni, W. E. Rudzinski, J. Control. release **70**, 1 (2001).
[42] E. B. Dickerson et al., Cancer Lett. **269**, 57 (2008).
[43] T. Patel, J. Zhou, J. M. Piepmeier, W. M. Saltzman, Adv. Drug Deliv. Rev. **64**, 701 (2012).
[44] A. Bolhassani et al., Hum. Vaccin. Immunother. **10**, 321 (2014).
[45] J.-M. Shen et al., Int. J. Pharm. **427**, 400 (2012).
[46] S. Parveen, S. K. Sahoo, Eur. J. Pharmacol. **670**, 372 (2011).
[47] A. Gupta et al., Biol. Med. **8**, 941 (2012).
[48] B. Tyler, D. Gullotti, A. Mangraviti, T. Utsuki, H. Brem, Adv. Drug Deliv. Rev. **107**, 163 (2016).
[49] H. Yuan et al., Mol. Pharm. **7**, 953 (2010).
[50] D. J. Hines, D. L. Kaplan, Crit. Rev. Ther. Drug Carr. Syst. **30**, (2013).
[51] V. Yardley, S. L. Croft, H. Ghandehari, J. Control Release **77**, 233 (2001).
[52] L. Cabeza et al., Eur. J. Pharm. Sci. **102**, 24 (2017).
[53] Y. Wang, S. Gao, W.-H. Ye, H. S. Yoon, Y.-Y. Yang, Nat. Mater. **5**, 791 (2006).
[54] S. Jin, J. C. Leach, K. Ye, Micro and Nano Technologies in Bioanalysis, 547, (Springer, 2009).
[55] H. Zhao et al., J. Mater. Chem. B **4**, 4060 (2016).
[56] M. Yu, J. Wu, J. Shi, O. C. Farokhzad, J. Control. Release **240**, 24 (2016).
[57] S. S. Lucky, K. S. Soo, Y. Zhang, Chem. Rev. **115**, 1990 (2015).
[58] S. Parveen, S. K. Sahoo, J. Drug Target. **16**, 108 (2008).
[59] E. E. Connor, J. Mwamuka, A. Gole, C. J. Murphy, M. D. Wyatt, Small **1**, 325 (2005).
[60] S. Roy, T. K. Das, G. P. Maiti, U. Basu, Mater. Sci. Eng. B **203**, 41 (2016).
[61] G. Schmid, Chem. Soc. Rev. **37**, 1909 (2008).
[62] Y. Pan et al., Small **5**, 2067 (2009).
[63] C. Wang et al., J. Mater. Chem. B **3**, 7372 (2015).
[64] L. Li et al., Theranostics **3**, 595 (2013).
[65] L. Gu et al., Nanoscale **10**, 15967 (2018).
[66] M. Mahmoudi, S. Sant, B. Wang, S. Laurent, T. Sen, Adv. Drug Deliv. Rev. **63**, 24 (2011).
[67] A. Kohut, A. Voronov, W. Peukert, Langmuir **23**, 504 (2007).
[68] A. Petri-Fink, B. Steitz, A. Finka, J. Salaklang, H. Hofmann, Eur. J. Pharm. Biopharm. **68**, 129 (2008).
[69] M. Barrow, A. Taylor, P. Murray, M. J. Rosseinsky, D. J. Adams, Chem. Soc. Rev. **44**, 6733 (2015).
[70] Y. Zhang, N. Kohler, M. Zhang, Biomaterials **23**, 1553 (2002).
[71] G. S. Demirer, A. C. Okur, S. Kizilel, J. Mater. Chem. B **3**, 7831 (2015).
[72] C. Cha, S. R. Shin, N. Annabi, M. R. Dokmeci, A. Khademhosseini, ACS Nano **7**, 2891 (2013).
[73] G. Hong, S. Diao, A. I. Antaris, H. Dai, Chem. Rev. **115**, 10816 (2015).
[74] H.-C. Wu, X. Chang, L. Liu, F. Zhao, Y. Zhao, J. Mater. Chem. **20**, 1036 (2010).
[75] J.-Y. Hwang et al., BNanoscale **5**, 487 (2013).
[76] M. Roldo, D. G. Fatouros, Annu. Reports Sect. C"(Physical Chem. **109**, 10 (2013).
[77] R. Alshehri et al., J. Med. Chem. **59**, 8149 (2016).
[78] N. Hoshyar, S. Gray, H. Han, G. Bao, Nanomedicine **11**, 673 (2016).
[79] M. Caldorera-Moore, N. Guimard, L. Shi, K. Roy, Expert Opin. Drug Deliv. **7**, 479 (2010).
[80] R. Zellner, Biological responses to nanoscale particles, (2015).
[81] L. Sui et al., Int. J. Pharm. **471**, 157 (2014).
[82] K. C.-W. Wu et al., Chem. Commun. **47**, 5232 (2011).
[83] N. Arsalani, H. Fattahi, M. Nazarpoor, Express Polym Lett **4**, 329 (2010).
[84] W. J. Gradishar et al., J. Clin. Oncol. **23**, 7794 (2005).
[85] J. D. Byrne, T. Betancourt, L. Brannon-Peppas, Adv. Drug Deliv. Rev. **60**, 1615 (2008).
[86] B. C. Mei et al., J. Mater. Chem. **18**, 4949 (2008).
[87] B. C. Mei, K. Susumu, I. L. Medintz, H. Mattoussi, Nat. Protoc. **4**, 412 (2009).
[88] J. A. Champion, Y. K. Katare, S. Mitragotri, J. Control. release **121**, 3 (2007).

[89] S. K. Murthy, Int. J. Nanomedicine **2**, 129 (2007).
[90] Y. Hong, Y. Rao, Biomed. Pharmacother. **114**, 108764 (2019).
[91] C. M. Dawidczyk et al., J. Control. Release **187**, 133 (2014).
[92] C. Li et al., Acta Pharm. Sin. B (2019).
[93] H. M. Redhead, S. S. Davis, L. Illum, J. Control. Release **70**, 353 (2001).
[94] Y. Zhao et al., J. Control. release **222**, 56 (2016).
[95] Y. Yang, Z. Wang, Y. Peng, J. Ding, W. A. Zhou, Front. Pharmacol. **10**, 10 (2019).
[96] X. Guo, F. C. Szoka, Bioconjug. Chem. **12**, 291 (2001).

[97] M. Kuruppuarachchi, H. Savoie, A. Lowry, C. Alonso, R. W. Boyle, Mol. Pharm. **8**, 920 (2011).
[98] M. A. Konerding, E. Fait, A. Gaumann, Br. J. Cancer **84**, 1354 (2001).
[99] J. M. Pantshwa, P. P. D. Kondiah, Y. E. Choonara, T. Marimuthu, V. Pillay, Cancers **12**, 213 (2020).
[100] S. K. Samal et al., Chem. Soc. Rev. **41**, 7147 (2012).
[101] Y. Yang, J. Lee, M. Cho, V. V. Sheares, Macromolecules **39**, 8625 (2006).
[102] S. Svenson, D. A. Tomalia, Adv. Drug Deliv. Rev. **64**, 102 (2012).
[103] Y. Gao, G. Gao, Y. He, T. Liu, R. Qi, Mini Rev. Med. Chem. **8**, 889 (2008).
[104] K. C. Wood et al., Bioconjug. Chem. **19**, 403 (2008).
[105] R. Qi et al., AAPS J. **11**, 395 (2009).
[106] B. Newland et al., J. Am. Chem. Soc. **134**, 4782 (2012).
[107] R. Huang et al., J. Res. Sci. gene Transf. its Clin. Appl. **11**, 754 (2009).
[108] S. S. Gill et al., Nat. Med. **9**, 589 (2003).
[109] O. Samsonova, C. Pfeiffer, M. Hellmund, O. M. Merkel, T. Kissel, Polymers **3**, 693 (2011).
[110] Y. Qian et al., Biomaterials **34**, 2117 (2013).
[111] S. A. Kulkarni, S.-S. Feng, Pharm. Res. **30**, 2512 (2013).
[112] D. R. Elias, A. Poloukhtine, V. Popik, A. Tsourkas, Biol. Med. **9**, 194 (2013).
[113] M. A. Dobrovolskaia, S. E. McNeil, J. Control. release **172**, 456 (2013).
[114] M. A. Dobrovolskaia, P. Aggarwal, J. B. Hall, S. E. McNeil, Mol. Pharm. **5**, 487 (2008).
[115] P. J. Murray, T. A. Wynn, Nat. Rev. Immunol. **11**, 723 (2011).
[116] I. Capjak, S. Š.Goreta, D. D. Jurašin, I. V. Vrček, Arch. Ind. Hyg. Toxicol. **68**, 245 (2017).
[117] S. Tenzer et al., Nat. Nanotechnol. **8**, 772 (2013).
[118] T. Niidome et al., J. Control. Release **114**, 343 (2006).
[119] F. Iversen et al., Theranostics **3**, 201 (2013).
[120] J. Fang, H. Nakamura, H. Maeda, Adv. Drug Deliv. Rev. **63**, 136 (2011).
[121] Q. Yang et al., Mol. Pharm. **11**, 1250 (2014).
[122] L. Zhang et al., Fitoterapia **83**, 678 (2012).
[123] P. L. Rodriguez et al., Science **339**, 971 (2013).
[124] C.-M. J. Hu et al., Proc. Natl. Acad. Sci. **108**, 10980 (2011).
[125] J.-G. Piao et al., ACS Nano **8**, 10414 (2014).
[126] J. Su et al., Adv. Funct. Mater. **26**, 1243 (2016).
[127] A. Parodi et al., Nat. Nanotechnol. **8**, 61 (2013).
[128] A. E. Nel et al., Nat. Mater. **8**, 543 (2009).
[129] G. Jiménez Sánchez et al., Int. J. Mol. Sci. **20**, 4618 (2019).
[130] T. Kong et al., Small **4**, 1537 (2008).
[131] P. Liu et al., Int. J. Nanomedicine **11**, 5003 (2016).
[132] A. Panariti, G. Miserocchi, I. Rivolta, Nanotechnol. Sci. Appl. **5**, 87 (2012).
[133] A. Gronewold, M. Horn, I. Neundorf, Beilstein J. Org. Chem. **14**, 1378 (2018).
[134] A. M. El Badawy et al., Environ. Sci. Technol. **45**, 283 (2011).
[135] L. K. Bogart, A. Taylor, Y. Cesbron, P. Murray, R. Lévy, ACS Nano **6**, 5961 (2012).
[136] C. Tassa, S. Y. Shaw, R. Weissleder, Acc. Chem. Res. **44**, 842 (2011).
[137] C. He, Y. Hu, L. Yin, C. Tang, C. Yin, Biomaterials **31**, 3657 (2010).
[138] R. Duncan, Nat. Rev. Drug Discov. **2**, 347 (2003).
[139] H. H. Gustafson, D. Holt-Casper, D. W. Grainger, H. Ghandehari, Nano Today **10**,

487 (2015).
[140] X. Liu, N. Huang, H. Li, Q. Jin, J. Ji, Langmuir **29**, 9138 (2013).
[141] W. Jiang, B. Y. S. Kim, J. T. Rutka, W. C. W. Chan, Nat. Nanotechnol. **3**, 145 (2008).
[142] J. A. Champion, Y. K. Katare, S. Mitragotri, Proc. Natl. Acad. Sci. **104**, 11901 (2007).
[143] B. D. Chithrani, W. C. W. Chan, Nano Lett. **7**, 1542 (2007).
[144] M. M. Arnida, A. Ray, C. M. Peterson, H. Ghandehari, Eur. J. Pharm. Biopharm. **77**, 417 (2011).
[145] D. Hristozov, I. Malsch, Sustainability **1**, 1161 (2009).
[146] L. Braydich-Stolle, S. Hussain, J. J. Schlager, M.-C. Hofmann, Toxicol. Sci. **88**, 412 (2005).
[147] A. Nemmar et al., Circulation **105**, 411 (2002).
[148] W. I. Hagens, A. G. Oomen, W. H. de Jong, F. R. Cassee, A. J. A. M. Sips, Regul. Toxicol. Pharmacol. **49**, 217 (2007).
[149] L. Yang, D. J. Watts, Toxicol. Lett. **158**, 122 (2005).
[150] K. Donaldson et al., J. aerosol Med. **15**, 213 (2002).
[151] H. J. Johnston et al., Crit. Rev. Toxicol. **40**, 328 (2010).
[152] Y.-W. Huang, C. Wu, R. S. Aronstam, Materials (Basel). **3**, 4842 (2010).
[153] W. Lin, Y. Huang, X.-D. Zhou, Y. Ma, Int. J. Toxicol. **25**, 451 (2006).
[154] W. Lin, I. Stayton, Y. Huang, X.-D. Zhou, Y. Ma, Toxicol. Environ. Chem. **90**, 983 (2008).
[155] S. Navakkode, C. Liu, T. W. Soong, Ageing Res. Rev. **42**, 86 (2018).
[156] Y. Wang, Y. Shi, H. Wei, J. Alzheimer's Dis. Park. **7**, (2017).
[157] M. Tang et al., Environ. Health Perspect. **116**, 915 (2008).
[158] Y. Cao et al., J. Inorg. Biochem. **126**, 70 (2013).
[159] T.-H. Tang et al., J. Biomed. Sci. **20**, 48 (2013).
[160] D. Lee et al., Int. J. Mol. Sci. **20**, 6309 (2019).
[161] N. Hanagata et al., ACS Nano **5**, 9326 (2011).
[162] M. I. Setyawati, C. Y. Tay, D. T. Leong, Small **11**, 3458 (2015).
[163] D. Wingett, P. Louka, C. B. Anders, J. Zhang, A. Punnoose, Nanotechnol. Sci. Appl. **9**, 29 (2016).
[164] U. Thonsri et al., Histol Histopathol **32**, 503 (2017).
[165] A. B. Chinen et al., Chem. Rev. **115**, 10530 (2015).
[166] X. Zhang, Q. Guo, D. Cui, Sensors **9**, 1033 (2009).
[167] X. Chen et al., Cell Res. **18**, 997 (2008).
[168] L. Fan et al., Tumor Biol. **37**, 7777 (2016).
[169] S. Wu et al., Talanta **156**, 48 (2016).
[170] H. Matsudaira, A. M. Ueno, I. Furuno, Radiat. Res. **84**, 144 (1980).
[171] R. S. Mello, H. Callisen, J. Winter, A. R. Kagan, A. Norman, Med. Phys. **10**, 75 (1983).
[172] E. Taha, F. Djouider, E. Banoqitah, Australas. Phys. Eng. Sci. Med. **41**, 363 (2018).
[173] J. Bernier et al., N. Engl. J. Med. **350**, 1945 (2004).
[174] G. E. Laramore et al., Int. J. Radiat. Oncol. Biol. Phys. **23**, 705 (1992).
[175] C. Wang et al., J. Int. Med. Res. **47**, 2832 (2019).
[176] E. Banoqitah, F. Djouider, Radiat. Phys. Chem. **127**, 68 (2016).
[177] P. Retif et al., Int. J. Nanomedicine **11**, 6169 (2016).
[178] Y. Liu et al., Phys. Medica **31**, 210 (2015).