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Functional Nanomaterials in Biomedicine: Current Uses and Potential Applications

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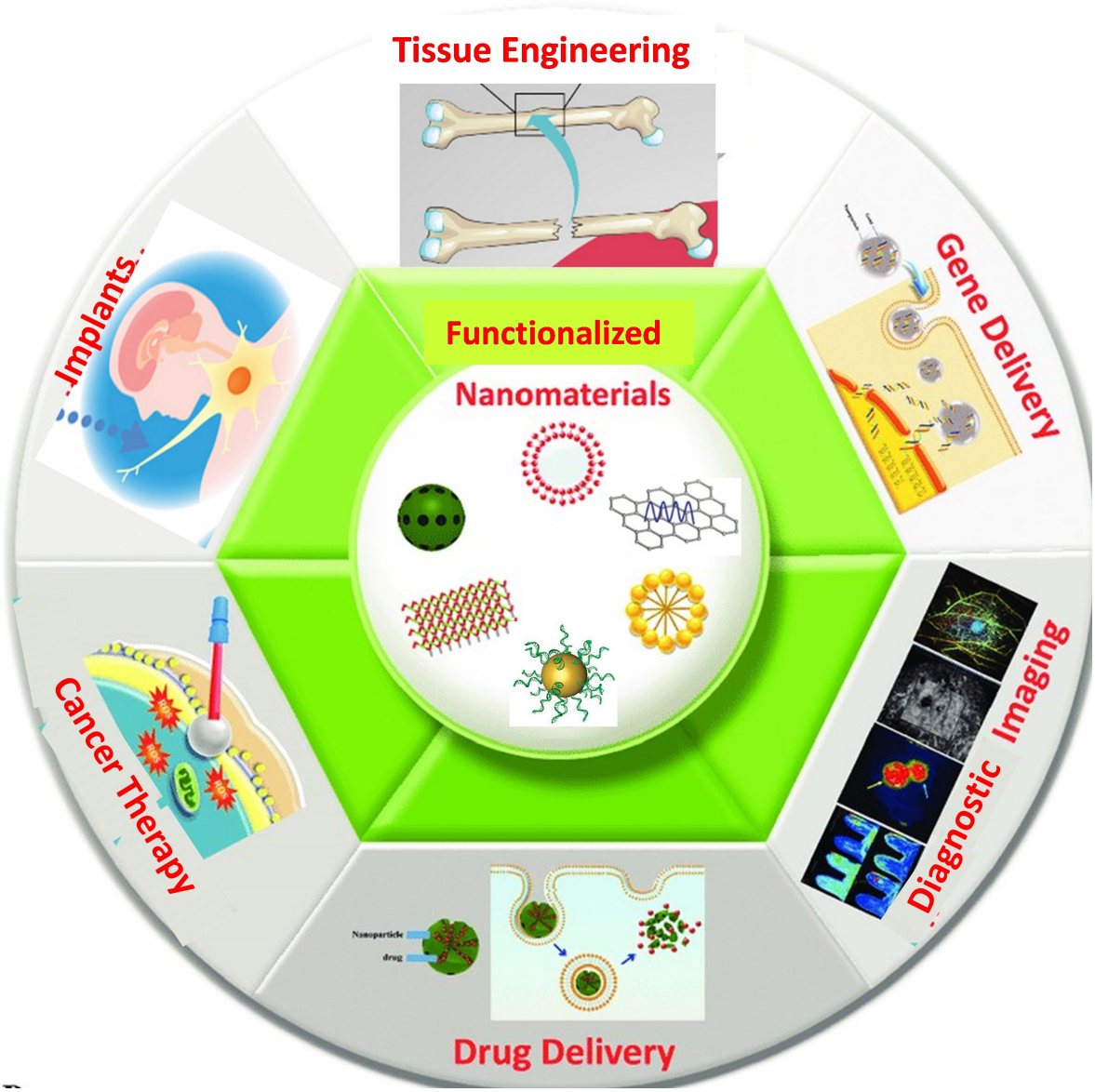

Nanomaterials, that is, materials made up of individual units between 1 and 100 nanometers, have lately involved a lot of attention since they offer a lot of potential in many fields, including pharmacy and biomedicine, owed to their exceptional physicochemical properties arising from their high surface area and nanoscale size. Smart engineering of nanostructures through appropriate surface or bulk functionalization endows them with multifunctional capabilities, opening up new possibilities in the biomedical field such as biosensing, drug delivery, imaging, medical implants, cancer treatment and tissue engineering. This article highlights up-to-date research in nanomaterials functionalization for biomedical applications. A summary of the different types of nanomaterials and the surface functionalization strategies is provided. Besides, the use of nanomaterials in diagnostic imaging, drug/gene delivery, regenerative medicine, cancer treatment and medical implants is reviewed. Finally, conclusions and future perspectives are provided.

1. Introduction

Due to their exceptional physicochemical properties arising from their high surface area and nanoscale size, nanomaterials have lately involved a lot of attention since they offer a lot of potential in many fields, particularly in pharmacy and biomedicine, for cancer treatment,[1] drug/gene delivery,[2] tissue engineering,[3] medical implants,[4] biological imaging,[5] etc. A large number of nanomaterials have countless potential to be applied in biomedicine, including nanoplates, nanotubes, nanoparticles, nanowires, a so forth.[6]

Besides, they must meet specific characteristics to be used in biomedical applications. Their potential cytotoxicity, which can be induced by their structure, chemical content, or features, for example, as well as their biocompatibility, have to be assessed.[7] Their colloidal stability should also be maintained under physiological conditions, ideally across a wide pH range.[8] As a result, it is critical to consider these criteria to ensure the safety, non-toxicity and biocompatibility of the nanomaterials. Specific interactions with biomolecules of interest are required to modify and functionalize the nanomaterial surface in order to meet these criteria.

The methods for creating, manipulating, and deploying functionalized nanoparticles (FNPs) open up exciting new opportunities for developing novel multifunctional biological devices.[9] Furthermore, functionalization protects nanoparticles (NPs) from agglomeration and makes them compatible in subsequent phases. As a result, FNPs can transport more efficiently after systemic injection and have better pharmacokinetic characteristics in vivo. FNPs can be deeply drive into tissues through narrow capillaries and epithelial coating, leading to improved therapeutic agent delivery to the targeted location.[10] Furthermore, the size of FNPs enhances exceptional physicochemical features such as solubility, diffusivity, immunogenicity, and the capacity to target the designated region with minimum diffusion to its surrounding.[11]

The NPs interface can be designed and applied in different ways. These approaches are classified as replacement, non-covalent, and covalent conjugations based on the primary concept of the type of functionalization interaction.[12] The interface between the nanoparticles and molecules attached is modified via the replacement approach, which comprises ligand exchange and ligand addition.[13] Non-covalent techniques rely on intermolecular forces such as ionic interactions for electrostatic particles, Van der Waals forces for bilayer encapsulation, and much complex interplays such as host-guest interactions.[14] Covalent attachment techniques have been proposed to alter the external functionalization of nanomaterials to bind molecular entities for biomedical purposes, hence giving the nanoparticles additional functionality.[15]

This article aims to provide specific examples to cover the different ways of NP functionalization. Before highlighting particular examples of each type of functionalization, the basis of the functionalization will be summarized. Despite some studies on nanoparticles, interface modification, and fabrication for medical and nanotechnological application have been reported,[1,2,16] the current paper reviews the different types of functionalization, classified by functional groups and particular uses. Moreover, the use of FNPs as a versatile tool in nanobiotechnology will be discussed. Various approaches to NP functionalization have been developed, and have attracted huge attention by their diverse implementation. Due to their beneficial characteristics such as biodegradability and biocompatibility in physiological mechanisms, wide availability, suitability for chemical treatment, and wide range of potential synthesis process from different sources, NPs have been extensively explored in the literature. This article offers novel insights on NP functionalization, focusing on their therapeutic, diagnostic, drug/gene delivery and tissue engineering applications. Besides, their potential cytotoxicity will be discussed. Finally, conclusions, forthcoming applications and future perspectives will be provided.

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2. Features of Functional Nanomaterials for Biomedicine

Drug delivery, gene therapy, tagging and tracking, hyperthermia, and medical imaging with various modalities such as magnetic resonance imaging (MRI), positron emission tomography (PET), and so forth use NPs.[17] It should be noted that biomolecular interactions rely on the chemical modification of the nanoparticle surface when using NPs for in-vitro or in-vivo applications. As a result, various types of targeting moieties have been implemented to be incorporated on the surface of nanoparticles, especially peptides,[18] aptamers,[19] antibodies,[20] and small molecules. Through a ligand-receptor interaction, such targeting moieties can allow nanoparticles to be embodied into cancer cells and tissues. To facilitate active targeting of NPs to receptors, which are located on the surface of the membrane, the nanoparticle surface can be tailored with targeting ligands, resulting in increased cellular internalization and/or selective absorption via receptor-mediated endocytosis.[21] Researchers are particularly interested in discovering new biomarkers and their relevant ligands in targeted medication administration. The binding of NPs to analytes, pathogens, and biomarkers might cause their signal to be amplified, making it easier to detect and image. When the scaffold surface is decorated with "bioactive cues" (FNPs that interact with cells to elicit a specific response), this is referred to as functionalization.[22]

The surface of NPs has been combined with a range of ligands, allowing them to be used in biomolecule and cell sensing, illness detection, and intracellular administration, as shown schematically in Figure 1. Chemists can easily make the suitable functionalities for use in clinics thanks to the ease of such functionalization.[23] Small ligands are a common selection for functionalizing NPs since they are simple to chemically bond to surfaces via functional moieties in their structure. Different types of surface-modified NPs by ligands/molecules including polymeric NPs, nanocapsules, micelles, dendrimers, liposomes, nanospheres and nanogels have been developed as multifunctional carriers capable of combining targeted drug delivery and imaging in the field of pharmaceutical applications.[24] Drugs

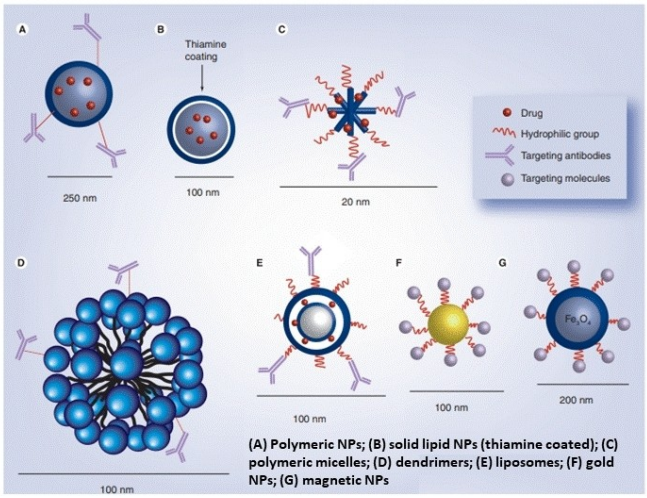

Figure 1. Different surface-modified NPs with ligand/molecules for active targeting. Adapted with permission from Ref. [24], Copyright 2009, Future Science Group.

may be absorbed at the NP surface or encapsulated within the particle. Furthermore, functionalization has been proven to protect NPs against agglomeration and make them biocompatible materials in other application stages.[25] Functionalization improves the NP physical, chemical, and mechanical characteristics, resulting in synergetic effects.[26] For example, Hirayama and coworkers[27] described the encapsulation and release of anti-malaria quinine drug from the surface of functionalized mesoporous silica nano carriers (MCM-41), and optimized the pH and thermal conditions, resulting in improved drug loading capacity due to the synergy between the nanoparticles and the silane coupling agents.

Polymers are suitable functionalizing agents because they create a physical barrier around the NPs, preventing the core of the NPs from coming into direct contact with biological receptors. Polymers can act as ligands and produce a physical barrier but with a reduced hydrodynamic radius. As a result, polymer coatings outperform small molecule ligands when imparting macromolecular system characteristics to the particle

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surface, similar to biological proteins. The use of polymers like polyethylene glycol (PEG) to coat nanoparticles improves passive tumor tissue targeting, increasing permeability and retention (EPR) effect. This effect is a debatable concept by which NPs tend to accumulate in tumor tissues much more than they do in normal ones. PEG and other polymer coatings decrease blood serum protein adsorption, lengthen circulation duration, and promote particle absorption into tumor tissues.[28] Biomolecule-coated NPs have features that are troublesome or inconceivable to attain with synthetic materials, such as excellent biomacromolecule distribution with little cytotoxicity. The synthesis of gold–thiol bonds to create oligonucleotide–AuNP conjugates was one of the first bio-nanotechnology examples described by Mirkin and Alivisatos.[28] Using gold nanoparticles synthesized by stacking cationic polyallylamine and anionic poly (acrylic acid) polyelectrolyte layers, Kleinfeldt and coworkers[29] developed a superhydrophilic and biocompatible coating that showed colloidal stability. Makvandi et al.[30] investigated the functionalization of various polymers (glyclusters, glydendrimers, glycopolymers) and nano-materials (silver oxide, copper oxide, iron oxide, zinc oxide, magnesium oxide, titanium oxide, selenium, nickel, and palladium) for water purification, food containers, fabrics, and medical applications. The benefits and drawbacks of polymer functionalization were investigated and explored in that study. Despite substantial development in nanotechnology and its therapeutic use, many obstacles were identified.

The manipulation of FNPs opens up novel routes for the progress of unexplored multifunctional instruments for biomedical and nanotechnological applications.[31] Nanotechnology applications have drawn a great deal of interest since the late 1980s.[32] Several methods of NPs functionalization for biomedical and nanotechnological applications are described below with the aid of particular examples.

A recent review[33] explored functionalized gold nanoparticles (AuNPs) in biomedicine. To reach this aim, the structure of AuNPs and their manufacture as well as functionalization routes were examined in detail. These NPs have been applied in biology, electrochemical technology, and radiation oncology. Multifunctionalization, that is, functionalization that enables to attain more than one quality simultaneously, offers extra value to these NPs due to synergistic effects. Multifunctional AuNPs have been found to be a feasible option in biomedicine for delivering anticancer drugs and antibiotics in order to combine photothermal and chemical therapy.[34] Figure 2 depicts several means of AuNPs functionalization by polymers to be used as sensing materials.[35]

The covalent approach consist of the "grafting" (chemical bonding) of polymeric segments to the NP surface, and can be implemented via "grafting to" or "grafting from (Figure 2). The first is based on the synthesis of a modified polymer susceptible to react with the functional groups on the NP surface. A shortcoming of this method is that the amount of polymer grafted to the nanomaterial is restricted, owed to the low reactivity and large steric barrier of the polymeric segments. Advantages of this method are the availability of many types of polymers and the easy synthesis (one-pot synthesis).[36] In the

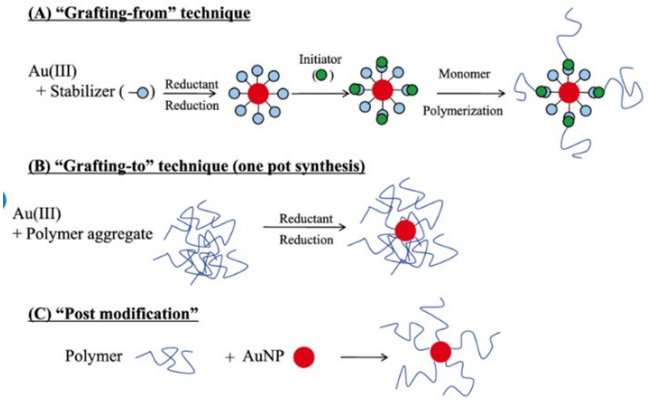

Figure 2. Representation of functionalization of AuNPs by polymers through "grafting from" (A), "grafting to" (B), and "post modification" (C) techniques. Reproduced with permission from Ref. [35], Copyright 2010, The Japan Society for Analytical Chemistry.

"grafting from" path the polymer is grown from the NP surface via polymerization of monomers. This approach is effective, allowing introduction of polymer chains with high density and a high grafting level, allows a precise control of the molecular weight of the incorporated polymers and a versatile structural design of the polymer layer.

The post-modification approach is the most frequent and the simplest method for preparing Au nanocomposites because mixing both of the as-prepared materials can eliminate undesired factors such as the dispersion of AuNP size and molecular weight. Drawbacks of this tactic are the low efficiency of polymer introduction of due to the steric hindrance of the conjugated polymers and unintended adsorption through functional groups in the polymers. For example, conjugation of AuNPs with polymers having SH-terminated groups form covalent-bonded nanocomposites. Also, surface-modified AuNPs with alternating polyelectrolyte layers of cationic polyallylamine and anionic poly(acrylic acid) were covalently bonded to papain via amide bond between the NH_2 groups of papain and the terminal COOH groups of the modified NPs, using carbodiimide as coupling agent. The resulting compound has been applied in bioanalysis and biopharmaceutical analysis.[37]

Despite the struggles performed, additional studies into smart drug delivery systems based on NPs, specially AuNPs, are necessary. Notwithstanding numerous publications, scarce clinically approved drug delivery nanosystems are nowadays available in the industry. Therefore, there is an urgent need to include animal model examination into clinical practice.[34] A recent study[38] used a cyclodextrin-based polymer to cargo phenylethylamine (PhEA) and piperine (PIP) onto gold nanostars (AuNSs); the potential of the compound for concurrent drug loading and SERS-based detection was assessed. Besides, the polymer contained AuNSs that were functionalized with PhEA and PIP, leading to a novel system with optimum dimensions and Z potential to be applied in biomedicine. Hybrid compounds comprising carbon nanomaterials and

AuNPs have also been designed. For example, Geng et al.[39] reported the synthesis of soluble fullerene-linked AuNPs via amination reaction of C_{60} with amino groups on the Au surface that were introduced by the reduction of $HAuCl_4$ with sodium borohydride ($NaBH_4$) in the presence of 4-aminobenzenethiol/1-hexanethiol molecules in a one-pot way. This approach enables to control the optical and photochemical properties of the nanoparticles. A recent review reported the preparation, functionalization, modification, and applications of nanostructured gold.[40] The feasibility of AuNPs to bind thiols, amines and polymers offers effective means that can be used for targeting and conjugating therapeutic agents such as antibodies, peptides, aptamers, drugs, siRNA, genes and so forth. By combining AuNPs with other nanoplatforms such as liposomes. therapeutic uses for cancer treatment are endowed. AuNPs have great interest in the field of radio-sensitization for oncology. In particular, the survival of mice with subcutaneous EMT-6 mammary carcinoma can be significantly enhanced by X-ray irradiation and activity with AuNPs.[41] In addition, researchers have revealed new methods of radio-sensitization for chemical improvement (DNA damage and radical production) and biological improvement in physical improvement (ROS-induced oxidative stress, inhibition of DNA healing and cell cycle disruption. The AuNP size and surface charge strongly conditions the efficacy of their cellular uptake.[42] The selective accumulation at tumour sites was assisted via circulating AuNPs with enhanced porous design and retention (EPR) effect aid. The cellular absorption of highly negatively charged surface AuNPs is very difficult. To increase cellular uptake, neutral or positively charged surfaces are helpful. By modifying the surface chemistry of AuNPs with coating molecules, cancer radiotherapy can be significantly enhanced. An overview of the biomedical applications of AuNPs is shown in Figure 3.

The exceptional combination of mechanical, optical and electrical properties offered by carbon nanotubes (CNTs) has fostered research for their use in many applications in the biomedical field.[43] They are an allotropic form of carbon reported for the first time by Iijima in 1991. They can be synthesized by different methods including electric-arc discharge, laser ablation and chemical vapour deposition (CVD) techniques.[44] CNTs can be described as a rolled-up graphene layer, occasionally closed at the end by fullerene caps. The number of concentric walls composing a CNT is an essential parameter that determines many properties. Increasing the number of layers increases the number of defects and thus makes them easier to modify and to functionalise, most of the time at the cost of a degradation of their physical properties. Biomedical applications of CNTs require a few challenges to be addressed. One is related to safety and involves the use of high purity CNT in order to avoid release of toxic ions. This is a practical challenge since high purity CNTs cannot be prepared at a large scale, hence a compromise between quality and quantity has to be made. Another is being able to attain good dispersions of CNTs in solvents and aqueous solutions. The strong hydrophobicity of CNTs make them difficult to disperse in a solvent but also to stabilize in suspension. This can be achieved through covalent and non-covalent functionalization (covalent typically by chemical oxidation and non-covalent by addition of a dispersing agent or a surfactant).[45]

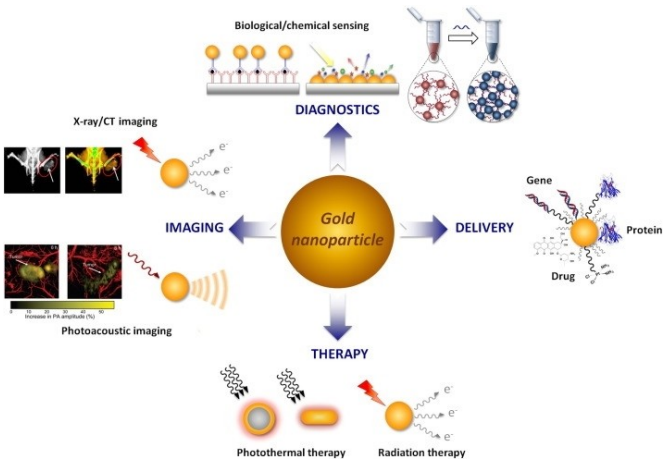

Figure 3. Representation of the biomedical applications of AuNPs. Reproduced with permission from Ref. [41], Copyright 2017, Elsevier.

Several researchers have considered using functionalized CNTs in electrochemical sensors.[46] They can also act as contrast agents in different bioimaging methods.[47] Functionalised and conjugated with various biomarkers can indicate the presence and localisation of targeted cells with a good spatial resolution.

Anticancer therapies based on CNTs have also been reported.[48] For instance, intra-tumoral injection of MWCNT suspension, followed by short laser excitation led to in tumour ablation in mice and enhanced survival.[48a] On the other hand, Wang et al.[48b] described the intravenous injection of SWCNT conjugated with anti-CTLA-4 by triggered immune reaction, which improved both cytotoxic activity and photothermal therapy, ensuing in the destruction of the residual nodules. The coupling of imaging methods with PTT was also reported to stimulate treatment of primary tumours and detection of connected lymph nodes in a single stage.[48c] Lately, MWCNT/AuNS hybrids improved photothermal conversion, making it possible to limit laser stimulation time during the therapy.[48d] To investigate the potential of the hybrid material for enhanced photothermal ablation of cancer cells, different concentrations of MWCNTs/AuNS were cultured with B16-F10 melanoma cells for 24 h, and then irradiated with a laser for different times at different power densities (Figure 4). A concentration of 0.32 nM, an irradiation time of 3 min and power density of 1.0 $W\,cm^{-2}$ were chosen as optimal parameters. The hybrids displayed better photothermal efficiency than the AuNSs alone, leading to many cancer cells detaching from the substrate. Furthermore, the combination of molecular delivery and PTT broadens the application range of this cancer therapy method. CNT-assisted PTT is nevertheless restricted by laser penetration depth in tissues and still needs to prove its efficacy for thicker samples.

Hydrogels are widely used in the biomedical field due to their biocompatibility and the low inflammatory responses. CNT-based hydrogels have been recently used for tissue engineering;[49] the inclusion of CNTs in this type of gels enhances the mechanical properties but also improves the

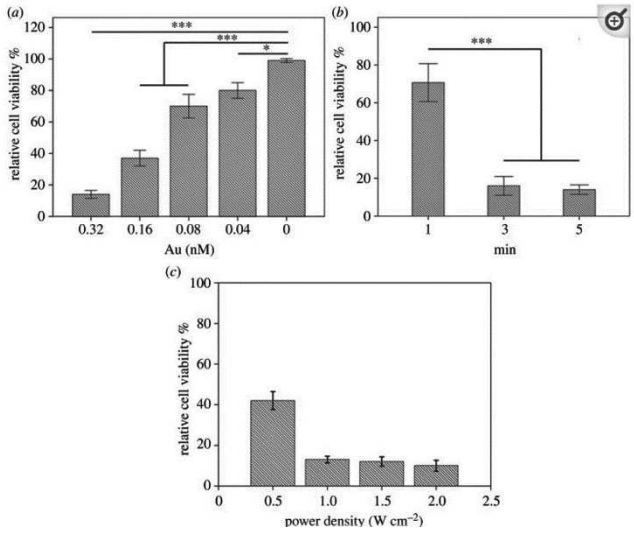

Figure 4. Relative viability of B16-F10 cells incubated with (a) different concentrations of MWCNTs/AuNSs after irradiation by an 808 nm laser (1.0 W cm^{-2}, 3 min), (b) 0.32 nM MWCNTs/AuNSs for different irradiation times (1.0 W cm^{-2}) and (c) 0.32 nM MWCNTs/AuNSs with different power density (3 min). Each value represents the mean ± standard error (n = 6). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Reproduced with permission from Ref. [48d], Copyright 2018, The Royal Society.

electrical conductivity, and the properties of the engineered scaffolds can be finely tuned by modifying the CNT concentration and/or orientation inside the hydrogel. For instance, the addition of CNTs effectively reinforced gelatin methacrylate (GelMA) hydrogels without reducing their porosity or hindering cell growth.[49a]

Several teams explored the prospect of electro-responsive CNT-based hydrogels for drug delivery.[50] For instance, Spizzirri et al.[50a] synthesised microspheres via polymerisation of gelatine containing MWCNT. Diclofenac sodium salt, a drug, was loaded by soaking the microspheres into a concentrated solution, and the application of a voltage caused the contraction of the microspheres and improved drug release efficiency. Further, the drug release behaviour depended on drug charge. The release rate of anionic drugs increased under voltage stimulation whereas cationic drugs were released quicker without electrical stimulation.[50b] The use of implantable CNT-based hydrogels for electrically remote controlled delivery has also been explored. The application of electrical pulses stimulates drug release.[50c,d]

Graphene and its derivatives, graphene oxide (GO) and reduced graphene oxide (rGO) are 2D carbon nanomaterials with exceptional flexibility, strong mechanical strength, large surface area, high resistance to degradation and optical transparency, which combined with their biocompatibility and antiviral properties make them appropriate for the design of selective and sensitive sensors of biomolecules.[3a] GO nanosheets comprising epoxide molecules on either side of the sheet and carboxyl, carbonyl and hydroxyl groups at the edges, are of special interest due to their size controllability, ability to tune their property by altering the oxidation level and high dispersibility in water. These carbon nanostructures have been reported to have strong antibacterial activities toward a broad range of pathogens.[51] In addition, recent studies have demonstrated that GO shows antiviral activity toward Virus-like pseudorabies viruses (PRV) and RNA viruses, which depends on the concentration and incubation time.[52] The antimicrobial action of GO is not well understood yet, but seems to be related to what it is called the "nanoknives' mechanism", also referred to as "penetration mode" or "insertion mode". The sharp edges of the GO layers act as blade, cutting and penetrating the microbial cell membrane with the consequent leakage of the cytoplasmic content and cell death.[53] Supporting this, many other research findings indicate the leakage of intracellular content, including DNA/RNA, as a result of the mechanical disruption of the cell membrane-derived by sharp-edge. rGO and GO show similar antiviral activity, pointing towards an insignificant influence of the surface functional groups.[54] The physical interaction of the viruses with their sharp edges seems also to be the leading cause for the antiviral activity. In addition, they are negatively charged, which allows electrostatic interaction with the positively charged viruses. The higher interactions result in the destruction and inactivation of the virus.

As the increasing antibiotic resistance of bacterial strains create critical health risks, replacement of conventional antibiotics with alternative antibacterial agents is highly encouraged. Carbon quantum dots (QDs) are 0D nanomaterials with fluorescence features, and also display antimicrobial and antiviral properties.[55] Their activity is attributed to the functional groups on their surface. Direct interaction of QDs with bacterial cells also contribute to the overall antibacterial activity. Mechanisms of antibacterial activity of QDs include ROS generation, disintegration of cell structure, and leakage of the cytoplasm because of DNA binding and modulation of gene expression (Figure 5).[55d] The electrostatic attraction of QDs to the bacterial cell depends on a number of factors including the surface charge of the QDs, their surface modification and the type of bacterial strains. On the other hand, QDs functionalized

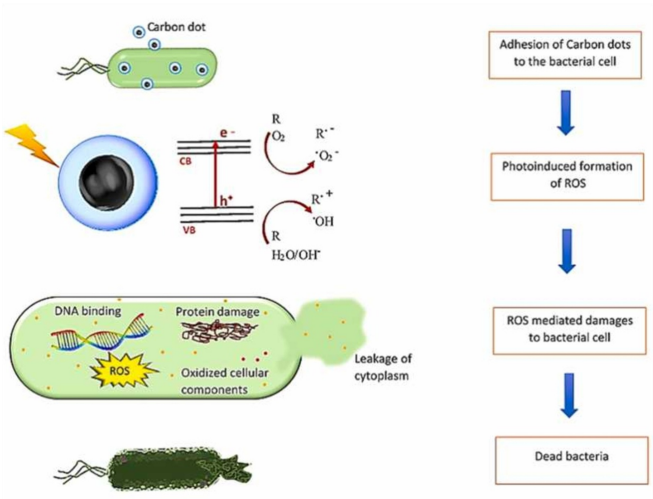

Figure 5. Representation of the antibacterial mechanism of QDs. Reproduced with permission from Ref. [55d], Copyright 2021, Elsevier.

with boronic acid proved antiviral efficacy against HCoV-229E Human Coronaviru, one of the viruses that cause the common cold.[56] Two pathways have been reported for their antiviral activity, namely the anchoring of QDs to the S-protein of viruses to prevent infectious contacts between host cells and viruses and the capacity of QDs to disrupt RNA genomic replication.

QDs also have great potential for cancer treatment. The selective anchoring of FR-positive tumor cells with folic acid was reported as a fast and simple mean for determining folate receptor expression in cancer cells. MKN 45, HT 29, and MCF 7 cancer cells were selectively marked using nitrogen doped QDs with folate coating. DNA-functionalized QDs have drawn substantial attention in sensing and imaging, as well as cancer therapy.[110] Covalent conjugation, electrostatic interaction, direct dative interactions, and other means for conjugating DNA to QDs have been summarized in the literature.[57]

Iron oxide NPs are an attractive family of nanostructures that have attracted much interest in the medical area since they present negligible toxicity, high biocompatibility, and inherent magnetic properties.[58] The most common approaches to produce hollow iron oxide NPs are the Kirkendall effect, chemical etching, galvanic substitution, nano template-mediated, and hydrothermal/solvothermal[59] Surface modification of iron oxide NPs with various covering substances such as dopamine (DOPA), polyethylene glycol with thiol end group (thiol–PEG), and poly(acrylic acid) (PAA) is achievable is essential for biomedical applications.[60] Magnetic nanoparticles (MNPs) have been widely investigated as MRI contrast agents to help in the detection, diagnosis, and treatment of cancers. The absorption of superparamagnetic iron oxide NPs (SPIONs) in the endothelial reticulum system (RES) can be used in medical imaging to detect liver neoplasms and metastases. Furthermore, ultra-small superparamagnetic iron oxide NPs (USPIONs) show promising utility in MRI exams for the identification of lymph node metastases that are 5–10 mm wide. By utilizing the distinct molecular fingerprints of these disorders, the future iteration of active targeting MNPs, which has recently been explored, has the capacity to enhance tumor detection and characterization.[61]

3. Cytotoxicity of Functional Nanomaterials

Numerous parameters including the nanomaterial shape, size, composition, concentration, crystalline structure, aspect ratio and surface functional groups influence strongly the cytotoxicity and uptake pathways of NPs.[62] The composition controls its interaction with cells, cellular uptake mechanisms and intracellular localization, and can provoke oxidative stress. For instance, AgNPs are more toxic than asbestos; CNTs provoke more harm to lungs than carbon black or SiNPs, while TiO_2 and Fe_2O_3 are regarded less toxic than asbestos.[62c] The crystalline structure also determines the level of toxicity. Rutile TiO_2 NPs (200 nm) generated oxidative DNA damage in the absence of UV light and also caused reactive oxygen species (ROS) production while anatase NPs of the same size did not cause this effect.[63] Another factor strongly influencing toxicity is the NP size. Smaller nanoparticles are able to pass through physiological barriers and can enter cells by phagocytosis, micropinocytosis, receptor-mediated endocytosis and other mechanisms.[62e] NPs ability to penetrate the cells is governed by various interactions such as van der Waals forces, steric interactions or electrostatic charges. NPs with bigger surface area tend to agglomerate in the liquid, interact with molecules, such as proteins and DNA and induce oxidation and DNA damage.[62b] It has also been shown that the shape (aspect ratio) conditions cellular uptake efficiency and may affect cell viability.[62c] Besides, surface functionalization conditions their distribution in biological systems. For instance, functionalization of AuNPs is of paramount importance in order to increase their cellular uptake, delivery capability, and optimize their distribution inside the body. Their effects on cytotoxicity, oxidant/antioxidant parameters, and DNA damage in HepG2 cells and the potential toxic effects of different polymeric coatings such as polyethylene glycol (PEG) and polyethyleneimine (PEI; molecular weights of 2,000 (low molecular weight [LMW]) and 25,000 (high molecular weight [HMW]) has been investigated.[62e] After incubating HepG2 cells with different concentrations of NPs for 24 h, half maximal inhibitory concentrations were determined as 167, 258 and 198 μg/mL for AuNPs, AuNPs/PEG, and AuNP/PEI LMW, respectively (Figure 6). Although intracellular ROS levels significantly increased in all nanoparticles, AuNPs as well as AuNPs/PEG did not cause any changes in oxidant/antioxidant parameters. However, AuNPs/PEI HMW induced oxidative stress as evidence of alterations in lipid peroxidation and protein oxidation.

4. Applications of Functional Nanomaterials in Biomedicine

Diagnostic medicine, healthcare services, vaccines and immunization treatments have been renovated and influenced by nanotechnology.[63] Chemical and physical functionalization,

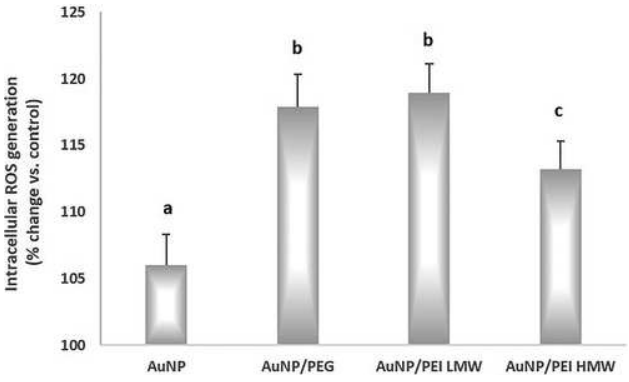

Figure 6. ROS generation after exposure to AuNPs y AuNPs coated with polyethylene glycol (PEG), low molecular weight and high molecular weight polyetherimide (PEI LMW and HMW). a,b,c Bars that do not share same superscripts are significantly different from each other. Reproduced with permission from Ref. [55d], Copyright 2021, Elsevier.

join biological agents with various NPs. The biomedical applications of NPs can be classified into different categories, as indicated in Table 1, where some representative examples for each type are provided.

4.1. Diagnostic applications

Nanomaterials are widely used in imaging modes, such as optical coherence tomography and magnetic resonance imaging (MRI). They are becoming essential to produce high-resolution, high-contrast images required for accurate and precise diagnostics, and can offer relevant information under preclinical and clinical circumstances. QDs are semiconductor nanocrystals commonly employed in optical imaging.[77] PbS QDs have been used for noninvasive scanning of septic encephalopathy in mice, suggesting that these nanomaterials can be applied to image a variety of vascular systems.[77b] On the other hand, magnetic nanomaterials integrating functional materials are named magnetic hybrid nanomaterials (MHNs). Such MHNs have attracted a lot of interest owed to their biocompatibility and the potential applications either as contrast agents or multimodal imaging probes.[77c] These MHNs combine magnetic nanomaterials with functional nanocomponents such as noble metal or isotopes, and can display not only superparamagnetism but also new characteristics that can be adapted in magnetic resonance imaging, computed tomography contrast modalities, positron emission tomography, and single-photon emission computed tomography.[77d] The combination of several nanomaterials provides synergistic effects, leading to higher sensitivity and spatial resolution than conventional materials.

Porous iron oxide nanoagents (PIONs) are magnetic nanomaterials that have the potential to be guided to tumor tissues by a magnetic field and can be used for MRI and photoacoustic imaging (PAI) both in vitro and in vivo (Figure 7). They have been combined with a plasmid vector (pDNA) to as a platform for photothermal therapy and diagnostic imaging. To test their diagnosis and treatment, Huang et al.[78] used a PAI machine to measure their signal, which was found to increase linearly with the increase of PIONs concentration under different wavelengths (Figure 7c). PIONs were found to display excellent and stable PAI ability. Further, the MRI signal showed a linear relationship with their concentration (Figure 7d).

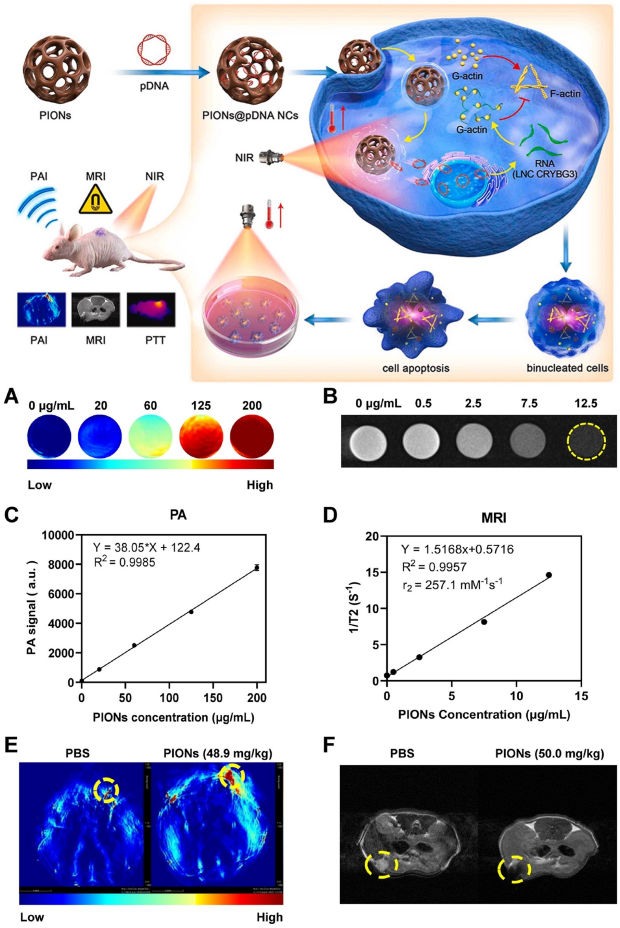

Figure 7. Top: Schematic illustration of PIONs loaded with the plasmid vector pDNA as a nanoplatform for photothermal therapy. Down: Imaging Property of PIONs in vitro and in vivo. (A) PAI and (B) MRI of PIONs at different concentrations. (C) PAI signal of PIONs vs. concentration showing a linear relation. (D) The inverse of the relaxation time (1/T2) of PIONs at different concentrations. (E) PAI and (F) MRI of PIONs in vivo. The data are expressed as mean ± standard deviation (SD). The error bar is derived from triplicate measurements. $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$.. Reproduced with permission from Ref. [78], Copyright 2022, Elsevier.

Table 1. Typical applications of functional nanomaterials in biomedicine.

Application	Type	Reference
Sensing	Metabolic biomarker	[64]
	Insulin	[65]
	Glucose	[66]
Diagnostic imaging	Tomography	[67]
	Magnetic resonance imaging	[68]
	Photothermal imaging	[69]
Therapy	Anticancer treatment	[70]
	Drug delivery	[71]
	Gene and stem therapy	[72]
Medical implants	Orthopaedic	[73]
	Cardiovascular	[74]
	Dental	[75]
Tissue engineering	Bone	[3]
	Cartilage	[76]

The development of nanoparticles with fluorescence characteristics for in-vivo imaging is currently in progress. Fluorescent NPs including fluorescent proteins, quantum dots, carbon dots, aggregation-induced emission NPs, and upconverting NPs (UCNPs) are powerful platform materials for in vivo imaging and will provide better penetration, sensitivity, and resolution.[79] In particular, UCNPs use trivalent lanthanide ions embedded in an appropriate inorganic host lattice to produce higher energy anti-Stokes luminescence. They present many attributes including zero autofluorescence background that improves signal-to-noise ratio, narrow emission bandwidths allowing easiness of multiplexed imaging, and high resistance to photobleaching, making them appropriate for long-term repetitive imaging.[79b] In addition, UCNPs are nonblinking, less light scattering, and

allow for deep tissue penetration since the excitation is in the NIR region. Recently, significant advances have been made by the use of nanochemistry that allows for nanocontrol of their optical properties to improve upconversion at a certain wavelength, surface modification for phase transfer, and surface coupling chemistry for ligands that target biomarkers.

4.2. Therapeutic applications

Functionalized nanomaterials such as superparamagnetic iron oxide nanoparticles (SPIONS), AuNPs, QDs and up- and down-conversion lanthanide NPs have revolutionized the biomedical field over the past few years due to their therapeutic properties.[80] They have been used to enhance already existing disease treatment modalities and have led to the growth of better therapeutic approaches for the advancement of the treatment of critical human diseases including cancers and related malaise. In photodynamic therapy, where the delivery of therapeutic agents should avoid toxicity on nearby healthy cells, SPIONs are capable of making photodynamic therapy (PDT) prodrugs and their associative targeting moieties tumor-specific via their exceptional response to external magnetic fields. Besides, functionalization can improve their properties for PDT.[80b] For instance, water-soluble surface-capped SPIONs have improved therapeutic heat efficient for the destruction of tumor cells under the influence of alternating current magnetic fields. Many biocompatible polymers, such as dextran, PEG, polyvinyl pyrrolidone (PVP), dendrimers, proteins as well as functionalized AuNPs have been reported to stabilize SPIONs in aqueous solution.[80d] Modification of a SPIONs surface is typically attained by direct addition of the coating agent during or after synthesis or by ligand exchange. The latter involves the interchange of a hydrophobic ligand on the surface of the SPION with a hydrophilic ligand in order to make the SPION water-soluble.

The light associated toxicity of PDT limits its applications. To decrease toxicity, a targeted platform that combines a second-generation PDT drug, Pc 4, with a cancer targeting ligand, and SPIONS has been designed.[80c] Carboxyl functionalized SPIONs were initially conjugated with a fibronectin-mimetic peptide (Fmp). Then the PDT drug Pc 4 was encapsulated into the ligand-conjugated SPIONs to generate Fmp–IO–Pc 4. Both IO–Pc 4 and Fmp–IO–Pc 4 reduced the size of xenograft tumors more effectively than free Pc 4. Therefore, they can enhance treatment efficacy and reduce PDT drug dose. The targeted IO–Pc 4 NPs have great potential to serve as both a magnetic resonance imaging (MRI) agent and PDT drug in the clinic (Figure 8).

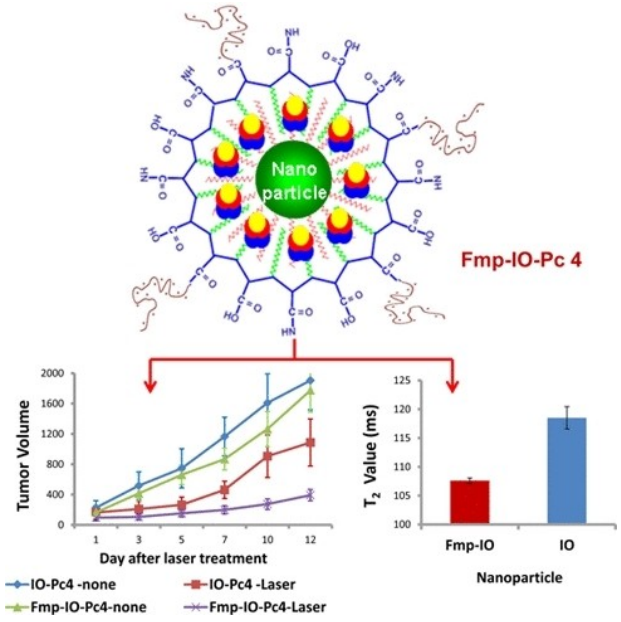

Figure 8. Top: Structure of water-soluble Fmp–IO–Pc 4. Down: effect of Fmp–IO–Pc 4 on tumor growth under laser treatment. n vitro MRI imaging experiment of Fmp–IO–Pc 4. Reproduced with permission from Ref. [80c], Copyright 2014, American Chemical Society.

4.3. Applications of functionalized nanomaterials in drug/gene delivery

The ability to include drugs or genetic materials such as plasmid DNA, RNA, and siRNA into functionalized NPs with little toxicity proves a new era in pharmacotherapy for delivering drugs or genes selectively to tissues or cells.[81] It is anticipated that the transfer of nanoengineering capability into disease therapy will offer constant and concentrated drug delivery to targeted tissues, minimizing systemic side effects and toxicity. Nanotechnology can be used to improve controlled drug release and sustainable drug delivery in solid tumors and on new drug therapies for age-related neurodegenerative disorders.

In particular, the unique properties of CNTs such as their high aspect ratio, enhanced conductivity, strength and biocompatibility, have led to their consideration to serve as novel drug and gene delivery carriers.[81a] CNTs are successfully taken up by numerous cell types via several mechanisms. CNTs have acted as carriers of anticancer molecules like doxorubicin (DOX), methotrexate (MTX), docetaxel (DTX), paclitaxel (PTX), and gemcitabine (GEM)), anti-inflammatory drugs, osteogenic dexamethasone (DEX) steroids, etc. Further, the easy surface functionalization of CNTs has stimulated their use to deliver different genes, such as plasmid DNA (pDNA), micro-RNA (miRNA), and small interfering RNA (siRNA) as gene delivery vectors for various diseases such as cancers. The key objective of CNT functionalization is not only to enhance the physical properties of CNTs like solubility and dispersibility but also to promote their biocompatibility. Aggregation and poor dispersibility make them more cytotoxic in the body.[81d] Surface functionalization is a good mean to lessen their cytotoxicity and improve their efficacy in drug delivery.[81e] For instance, SWCNTs have been conjugated with human serum albumin (HSA) for intracellular transportation of PTX.[82], leading to improved antitumor activity of PTX in vitro. SWCNTs can be conjugated with lipids and natural amino acid-based dendrimers for effective delivery of nucleic acids.[83] Also, functionalized SWNTs

were used to deliver siRNA for gene-based cancer therapy. SWNTs noncovalently functionalized by 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), a cationic molecule, strongly interacted with the negatively charged siRNA, and inhibited the growth of prostate cancer cells.[84] Nonetheless, despite all these potential, the most important concern raised nowadays is CNT nanotoxicology and the environmental effects of CNTs, mostly because of their non-biodegradable state. A summary of functionalized nanomaterials used for drug/gene delivery is shown in Table 2. A schematic representation of the mechanism of functionalized SWCNTs as drug carriers in shown in Figure 9.[85]

On the other hand, SPIONs constitute robust nanoplatforms since they can achieve high drug loading as well as targeting abilities stemming from their remarkable properties magnetic and biological properties.[95,97] They can achieve the highest drug targeting efficiency among carriers, since an external magnetic field locally applied to the target organ enhances the accumulation of magnetic nanoparticles in the drug site of action. Moreover, theranostic multifunctional SPIONs make simultaneous delivery and imaging possible. In spite of these favorable qualities, there are some toxicological concerns, such as oxidative stress, unpredictable cellular responses and induction of signaling pathways, alteration in gene expression profiles and potential disturbance in iron homeostasis, that need to be carefully considered.

Table 2. Applications of functional nanomaterials in drug/gene delivery.

Nanomaterial	Drug/Gene	Reference
SWCNT–HSA	PTX	[82]
SWCNT–dendrimer	siRNA	[83]
SWCNT–DOTAP	siRNA	[84]
Fe_3O_4–GO	DOX	[86]
Silk–GO	DOX	[87]
$CaCO_3$–alginate	Insulin	[88]
Folic acid–AuNPs	DOX	[89]
PEG–Folic acid–AuNPs	DTX	[90]
Hyaluronic acid–Si/Fe_3O_4 NPs	DOX	[91]
Fe_3O_4–SA–PVA–BSA	DOX	[92]
PLGA–Fe_3O_4	5-Fluorouracil	[93]
PLGA–Fe_3O_4	DOX	[94]
Fe_3O_4 conjugate oleate/oleylamine	Chromone	[95]
Fe_3O_4/DPA–PEG–COOH	Dextran	[96]
Thiolated starch-coated Fe_3O_4	Isoniazid	[97]
Arginine–NCQDs	EGFP gene	[98]
Zn-doped Fe_3O_4	DOX and siRNA	[99]

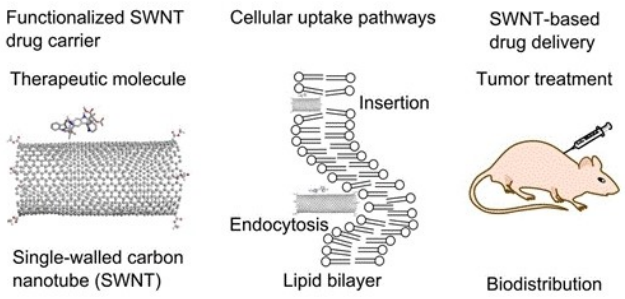

Figure 9. Representation of functionalized SWCNTs as drug carriers. Reproduced with permission from Ref. [85], Copyright 2017, Elsevier.

4.4. Applications of functionalized nanomaterials in regenerative medicine

Regenerative medicine seeks to replace tissue or organs that have been damaged by disease, trauma, or congenital issues, vs. the current clinical strategy that focuses primarily on treating the symptoms. The tools used to carry out these outcomes are tissue engineering, cellular therapies, as well as medical devices and artificial organs.[100]

Bone is composed of collagen and inorganic hydroxyapatite, with a hierarchical structure ranging from nanoscale to macroscale (Figure 10). Taking into account the severe limitation in traditional therapies, nanomaterials offer some novel approaches for bone regeneration. Nanostructured scaffolds provide a handier structural support approximation to native bone architecture for the cells and control cell proliferation, differentiation, and migration, which results in the formation of functional tissues. Several groups[101] have tried to manipulate the mechanical properties (e.g., stiffness, strength, and toughness) of scaffolds through the design of nanostructures (e.g., the inclusion of nanoparticles or nanofiber reinforcements in polymer matrices) to mimic bone's natural nanocomposite architecture. For instance, chitosan/nanohydroxyapatite (CS–nHA) scaffolds have been developed, and it was reported that this hybrid scaffold could encourage the proliferation of bone marrow mesenchymal stem cells (BMSCs), and their molecular mechanism both in vivo and in vitro was explored.[101e] In addition, nanohydroxyapatite can be combined with other polymeric materials such as PEG, polycaprolactone (PCL), and polyglycolic acid (PLGA), which have displayed

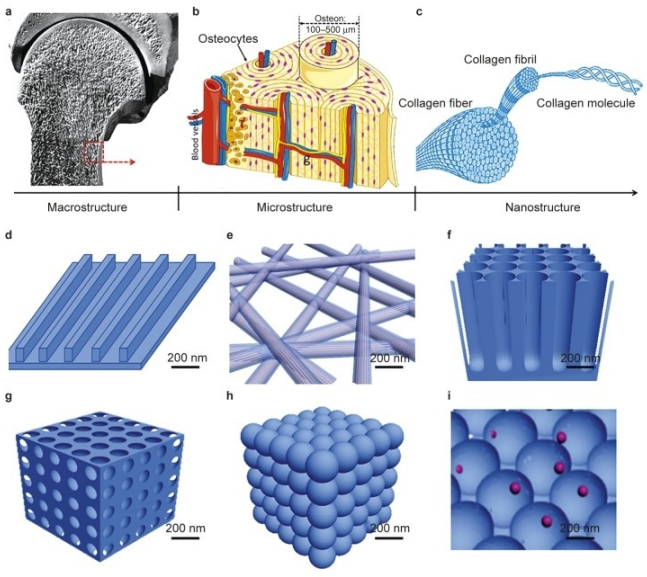

Figure 10. The microstructure and nanostructure of bone and the nanostructured material used in bone regeneration. Reproduced with permission from Ref. [100b], Copyright 2015, Springer Nature.

improved effects in bone regeneration/repair.[102] Representative functionalized nanomaterials that have been designed for tissue engineering/regenerative medicine are summarized in Table 3.

Overall, the applications of nanomaterials in tissue engineering are very important for the repair or regeneration of destructed tissue. Over the last years, progressively more scholars try to develop new biomaterials using different combinations of several nanomaterials. However, when these nanomaterials are used in tissue engineering to substitute damaged organs, concerns of the sensitivity of implanted materials, the following immune response, the possible toxicity, the impact on reproduction and even the effect on fetal development, etc., have to be cautiously analyzed.

Table 3. Applications of functional nanomaterials in regenerative medicine.

Nanomaterial	Tissue/cells	Reference
CS–nHA	BMSCs	[101e]
PLGA/PLGA–nH	MSCs	[102a]
WS_2/PLA/nHA	Bone	[102b]
CS–nHA–PEG	Bone	[102d]
nHA/PCL–PEG–PCL	Osteoblasts	[102e]
PEG–Vitamin D–AuNPs	Osteoblasts	[103]
Silk–nHA–AuNPs	Bone	[104]
Collagen–AuNPs	Chondrocyte	[105]
CS/κ-carrageenan/AuNPs	MG-63	[106]
CS/pectin/AuNPs	MC3T3-E1	[107]
HA/graphene	Osteoblasts	[108]
GO/CS	Bone	[109]
GO/CS	Cartilage	[110]
PPF/GO	Bone	[3a]
CS/Fe_3O_4	Chondrocyte	[111]

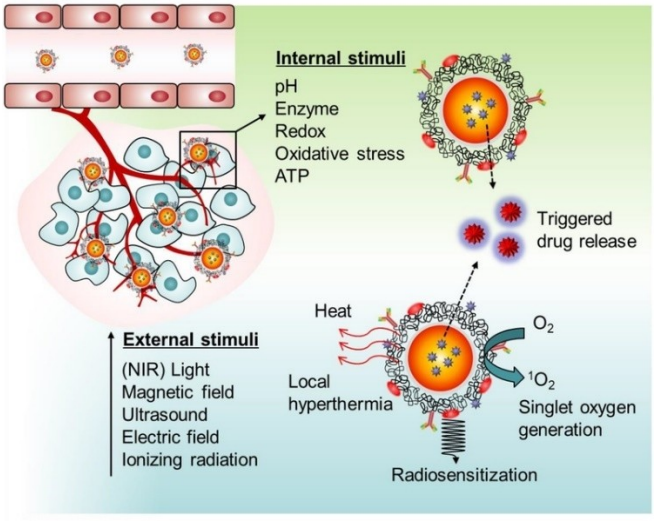

Figure 11. Internal and external stimuli for triggering therapeutic effects of systemically delivered nanosystems. Reproduced with permission from Ref. [112], Copyright 2018, Elsevier.

4.5. Applications of functionalized nanomaterials in cancer therapy

Multifunctional NPs have been designed for targeted cancer therapy by modulating their physicochemical properties to be delivered to the target and activated by internal and/or external stimuli. Stimuli-responsive nanosystems can achieve favorable tumor targeting and enhance targeted cancer therapy. In this regard, smart nanosystems can be responsive to external stimuli (e.g., light, magnetic field, ultrasound, and electric field) and internal stimuli in the tumor microenvironment (e.g., pH, enzyme, redox potential, and oxidative stress), as schematized in Figure 11.

pH gradients are widely used, in particular those that utilize either slightly acidic extracellular pH environment (pH ≈ 6.8) of solid tumor tissue or intracellular pH of cancer cells (*i.e.*, endosomal and lysosomal pH = 5.5–6.0), compared with that in circulating blood (pH ≈ 7.4). Negatively charged NPs composed of HA–polypyrrole (PPy) and positively charged DOX were developed by charge-charge interactions.[113] These interactions were altered in acidic pH after endocytosis to cancer cells, where HA is protonated and its negative charge is reduced. This triggered the release of the drugs from the NPs and spontaneous turn-on of its optical properties. PEGylated PEI linked by Schiff base (PEG-s-PEI) was designed to render pH-sensitive nanoassemblies through multiple interactions with indomethacin and DTX.[114] They exhibited better pH-sensitivity at extracellular pH of tumor microenvironment, compared to normal tissues, thereby long circulated in blood but were highly phagocytosed by tumor cells.

Surface modification of NPs has been demonstrated to produce targeted accumulation in tumor tissue due to the EPR effect. Tumors have more permeable vasculature, a poorly defined lymphatic system, and various substances that help in increased targeting, as contrasted to normal tissue, such as VEGF and basic fibroblast growth factor. In cancer immunotherapies, NPs can keep track of critical immune cells during metastasis. Table 4 includes representative applications of functional nanomaterials in anticancer therapy.

Table 4. Applications of functional nanomaterials in anticancer therapy

Nanomaterial	Drug	Reference
HA–PPy	DOX	[113]
PEG-s-PEI NPs	DTX, indomethacin	[114]
Ce6-conjugated HA–GO	DOX-loaded liposomes	[115]
PEG–biotin–AuNPs	PTX	[116]
Aptamer/SPION	Epirubicin	[117]
phenylboronic acid–ZnO	Curcumin	[118]
Fe_3O_4–rGO	Camptothecin	[119]
Fe_3O_4–glycerol	PTX	[120]
Fe_3O_4–PLGA	DOX and verapamil	[121]
CD–MSNPs	DOX	[122]
MSNPs–galactose	Camptothecin	[123]
FA–CuNPs	Cytochrome C	[124]
FA–MoS_2	DOX	[125]
Peptide–SeNPs	DOX	[126]

5. Summary and Outlook

Progress in the use of functionalized NPs for drug delivery, imaging, tissue engineering and cancer treatment has been reviewed. The unique advantages of nanomaterials such as nanoscale size, high surface area, controllable structure, exceptional optical and physical properties, good biocompatibility, easy surface functionalization and so forth, may allow numerous nanomaterials, both organic such as CNTs, graphene, QDS, and inorganic like silica, Fe_3O_4, TiO_2, ZnO, etc. Preliminary clinical results have shown that functionalization of NPs with specific recognition surface moieties results in improved efficacy and reduced side effects, due to properties such as targeted localization in tumors and active cellular uptake. A prerequisite for progressing in this research area is the development of novel chemical methods to conjugate chemical moieties onto NPs in a safe and reliable manner. The NPs surface modification is a powerful strategy to improve uptake and biocompatibility, as corroborated by the huge amount of scientific papers published on this topic. Studies demonstrate that the conjugation of molecules on the NPs surface can effectively enhance biocompatibility both in vivo and in vitro, due to the modification of surface charge and to the inactivation of reactive chemical groups that can affect cellular membrane stability. Besides, the addition of specific molecules can improve NPs passive and active uptake, reducing toxicity in vivo and allowing high precision therapy and/or diagnosis. Thus, the application of NPs can not only benefit cancer treatment, but also contribute to wound healing, anti-inflammation, and the recovery of other diseases. In addition, intelligent and advanced nano-based technologies can provide specific physicochemical properties, which can aid fixing key issues related to the treatments of viral infections like SARS-CoV-2.

The binding of molecules on the NP surface can be achieved via covalent and non-covalent approaches. The first is widely used to bind proteins, antibodies, aptamers and peptides exploited to enhance uptake and to perform active targeting, while the second is frequently used for loading of drugs and for all molecules that must be released in the cells. Even though remarkable improvements have been attained, this research area is still in its early stages, and significant efforts are needed in order to be able to scale up the functionalization approaches developed at the laboratory level.

The main issue of using functionalized NPs clinically is their potential toxicity and side effects owing to their long body retention time. Some important points should be taken into account to methodically assess the toxicity and side effects of the functionalized NPs for their further clinical translation, namely their biodistribution in the different organs, their metabolic pathways, their acute toxicity, chronic toxicity, genotoxicity, and reproductive toxicity. Their biodistribution and toxicity should be first evaluated in different animal models. If the results are satisfactory, they should be further evaluated in clinical trials. In addition, the development of biodegradable NPs will be helpful for their potential clinical translation. NPs with small size be removed from bodies via urine in a short period of time. Thus, the size range of NPs should be further reduced: developing renal clearable functional nanomaterials with ultrasmall size can be a promising option. The foremost clearance pathway for NPs with larger sizes is likely hepatobiliary and feces excretion, being their clearance ability related to their surface properties or materials components. For example, NPs with coatings comprising an amphiphilic polymer followed by three crosslinked amphiphilic polymeric layers provided high biocompatibility and $>90\%$ excretion within 2 weeks of intravenous administration.[127] Another crucial issue is their biodegradability. Some inorganic NPs such as MnO_2, $CaCO_3$, $Ca_3(PO_4)_2$, and transition-metal dichalcogenide can be degraded into ions.[128] The biodegradability of functional NPs can be tailored via doping with inorganic species, such as calcium-doped silica NPs, integration of organic moieties, or the introduction of cleavable organic molecules or biodegradable polymers. On the other hand increasing the accumulation of NPs in targeted disease-affected tissues remains a significant challenge and is yet to be solved. For certain diseases, local injection may be considered an alternative drug delivery method for systemic administration.

In summary, functional nanomaterials show great potential for improving the therapeutic outcome of various diseases. Nevertheless, their potential toxicity and side effects still hindered their clinical translation. Most of the nanomaterials described in this review are still at the preclinical stage and further research about their long-term toxicity and biodegradation in the body are necessary to guarantee their safety. Thus, only a few nanomaterial-based nanomedicines are approved by FDA for marketing. For example, ultramicro SPIONs were approved by FDA in 2009 for iron-deficiency anemia. However, there are some novel formulations in the clinical trials. For example, PEGylated AuNPs are being tested for prostate cancer treatment. The market is likely to be pushed by a potential supply of nanotechnology-based products and related nanotechnology-based equipment, with significant growth potential in the following years. Throughout the next decade, it is expected that about 40% of products will be in phase II clinical trials, resulting in commercialization.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords: Biomedical applications · functional nanomaterials · drug delivery · tissue engineering · cancer treatment

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