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Review

Smart nanoparticles in biomedicine: an overview of recent developments and applications

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Simple Summary: Over the last decades, nanotechnology applied in medicine (nanomedicine) had sparked great interest from the scientific community thanks to the possibility to engineer nanostructured materials, including nanoparticles for a specific application. This is a continues evolving field that includes a huge variety of nanosystems and every day new scientific researches are published. In this context, this review is proposed to better explain how to design a specific nanoparticle and to clarify the relationship between the type, size and shape of NPs and the specific medical application, especially for tumour theranostic. Therefore, it was born from the necessity to exploit this aspect and to guide any researcher that would like to undertake nanoparticles medical research.

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Abstract: Nanotechnology is an emerging field of modern science based on the use of nanoparticles (NPs) with a huge potential in many sectors, including nanomedicine. Their small size confers them unique properties because they are subject to physical laws that are in the middle between classical and quantum physics. In this context, NPs project plays a pivotal role because the composition, size, shape and surface proprieties need to be carefully considered for their optimal design and application. As reported in this review, NPs are classified in inorganic (metallic NPs; quantum dots; carbon-based nanostructures; mesoporous silica nanoparticles) and organic (liposomes and micelles, dendrimers and polymer nanoparticles) ones. Here, we report an accurate description of the potential of each NPs type focusing on their multiple areas of application like theranostics drug delivery, imaging, tissue engineering, antimicrobial techniques and nanovaccines, and therefore they represent a promise to revolutionize the new era of nanomedicine, especially in cancer research.

Keywords: Nanotechnology 1; Nanomedicine 2; organic and inorganic nanoparticles 3; drug delivery 4; antitumour therapy5; tissue engineering 6.

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1. Introduction

Nanoscience represents one of the most exciting fields of modern science, with a highly interdisciplinary character; indeed, it develops by combining different doctrines like chemistry, biology, physics and engineering, taking advantage of their principles and processes. It is based on understanding and knowledge of the matter properties on the nanometric scale (between 1 and 100 nm). The realization of materials, systems and apparatuses on this size scale determines nanotechnology [1].

The term "nanotechnology" was first defined by Norio Taniguchi of Tokyo Science University in 1974 [2]. In the 1980s, the idea of nanotechnology as deterministic, rather than stochastic, handling of individual atoms and molecules was conceptually explored in depth by Dr K. Eric Drexler, called it Molecular Nanotechnology (MNT)[3]. It is a continually evolving field that finds application in many productive sectors: it is widespread the use of nanoparticles for cosmetics, coating and paints, but also high tech with the production of nano-hard disks or memory chips. One of the significant applications concerns the biomedical environment (Nanomedicine) principally for tissue engineering [4], [5] and drug delivery system (DDS) [6].

Nanoparticles (NPs) are dispersion solution of atomic aggregates or solid particles with a size between 1 and 300 nm and specific properties, like the high surface to mass ratio. Furthermore, the small size permits them to circulate more freely in the human body. It confers some unique chemical, magnetic, mechanical and biological properties that can increase biocompatibility and cellular uptake. The possibility to engineer their surface permits multifunctional applications, especially in the clinical environment for diagnosis and therapies[7], [8] (Figure 1).

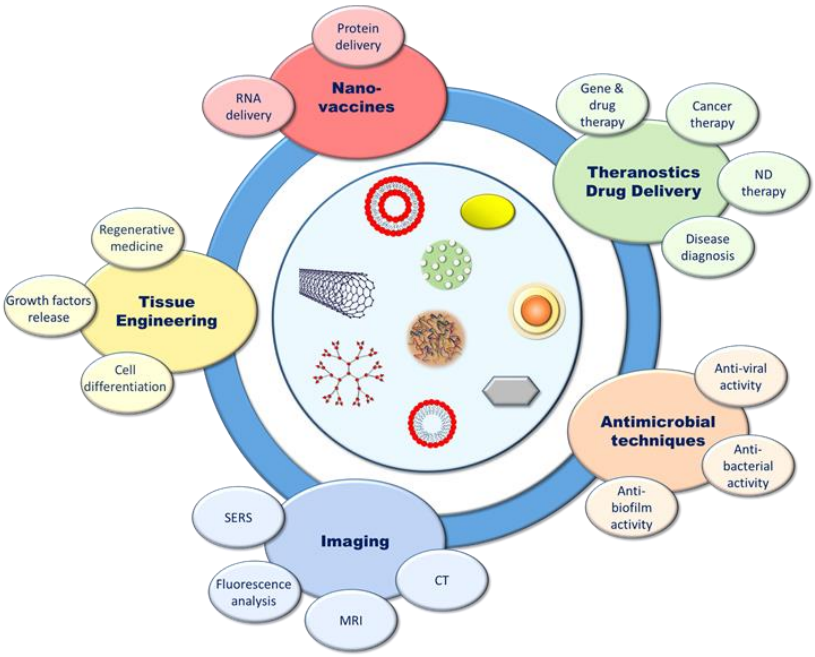

Figure 1. Nanoparticles applications in biomedicine. Depending on the material, size, shape and functionalization, NPs can be employed in: nano-vaccines both for protein and RNA delivery; Theranostics drug delivery that includes treatment and diagnosis of many pathologies like cancer and neurodegenerative diseases (ND); Antimicrobial (anti-viral, bacteria and biofilms) techniques; Imaging for CT, MRI, SERS and fluorescence analysis; Tissue engineering in regenerative medicine for growth factors release or cell differentiation.

NPs theranostic applications are amply employed thanks to the possibility to conjugate them, for example, with fluorescence probes for bioimaging studies: this can help for the detection of tumour masses or specific features of other diseases.

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On the other hand, they can act like vectors able to carry biological molecules (i.e. drugs) to a specific tissue and release them with a controlled mechanism. All these characteristics make them optimal candidates for DDS in many pathologies including neurodegenerative diseases (NDs) and tumours. Although many progressions in the last decades, cancer remain one of the most devastating diseases globally causing 9.6 million deaths in 2018 (Data from OMS), because the tumour heterogeneity limits the formulation of a common therapy. Therefore, conventional drug administration systems (CDASs) (parenteral, oral, cutaneous or topic) for diseases and, in particular, the cancer chemotherapy, can induce side effects because of their nonspecific action: indeed, they act both on healthy and malignant cells[9], [10]. Due to the high capability to divide of the tumour cells out of control, it is based on the destruction of all the rapidly dividing cells, unfortunately including also the body's other rapidly proliferating cells, such as in the hair follicles, myelopoietic bone marrow precursor cells and intestinal epithelial cells, inducing high side effects [11]. Therefore, the knowledge or definite cancer physiology and structure can be the starting point to design engineering NPs for a specific tumour targeting.

Furthermore, the drug dilution in the bodily fluids limits its absorption in the target tissue so that it is necessary to administer substantial doses to have a high local concentration. On the other hand, the use of nanosystems as drug delivery system permits a controlled release of the conjugated drug, depending on physiological conditions of the targeted site (site specific-targeting) and modulation during the release time (temporal modulation), related to the physical properties of the microenvironment [12].

Disease treatment is only one of the multiple applications in which NPs can be involved. Depending on their nature, NPs can present antibacterial or antiviral properties, antiangiogenetic and antineoplastic effects and they are abundantly employed in tissue engineering to promote tissue differentiation thanks to the possibility to local delivery of bioactive (growth factors, chemokines, inhibitors, cytokines, genes etc.) and contrast agents in a controlled way.

Furthermore, in the last decades, the use of different kinds of NPs as a delivery system in vaccines sparked great interest from the scientific community, thanks to their potential to improve vaccine efficacy and reduce risk of the attenuated vaccines. The encapsulation protects the antigens from early proteolytic degradation, permits a controlled antigen release, and helps antigen uptake and processing by antigen-presenting cells. Moreover, the possibility to obtain a specific targeting can improve vaccine formulation [13].

Therefore, the project of nanoparticles systems with a specific focus on the choice of the size, shape, composition (material) and surface properties plays a pivotal role to optimize their use in biomedical applications.

2. 1. Project of nanoparticles

Functionalization on nanoparticles plays a crucial role in the NPs' action in a wide range of delivery applications including diseases treatment like tumours, neurodegenerative and metabolic pathologies, such as bioimaging, tissue engineering, nano-vaccines and antimicrobial techniques (Figure 1). The engineered nanomaterials can be synthesized by two different approaches, top-down and bottom-up, that can also be used in a complementary way (Figure 2).

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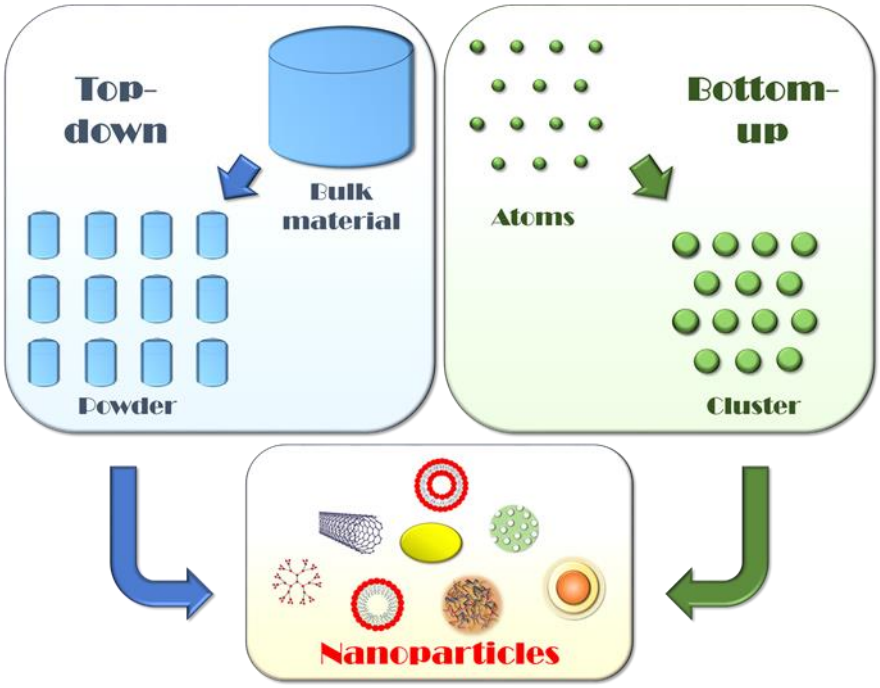

Figure 2. Representation of the nanoparticles synthesis process. Top-down: from macrostructures (bulk materials) to NPs; Bottom-up: from atoms to NPs.

Top-down is a physical approach that reduces macrostructures, named bulk materials, through incisions, grindings and cuttings [14]. On the other hand, bottom-up is a chemical approach that brings NPs production starting from atoms or molecule aggregates [15]. It is the typical synthesis mechanism adopted in the biomedical field because it permits specific control of the process to obtain nanostructures with the desired properties [16]. Starting from this, the size, the shape, the composition (material) and the surface properties must be considered and analysed to increase the circulating half-life, the biocompatibility, the drug loading and corresponding site-specific release and the definite addressing [17].

3. Size

Morphological characteristics, like size and shape, play a pivotal role in nanoparticle-based drug delivery. Size change in the nanoscale influences physical properties (like optical absorption or melting points that decrease in a size-dependent way), chemical reactions (like thermodynamic features) and magnetic properties especially for metal NPs and electrical properties.

NPs size needs to be chosen with particular attention because nanosystems have to be small enough to escape the capture from the cells of the mononuclear phagocyte system (i.e., in the spleen and the liver) and big enough to avoid their rapid leakage into blood vessel following by clearing in the kidneys [18]. Depending on the administration technique, NPs cytotoxicity and adsorption across the epithelial barrier is related to their size. For example, inhalation enables penetration in the lung parenchyma, showing a different localization on the respiratory tract. On the other hand, as reported by Braakhuis *et al.*, the cytotoxic effect in rats of inhaled silver NPs is related to their dimension: NPs of 18 and 34 nm induced cell damage in a concentration-dependent way. Simultaneously, there was not dose-dependent toxicity of 60 and 160 nm NPs[19]. Many studies have evaluated the NPs pharmacokinetics (*in vivo* distribution), revealed a size-dependent different organ distribution as assessed by Ibrahim and colleagues: 5nm gold nanoparticles (AuNPs) preferentially address to the liver, while bigger AuNPs of 20 and 50nm localized on the spleen [20], [21]. De Jong *et al*. also had analysed AuNPs

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size-dependent tissue distribution reporting an exclusive localization of 10nm AuNPs in testis, thymus, heart and brain [22].

A decrease of NPs dimension corresponds to a higher surface area to volume ratio, suggesting that more conjugated drugs could be associated with or near the NPs surface, leading to faster drug release [23]. Furthermore, cellular uptake is also depending on size [24]. NPs are internalized faster and 15-250 times more than microparticles of 1-10 μm rage [25] through many mechanisms: large NPs are generally involved in micropinocytosis; 100 nm NPs in clathrin-mediated endocytosis, while 15-80 nm NPs in caveolae-mediated endocytosis [26], [27].

The optimal NPs size must be ranged between 1 and 100 nm, especially to cross the blood-brain barrier (BBB) as suggested by neurodegenerative disease studies, including Alzheimer, Parkinson or glioma. The biggest problem with treating cerebral pathologies is the impossibility or high limits of common drugs to pass through the BBB. Their conjugation with NPs of different natures (i. e. polymeric, inorganic or liposomes) permits them to cross the BBB by active (receptor-mediated or adsorption-mediates endocytosis or carrier-mediated transport) or passive (diffusion through endothelial cells) transport mechanisms [28]. For example, one of the most used drugs for Alzheimer disease is the anti-amyloidogenic drug curcumin, but it is unable to cross the BBB. For this reason, Barbara *et al*. encapsulated it in PLGA (polylactide-co-glycolic-acid) nanoparticles modified with g7 ligand that permits the BBB crossing. An intensive decrease of Aβ aggregates in response to curcumin loaded NPs was registered, suggesting a possible approach in the treatment of Alzheimer disease [29].

This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation, as well as the experimental conclusions that can be drawn.

4. Shape

Nanoparticles shape confers peculiar features that influence blood lifespan, macrophages uptake and cell membrane interaction. Generally, nanoparticles are injected into the blood vessels and are subjected to Brownian motion and convective forces, inducing rotation and rolling, especially for oblate-shaped NPs compared to spherical ones [30]. In fact, blood circulation is depending on nanosystem shape, as suggested by Geng and co-workers that showed that polymer filomicelles persisted in the circulation of rodents about ten times more than their spherical counterparts (more than one week against 2-3 days), probably due to the possibility to align to the blood fluid [31]. Zhao *et al*. also confirmed this data, which reported the more prolonged bloodstream circulation of the long rod mesoporous silica nanoparticles (NLR) compared with the short rod (NSR) and spherical (NS) ones [32]. They also investigated the body biodistribution after rat oral administration; although the liver and kidney took up all the NPs, NLR had the longest residence time in the gastrointestinal.

The major part of the nanoparticles nonspecific clearance depends on the mononuclear phagocytic system (MPS) of the spleen and liver. Their retention for a long time can induce an inflammation state [33], [34]. Therefore, their blood lifetime is improved by evoking the macrophages phagocytosis in the reticuloendothelial system to reach the target tissue. Many strategies have been adopted to avoid the MPS. The most diffused is the functionalization with polyethylene glycol (PEGylation) that permits the formation of a hydrating layer due to the association with water molecules [35]. In this manner, it prevents the NPs aggregation and interaction with blood components, like opsonins, prolonging systemic circulation time [36]. PEGylated particles' behaviour is related to PEG molecular weight and its surface density that influences its superficial confirmation [37], [38]. Another approach consists of the nanoparticles "mimetic effect" by conjugating them with "self" molecules, like CD47 peptides [39], or coating them with cell membranes extracted from autologous leukocytes [40] and red blood cells [41].

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Cellular uptake and internalization are depending on the NPs aspect ratio (AR) and the contact angle. Elongated rod nanosystems with very high AR attached better to cell membrane than spheroidal or rod NPs with lower AR, but are phagocytosed less efficiently [42]. This probably is related to the alignment of the longer axis parallel to the cell membrane: in this case, its internalization is more difficult compared to sphere shape nanoparticles. The geometry of the initial contact of the NP with the macrophage (tangent angles) determinates the cell response: the cell starts to remodel the actin cytoskeleton to cover and engulfed the nanosystem only when is the smaller axis of the oblate-shaped NP to contact the cell membrane. On the contrary, an incorrect interaction, depending on the local particle shape, fails to correctly organize the actin, inducing a simple spreading without any internalization [43], [44]. Shape-dependent different macrophages uptake is also attributed to the different endocytosis pathways: spherical gold nanoparticles are generally internalized by clathrin- and caveolin-mediated endocytosis, while the cylindrical ones by clathrin-mediated endocytosis. Moreover, the elongated shape induces a more efficient interleukin 6 inflammatory response than the shorter rod or spherical [45]. Shape-related differential uptake grade was also individuated in another kind of cell such as the tumour cells. For instance, breast cancer cells show a preferential uptake of rod nanoparticles, followed by dish and spheres [46].

Furthermore, the shape can also influence specific nanosystems features. Xu and co-workers reported a relation between morphology and reaction rate of silver NPs (AgNPs): the reaction rate of nanocubes was found 14 times higher than that of triangular ones and four times more than the semi-spherical ones [47]. AgNPs morphology also influences their antibacterial effect: naocomplexes with a higher specific surface area resulted in more toxic for bacteria than smaller ones due to the difference in the Ag ion release depending on the shape [48], [49]. On the other hand, shape plays a pivotal role in mechanical properties and adhesion with hydrogel materials, as suggested by Arno ad co-worker. Analysing the interaction between polymeric NPs and calcium-alginate hydrogels, they found an increase in both the adhesion and the material's mechanical strength concerning spherical or cylindrical counterparts [50].

5. Nanoparticles material

Depending on the material used, nanoparticles can be classified into inorganic and organic ones. As reported in figure 3, the first one includes metal NPs (MNPs), Quantum Dots (QDs), carbon-based nanostructures (CBNs) and mesoporous silica NPs (MSNs); while liposomes and micelles, dendrimers and polymeric NPs represent the organic ones.

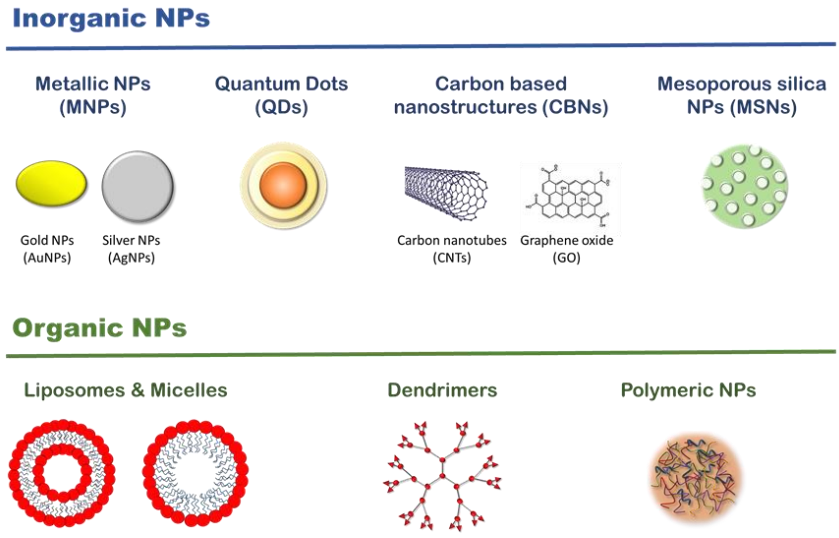

Figure 3. Nanoparticles classification: Inorganic NPs include metal NPs (gold and silver), Quantum Dots (QDs), carbon-based nanostructures (CBNs) and mesoporous silica NPs (MSNs); Organic NPs collect liposomes and micelles, dendrimers and polymeric NPs.

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5.1. Inorganic nanoparticles

Inorganic nanoparticles play a fundamental role in modern materials science due to their unique physical characteristics like size-dependent optical, magnetic, electronic, and catalytic properties. They can be quickly and cheaply synthesized and mass-produced and, for this reason, they can also be more readily used for many applications. These inorganic nanoparticles include metallic ones like gold and silver, quantum dots (QDs), carbon-based nanostructures (CBNs) and mesoporous silica nanoparticles (MSNs) [51].

5.1.1. Metallic nanoparticles: gold and silver nanoparticles

Metallic nanoparticles (MNPs) are amply employed in biomedical applications such as targeted drug delivery, hypothermia, bio-imaging and magnetotherapy [52] (Table 1). They can be used as imaging probes in many techniques like ultrasound (US), X-ray, computed tomography (CT), positron emission tomography (PET), magnetic resonance imaging (MRI), optical imaging, and surface-enhanced Raman imaging (SERS) [53].

In this contest, gold nanoparticles (AuNPs) have attracted interest recently for their use as CT or imaging contrast agents due to their high X-ray attenuation, simple synthesis, surface properties and biocompatibility [54]. They present peculiar absorption and scattering properties like surface plasmon resonance (SPR) that can be tuned by controlling the specific size and shape (sphere, rod and clusters), depending on the synthesis method [55]. AuNPs can be produced by physical (like the microwave and ultraviolet irradiation or laser ablation), chemical (like Turkevich method that consists in the reduction of gold chloride with sodium citrate), and biological (plants and microorganisms mediated) ways [56]. The last one is relatively new and attracted great attention because eco-friendly and microorganisms can adsorb gold atoms and collect gold nanoparticles by secreting enzymes involved in the enzymatic reduction of gold ions [57].

AuNPs are widely used in academic research for tumour treatment [58]. Some studies revealed their potential in limiting angiogenesis and tumour progression, as Li and colleagues suggested that demonstrated the AuNPs effect inhibition of epithelial-mesenchymal transition and tumour vasculature normalization [59], [60].

Furthermore, their functionalization with targeting molecules permits their specific cancer mass penetration to release the associated anticancer drug [6]. The possibility to target AuNPs to tumour site permits its detection in live animals because they can amplify the Raman scattering efficiencies of adsorbed molecules. In this contest, Qian *et al*. conjugated AuNPs to the ScFv antibody that recognizes the epidermal growth factor receptor, overexpressed in many types of human malignant tumours, for *in vitro* and *in vivo* tumour targeting [61]. The targeting features also permit selective delivery of drugs in cancer cells, as Du and co-workers had described. They designed AuNPs carrying an aptamer able to recognize PrPC prion protein expressed on the surface of human bone marrow neuroblastoma cells. Furthermore, the anticancer drug doxorubicin was conjugated to AuNPs-aptamer complex. Data suggested a specific targeting exclusively on the tumour cells and an apoptotic effect due to the doxorubicin release (Du *et al*. 2014).

Furthermore, AuNPs show an optical scattering that can be exploited for the nanophotolysis technique using a short-pulse laser. Inside tumour cells, AuNPs can absorb light and convert it into heat that leads to thermal explosion if the threshold laser flow is in the range of 25–40 mJ/cm2, as suggested by Letfullin and colleagues. [62]. Many studies suggest this approach for breast cancer therapy because it is an effective way to kill tumour cells selectively without affecting healthy ones. Laser photothermal therapy and drug delivery capability of AuNPs can be associated to work cooperatively. In this contest, Banu *et al*. synthesized AuNPs conjugated with the anticancer drug doxorubicin and targeted to human folate receptor overexpressed by very aggressive breast cancer cells.

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Data reported amplification of the released drug's killing into cancer cells by photo-excited using laser light for mediating hyperthermia [63].

All the AuNPs features make them an optimal candidate also for vaccine formulations. Peptide–AuNP conjugates can be internalized by macrophages resulting in their activation and AuNPs of 8-17 nm size bring a strong antibody response with low cytotoxicity [64], [65]. In this contest, Tao and colleagues had formulated AuNPs conjugated to the highly conserved extracellular region of the matrix 2 protein (M2e) of influenza A virus. M2e-AuNP conjugates were introduced in mice by intranasal vaccination and brought to M2e-specific IgG production with partial protection that was increased to a total one by the adding of CpG (cytosine-guanine rich oligonucleotide) adjuvant [66].

In the bloodstream, AuNPs can be recognized from the plasma (opsonization) and processed by the reticuloendothelial system (RES). To prevent this process, AuNPs can be functionalized by adding PEG (PEGylation) that prolongs their blood circulation [67].

PEGylation is also adopted for silver nanoparticles (AgNPs) because it increases their human cell biocompatibility and inhibits platelet aggregation underflow conditions [68]. Functionalization of AgNPs can also improve their typical features, like the antibacterial and antiviral activity. When AgNPs interact with microorganisms, release Ag^+ ions that can interact with the negatively-charged cell walls due to carboxyl, phosphate, and amino groups and alter the cell permeability and lead to cell death [69]. Size and shape influence the antibacterial effect of silver nanoparticles: Hong *et al*. reported a higher antibacterial effect to *E. coli* of silver nanocubes to spheres and wires [70]; while their antimicrobial activity decreases with increasing particles size, as reported by Raza and colleagues [48], [71]. Many studies suggest an AgNPs role in inhibiting bacterial biofilm formation and EPS (Extracellular polymeric substances) production, mainly when associated with the plant-derived drug-like quercetin [72], [73].

Furthermore, Elechiguerra *et al*. reported a specific role of AgNPs size in interaction with HIV-1 virus: they have shown an exclusive interaction with gp120 glycoprotein of particles in the range 1-10 nm [74]. AgNPs also act on eukaryotic cells as antineoplastic drugs by inducing apoptosis. They increase the ROS levels with the following reduction of mitochondrial membrane potential, release of cytochrome C into the cytosol, JNK activation and translocation of Bax to mitochondria [75]. This occurs, for example, in Dalton's lymphoma ascites (DLA) cell lines *in vitro* and *in vivo* as reported by Sriram end colleagues: AgNPs treatment reduces the volume of ascitic fluid in tumour-bearing mice by 65%, increasing their survival time by about 50% in comparison with tumour controls [76].

5.1.2. Quantum Dots (QDs)

Quantum Dots (QDs) are very small (2-10 nm) nanoparticles or nanocrystals with an inorganic core of semi-conductor of group II/IV (e.g., Cadmium/Selenium, Cadmium/Technetium) and an aqueous organic coated shell (e.g., zinc sulfide, cadmium sulfide). Typically, their semiconducting nature confers unique optical and electronic properties. Depending on the core structure's and composition, QDs can emit different colours over a wide spectral range if excited by the same light source. Therefore, they are amply employed as fluorescent probes in cellular and *in vivo* molecular imaging [77]. On the other hand, the outer shell can be functionalized by conjugating different molecules like peptides, protein or DNA acting as diagnostic and therapeutic agents for cancer diagnosis, photodynamic therapy cell labelling and biosensors [78], [79] (Table 1). For instance, conjugation with specific antibodies permits a specific tumour targeting so that Ab-modified QDs can be used for the detection of primary tumours (such as ovarian, breast, prostate or pancreatic cancer), as well as local lymph nodes and detached metastases [80]–[82].

Despite their extraordinary potential as fluorescence probes, QDs present some biomedical applications limitations because of their high toxicity for eukaryotic cells:

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cadmium could cause interferences in DNA repair or stimulate free radical synthesis [83], [84]. This aspect can be exploited in infectious diseases treatment as proposed by Ristic and colleagues. They synthesized graphene quantum dots (GQD) nanoparticles that present higher biocompatibility for eukaryotic cells and antibacterial activity in infectious diseases. If photoexcited, GQD generates reactive oxygen species due to increased propidium iodide cellular uptake. In this way, they kill two strains of pathogenic bacteria, methicillin-resistant *Staphylococcus aureus* and *Escherichia coli* [85].

QDs potential toxicity can also be limited by surface modification, like adding PEG [86] or carbohydrate to QDs [87], [88]. In this contest, recently, cadmium-free QDs (Cd-free QDs) made of indium/palladium were amply used because of their higher biocompatibility [89], [90]. Many studies reported Cd-free QDs for cellular and targeted drug delivery for cancer treatment like proposed by Mathew *et al*. that synthesized folic acid (FA) conjugated carboxymethyl chitosan coordinated to manganese doped zinc sulfide quantum dot (FA–CMC–ZnS:Mn) nanoparticles encapsulated with the anticancer drug 5-Fluorouracil (5 –FU). Their studies had demonstrated targeting, controlled drug delivery and also imaging of cancer cells. *In vitro* drug release studies showed a drug release sustained through slow degradation of CMC [91]. Overall, *in vitro*, the QDs application had given many results, especially cellular pathways' undertraining. Still, the transfection *in vivo* systems presents some limitations due to the RES system block and side effects even if they do not have heavy metals. PEGylation can reduce the liver and spleen uptake increasing their clearance [92]. Therefore, the length and the molecular weight of the PEG and the degree of substitution can modulate the circulation half-life: for example, mPEG-5000 coated QDs circulates longer in mice than mPEG-750 coated QDs that were completely cleared from the bloodstream after 1 hour of injection [93].

5.1.3. Carbon based nanostructure

Carbon-based nanostructures (CBNs) are amply employed in many biological applications like bioimaging, drug delivery, tissue engineering, diagnosis and cancer therapy due to their unique features, including thermal, mechanical, electrical, optical and structural properties [1], [94], [95] (Table 1). CBNs include graphene oxide (GO) and carbon nanotubes (CNTs). Graphene is a single-atom-thick, two-dimensional sheet of hexagonally arranged carbon atoms isolated from its three-dimensional parent material, graphite [96]. The oxide form consists of single-atom-thick carbon sheets with carboxylate groups on the periphery, where they provided pH-dependent negative surface charge and colloidal stability [6], [97]. The basal surfaces contain hydroxyl (-OH) functional groups, which were uncharged but polar [98]. The basal planes also included unmodified graphene domains that were hydrophobic and capable of steaking (π-π) interactions relevant to biological molecules' adsorption like nucleic acid. The bi-dimensional and planar nature offers a large surface area to interact with small interference RNA (siRNA) for drug delivery applications [99]. siRNA gene therapy can also be combined with photothermal therapy, as proposed by Yin *et al*. for pancreatic cancer. They had developed PEGylated graphene oxide nanosheets conjugated with the tumour targeting molecule folic acid to co-deliver two siRNA causing apoptosis, proliferation inhibition and cell cycle arrest. The synergistic combination of gene silencing and NIR light thermotherapy *in vivo* mouse model showed tumour volume growth inhibition by >80% [100]. Furthermore, this characteristic makes them optimal candidates as biosensors for electrochemical detection of DNA bases [101]. Another significant feature of graphene nanoparticles is the capability to promote the growth, proliferation and differentiation of MSCs (Mesenchymal stem cells), NSCs (neural stem cells), and iPSCs (Induced Pluripotent Stem Cells) into tissues of various lineages [102]–[104]. Therefore, its possible employment in tissue engineering and regenerative medicine had generated significant interests thanks to the further possibility of combining it with other materials like Poly-L-lactide (PLLA) [105]. For example, 3% wt of graphene added to PLLA scaffolds

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facilitate the differentiation of BMSC (Bone marrow-derived mesenchymal stem/stromal cells) and increases the calcium deposition and formation of collagen type I [1], [106].

Carbon nanotubes (CNTs) are also amply used in biosensing applications, thanks to their unique features like high aspect ratio, stability and thermal and electrical conductivity, strong mechanical strengths and fast electron-transfer rate [107]–[109]. They originate by wrapping graphene into a cylinder structure forming a tubular structure of 1-2 nm of diameter: the rolled sheets can be single (single-walled carbon nanotubes-SWCNTs), double (double-walled carbon nanotubes DWNTs) or more than two (multi-walled carbon nanotubes –MWCNTs) [110], [111]. Their limit solubility on all solvents generates toxicity problems that can be solved by chemical modification with peptides, proteins, nucleic acid and therapeutic molecules that can increase cellular uptake and drug release when they are used as drug delivery systems [111]–[113]. Su and colleagues conjugated iRGD- polyethyleneimine (PEI) and candesartan (CD) to develop MWCNTs targeting the tumour endothelium and lung cancer cells (by recognition of $\alpha v\beta 3$-integrin and AT1R). Additionally, plasmid AT2 (pAT2) was assembled to form iRGD-PEI-MWNT-SS-CD/pAT2 complexes. Co-delivery of CD and pAT2 synergistically inhibited angiogenesis by downregulating VEGF (vascular endothelial growth factor) and inducing tumour growth suppression in A549 xenograft nude mice [114]. Moreover, the nature of CNTs make them ideal elements for tissue engineering: for instance, Vaithilingam *et al*. introduced multiwalled CNTs to 3D scaffolds to make them conductive to stimulate human pluripotent stem cells to differentiate into cardiomyocytes and modulate their behaviour [115].

5.1.4. Mesoporous silica nanoparticles (MSNs)

Mesoporous silica nanoparticles (MSNs) present a polymeric structure of siloxane (-Si-O-Si-O-) rich in silanol (Si-OH) groups on their surface that can be modified by conjugation with biological molecules to obtain multifunctional nanoconjugates [116]. The controlled chemical synthesis permits to regulate their morphology, pore distribution, size and biodegradability [117]. Parameters like pH, surfactant, silica precursor and temperature can modulate nanoparticle size and shape that play a crucial role in cellular uptake and immune escape. For example, the maximum cellular internalization occurs at the size of 50 nm, while phagocytic cells prefer bigger MSNs as suggested by Lu *et al*. and Vallhov *et al*. [118]–[120].

Generally, MSNs are highly biocompatible because they degrade to silicic acid that is naturally present in body fluids and connective tissue, such as hair, nails, bone, skin, and tendons, and are rapidly eliminated through urine [121], [122] (Table 1). In this contest, MSN circulating time can be highly regulated: surface modification like the PEGylation can prolong their permanence in the bloodstream, while the increase of their pore size or the adding of metal ions can accelerate their elimination from the body [123], [124].

Furthermore, the significant amount of pores and channels that confers a high surface to volume ratio permits to accommodate a large number of biological molecules, including therapeutic agent or drugs that make them optimal candidate as drug delivery carriers for tumour therapy as suggested by Duo *et al*. They synthetized Doxorubicin-loaded MSNs coated with polydopamine (PDA) to obtain a pH-sensitive drug release and with PEG to increase stability and biocompatibility of the nanosystems. *In vitro* and *in vivo* analysis in the breast cancer model had suggested a higher cellular uptake and a controlled drug release with an improved anticancer activity than a free drug [125].

Silica can also reduce the toxicity of other nanoparticles contain, for example, heavy metals like gadolinium (Gd) that is typically used as a contrast agent on magnetic resonance imaging (MRI) [126]. In the same manner, their functionalization can occur for

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other bio-imaging applications such as optical imaging, position emission tomography (PET), computed tomography (CT) [127], [128] [129].

To limit the aggregation and increase the drug loading to gold nanoparticles, Zhang *et al*. synthesized a mesoporous silica shell coated AuNPs and conjugated with doxorubicin to the nanoparticle as an anticancer agent [130]. On the other hand, Ramasamy and colleagues developed AuNPs coated with silica to deliver the antibiofilm agent cinnamaldehyde for the eradication of bacteria, while Chen *et al*. individuated the ability of silica to amply their photoacoustic intensity [131], [132]. Moreover, MSNs could be incorporated on scaffolds for tissue engineering, especially for bone tissue engineering: MSNs loaded with bioactive factors can be combined with scaffolds to improve repair efficacy [133]. MSNs can release Si ions that can influence stem cell behaviour, especially in the expression of genes involved in differentiation and osteogenesis [134].

Moreover, mesoporous silica nanoparticles present an interesting potential as vaccine adjuvant as suggested by Oliveira and colleagues that had investigated their vector ability against the parasite *Schistosoma mansoni*. They had developed MSNs associated with SWAP (Soluble Worm Antigenic Preparation) to test their higher immunization activity compared to a conventional immunization system (SWAP-associated aluminium salt) [135]. In this scenario, MSNs pore sizes play a key role in the presentation of peptide-MHC I complexes to $CD8^+$ T cells, as suggested by Hong *et al*. They had shown that the association of ovalbumin (OVA) tumour antigen with MSNs enhanced both antibody and T cell responses and, in particular, the large-pore MSNs had shown strongest antitumor effects and immune response. Nanosystems, indeed, facilitated OVA escape from lysosomal degradation for MHC I restricted [136].

5.2. Organic nanoparticles

In the last years, many researchers have focused their studies on the possible use of organic nanoparticles (ONPs) in different sectors, especially in the biomedical ones. The organic nature of these systems, indeed, highly reduces their toxicity and therefore side effects. There are different types of ONPs depending on their composition and structure like liposomes and micelles, dendrimers, polymeric NPs and nanogels.

5.2.1. Liposomes and micelles

Liposomes are vesicles constituted by a self-assembled phospholipid bilayer that assumes a spherical shape delimiting an aqueous core of 50-1000 nm of diameter [137]. Depending on the bilayer's number, it is possibly classified in small or large unilamellar (ULVs) and multilamellar (MLVs) vesicles in which the layers are separated by aqueous spaces [138]. This unique structure permits to carry of both hydrophilic and hydrophobic molecules: the hydrophilic ones are localized in the inner core or between the bilayers, while the hydrophobic molecules are associated with the phospholipid membranes. This lead to a multidrug loading and, consequently, a possible sequential drug release from the two different compartments. In fact, they are primarily employed in the drug delivery system because they can fuse with the plasmatic membrane and release the drug inside the cell [139]. Furthermore, photosensitizers' inclusion into the liposomes permits a light-induced cargo release (light-induced liposome technology) [140]. Instead, micelles, are characterized by a single lipid layer that defines a spherical structure with a hydrophobic core, fundamental for transporting lipophilic molecules like many antitumor drugs [141].

Thanks to their nature, liposomes and micelles are non-toxic, biocompatible, biodegradable and non-immunogenic (Table 1). Their chemical-physical properties can be accurately modified by mixing different lipids molecules and changing the superficial charge, size and functionalization [142].

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Even if they have a good distribution in the organism, they present some advantages like the low solubility and half-life, the possibility to leak the loaded drugs especially when they occasionally change lipid components with High or Low-density lipoprotein (HDL and LDL respectively) and thus modify their size and composition, but also their accumulation into the tissues [143]. Additionally, surface modification like PEGylation can elongate the circulating time in the bloodstream while the addiction of targeting molecules can improve targeted delivery [144]. In this contest, PEGylated liposomes are amply studied in the "Trojan Horse Liposome" (THL) technology for transvascular non-viral gene therapy of the brain. Cationic liposomes of THL carrying non-viral gene expression plasmid are functionalized with specific antibodies able to recognize antigens on the blood-brain barrier (BBB) and, in this way, permits its crossing [145]. The encapsulation of genetic material in cationic liposomes limits their degradation from ubiquitous nucleases. The exogenous gene is expressed within one day of a single intravenous administration, as Jiang end colleagues had demonstrated that THL treatment reduced tissue inclusion bodies in the brain and peripheral organs [146], [147]. This technique is also applied in neurodegenerative diseases like Parkinson's disease [148] and tumour therapy as described by Zhang and colleagues. They reported that monoclonal antibody-targeted THLs carrying a siRNA knocking down the EGFR (epidermal growth factor receptor) was capable of increasing the survival time of mice with intracranial brain cancer [149]. Liposomes and micelles are the most primarily studied vector also for drug targeting to macrophages in the treatment of diseases like salmonellosis, leishmaniosis, tuberculosis, rheumatoid arthritis and obviously cancer [150]–[154]. Furthermore, liposomes can mimic pathogens features, inducing humoral and cellular immune responses, thanks to their capability to present antigen to APCs (Antigen-Presenting Cells) and, therefore, they can be optimal candidates as vaccines. Depending on the saturation grade of the lipids, they could induce Th2 (if they are unsaturated) or Th1 (if they are saturated) response [155]. For example, Huang and colleagues reported the improved efficiency of the Pfs230 malaria transmission-blocking antigen candidate, when it was incubated with liposomes containing cobalt-porphyrin-phospholipid (CoPoP) and the synthetic monophosphoryl lipid A (PHAD). They had shown an increase of anti-Pfs230C1 IgG response in mice also after 250 days and also the immunization of rabbits that inhibited parasite transmission [156]. On the other hand, lipid NPs can encapsulate mRNA for nucleic acid-based vaccines: liposomes protect the mRNA from enzymatic degradation and help cell uptake and intracellular release of the mRNA in target cells. Espeseth *et al*. had demonstrated a higher cellular immune response of mRNA/lipid NPs comparing to the protein-based vaccine. They tested lipid NPs-encapsulated mRNA vaccine encoding RSV F (Respiratory syncytial virus) protein on rodent animals highlighting the total absence of vaccine enhanced respiratory disease (VERD) that generally compare after protein immunization [157].

5.2.2. Dendrimers

Dendrimers are nanovectors with a spherical shape constituted by polymeric macromolecules that are capable of self-assembling. They present three different parts: a central hydrophobic core available for the encapsulation of drug molecules; ramification repeated units named "dendrons" that determinates the generation of the dendrimer and its globular structure; hydrophilic functional groups at the outer side that can be conjugated with specific molecules for the complex formation or other functionalizations [158]. Their synthesis is based on the polymerization process of the ramification units from the surface to the core (convergent synthesis) or vice versa (divergent synthesis) and it can be patterned to control the drug release [159]. Thanks to this globular shape, they present a high drug loading ability through both covalent and noncovalent bonds, low polydispersity, reproducible pharmacodynamics and pharmacokinetic behaviour (Table 1). Moreover, the positive charge on their surface due to amino groups' presence permits the

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interaction with nucleic acid-like siRNA or small DNA and the association with cell membranes with cellular uptake [160][161].

On the contrary, the cationic charge makes them toxic both for prokaryotic and eukaryotic cells, so that they are rapidly eliminated from the bloodstream by the mononuclear phagocyte system [162]. Therefore, generally, they are modified with molecules like PEG able to shield the positive charge, improving circulation time and make them more biocompatible even if it depends on PEG molecular weight, degree of PEGylation and tested cell lines [163]. The antibacterial activity is related to the ratio of surface cationic charge to hydrophobicity. It is probably mediated by disrupting the bacterial outer and inner membrane due to positive charges of terminal amino groups, as suggested by Kannan *et al*. [164]. Furthermore, Calabretta *et al*. had demonstrated this effect against both gram-positive and negative bacteria even after PEGylation [165]. On the other hand, their functionalization with anionic groups, such as acid or sulfonate residues, permits limiting the eukaryotic cell toxicity and determinates artificial mimics of the anionic cell surfaces, to exploit an antiviral function. Based on the virus-cell interaction depending on the binding to the cell membrane's sulphated residues, dendrimers can compete with cells for binding of virus stopping the infection [166].

There are over 100 families of dendrimers depending on their functionalization moieties and on the initiator cores (carbon, nitrogen and phosphorus). They are classified as polyamidoamine (PAMAM), polypropylenimine (PPI), carbosilane (CBS), poly-L-lysine (PLL) and phosphorus dendrimers [167]. PAMAM and PPI are amply employed in pharmaceutical sciences and biomedical engineering thanks to the possibility to work as a delivery system and overcome drug resistance. In this contest, they are amply used in the treatment of infectious diseases like malaria, leishmaniasis, schistosomiasis, toxoplasmosis, HIV, meningitis, hepatitis, herpes and especially in tumour therapy [168]–[170] (Table 1). Easy surface and core modifications with DNA, siRNA, plasmids, peptides, antibodies or drugs make them optimal candidates for drug delivery, especially for brain tumours like glioma, because they can cross the blood-brain barrier (BBB) and deliver biological molecules in a controlled way like proposed by Lu *et al*. They had formulated PAMAM dendrimers conjugated with PEG, Arg-Gly-Asp (RGD) tripeptide for the tumour targeting and to the anticancer drug arsenic trioxide (ATO): the use of dendrimer vector permits to the dug to cross the BBB and enhance its antitumor effect glioma [171], [172]. The capability to incorporate many biological molecules and the ramified structure are optimal tissue engineering applications [173]. In this contest, dendrimers can act as a polymerizing agent in hydrogel scaffolds and simultaneously can release growth factors in a controlled manner. In particular, they are amply used in bone tissue engineering as reported by Oliveira *et al*. that showed an increase of the ectopic early osteogenic differentiation of rat bone marrow stromal cells in osteoblasts in HA (hydroxyapatite) and SPCL (starch–polycaprolactone) scaffolds in the presence of Dex-loaded CMCht/PAMAM dendrimer nanoparticles (dexamethasone-loaded carboxymethylchitosan/poly(amidoamine) dendrimer) [174].

5.2.3. Polymer Nanoparticles

Polymeric nanoparticles (PNPs) present a size range from 1 to 1000 nm and are classified into nanocapsules and nanospheres [175]. The first one is constituted by an oily core in which is retained the drug or the biological molecule carried, surrounded by a polymeric shell that controls the drug release mechanism. On the other hand, nanospheres present a polymeric matrix able to encapsulate the drug: in this way, the drug is carried both in the inner and outer parts. Based on the polymer source of origin, it is possible to individuate natural and synthetic polymers [176]. Natural polymers include sodium alginate [177], albumin [178], chitosan [91], polypeptides [179], cellulose [180], inulin [181] and gelatin [182]. On the other hand, some examples of the synthetic ones are poly (lactide coglycolides) (PLGA) [183], [184], polyglycolides (PGA) [185], poly (malic

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acid) (PMLA) [186], poly (methyl methacrylate) (PMMA) [187], polyacrylamide (PAM) [188], poly (N-vinyl pyrrolidone) (PVP) [189], polyorthoesters (POE) [190], poly (methacrylic acid) (PMAA) [187] and poly-L-lactide (PLLA) [191], [192] that can be modified with particles like Hydroxyapatite (HA) for bone tissue engineering applications [193]. Generally, PNPs can be formulated by direct monomeric polymerization or dispersion of pre-existed polymers. The polymerization process can be obtained from monomers, by different preparation techniques like emulsion, miniemulsion or microemulsion, interfacial, controlled/living radical (C/LRP). At the same time, polymer dispersion can be developed by solvent evaporation, nanoprecipitation, salting out, dialysis, supercritical fluid technology (SCF) [194]. On the other hand, amphiphilic copolymers with distinct hydrophobic and hydrophilic segments can self-assemble to form micelles in an aqueous solution, wherein water-insoluble elements form the core and hydrophilic components form the corona [195].

Generally, polymers present themselves in many functional groups permitting the conjugation with biological molecules involved in specific targeting or a controlled drug release mechanism. Adamo *et al*., for example, designed PVP nanogels bringing both folic acid, for a specific tumour targeting, and the pro-apoptotic Bcl-2 siRNA through a redox-sensitive linker. Their data suggest a selective death induction only on cancer cells [196], [197]. Their biodegradability and biocompatibility make them optimum candidates for the treatment of cancer and neurodegenerative disorders and cardiovascular diseases (CVD) [198]. By investigating the use of nanoparticles for Alzheimer's disease (AD), Carradori and colleagues had demonstrated the therapeutic efficacy of PNPs conjugated with the antibody against Aβ1-42 peptide to reduce soluble forms of Aβ and rescue memory in AD mice [199]. On the other hand, Tan and co-workers had developed PNPs to encapsulate the Apomorphine (AMP) drug commonly used in Parkinson's disease. In this manner, AMP was protected by oxidation preventing toxic form formation and crossing the BBB [200]. Moreover, many studies suggest their application for ischemic protection since passive targeting may be doable because the blood-brain barrier's permeability increases upon ischemia [201]. Zamanlu end colleagues, for example, had formulated PEGylated PLGA nanoparticles conjugated with tissue plasminogen activator (tPA) for the treatment of ischemic stroke: circulating time and thrombolytic activity were increasing by association with the nanocomplex [202]. PLGA and the other PNPs can be functionalized to obtain a sustained, spatial, and temporally controlled delivery of growth factors involved in cell growth and differentiation. They can be encapsulated with cells into solid scaffolds or hydrogels to elaborate 3D structures for tissue engineering, as proposed by Nie and Wang [203]. They encapsulated BMP-2 plasmid DNA/chitosan nanoparticles into PLGA/Hydroxylapatite (HAp) composite scaffolds for bone tissue engineering. Testes on human marrow stem cells (hMSCs) suggested a higher cell attachment, higher cell viability and increased DNA release rate due to the incorporation of HAp nanoparticles.

Furthermore, in the last decade, many studies had dedicated to polymeric nanoparticles application as a controlled-release vaccine delivery system (Table 1). Some parameters like surface charge and antigen loading can influence the immune responses as suggested by Gu and colleagues that had tested PLGA NPs positively or negatively charged conjugated to ovalbumin (OVA) by adsorption or encapsulation. They reported that the negative charge facilitated the cytoplasmic antigen delivery by inducing the activation of dendritic cells in lymph nodes 5 days after the primary vaccination. On the other hand, when the antigen was encapsulated, more potent and long-term antigen-specific antibody responses were registered, compared to those of antigen-adsorbed nanoparticles [204]. Moreover, NPs immunogenicity can be improved by the adding of adjuvants like chitosan or glycol chitosan. *In vivo* studies suggest that glycol chitosan induce significantly higher systemic and mucosal immune response compared to only chitosan or nanoparticles alone [205].

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Table 1. Advantages, disadvantages and biomedical applications of each type of nanoparticles.

NPs		Advantages	Disadvantages	Applications in nanomedicine	Ref
Metallic NPs	AuNPs	• Biocompatibility • Easy to synthesize and conjugate to biological molecules • High X-ray attenuation • SPR	• Not biodegradable • Nanoparticles aggregation	• Drug delivery • Tumour therapy • Limitation of angiogenesis and tumour progression • Bio-imaging • Nanophotolysis technique • CT imaging contrast agent • Nano-vaccines	[59], [61]–[63], [66], [67], [206], [207]
	AgNPs	• Easy to synthesize • Antibacterial and antiviral activity • Anti-inflammatory and antitumor capacity • Antiangiogenic effects	• Toxic at higher concentrations • Various ecological problems if released into the environment	• Drug delivery • Antiviral and antibacterial activity (inhibition of bacterial biofilm formation and EPS production) • Antineoplastic effect	[68]–[71], [73]–[76]
Quantum Dots (QDs)		• Imaging properties • Capability to conjugate different biological molecules	• Toxic effect of metal core • Nanoparticles aggregation	• Drug delivery • Bio-imaging • Cancer diagnosis and treatment • Theranostic application • Photodynamic therapy • Biosensors	[77], [78], [82], [83], [85], [89], [91],

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				[92]
Carbon-based nanostructures (CBNs)	• Easy to synthesize and conjugate to biological molecules • Large surface area • Protect entrapped molecules	• Not biodegradable • Potential material toxicity • Poorly soluble in water	• Drug delivery • Bio-imaging • Cancer diagnosis and treatment • Tissue engineering • Photothermal therapy • Biosensors	[1], [94], [95], [99], [100], [104], [106], [107], [112], [114], [115]
Mesoporous Silica Nanoparticles (MSNs)	• High surface to volume ratio to conjugate with biological molecules • Stability • Easy control of morphology, pore distribution and size • Biocompatibility	• Not biodegradable • Potential cell lysis caused by silanol groups interacting with membrane lipids	• Bio-imaging • Drug delivery • Cancer treatment • Tissue engineering • Nano-vaccines	[117], [122], [125], [127], [131], [134], [136]
Liposomes and micelles	• Biocompatibility • Biodegradable • Amphiphilic • Longer duration of circulation	• Low solubility and stability • Tends to agglomerate • Some may be allergic • May trigger an immune response	• Drug delivery • Cancer treatment • Neurodegenerative disease treatment • Trojan Horse Liposome (THL) technology (E.g.. to cross BBB) • Nano-vaccines	[139], [142], [143], [145], [149], [150],

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				[156]
Dendrimers	• High drug loading ability • Low polydispersity, • Reproducible pharmacodynamics and pharmacokinetic behaviour • High cellular uptake • Capability to cross BBB	• Immunoreaction • Haematological toxicity • Toxicity for prokaryotic and eukaryotic cells	• Drug delivery • Cancer treatment • Antiviral and antibacterial activity • Tissue engineering	[160], [162], [166], [169], [171], [173], [174]
Polymer nanoparticles	• Biocompatibility • Biodegradable • Variety for chemical composition • Stability	• Inflammatory response • Nanoparticles aggregation depending on the polymer used	• Drug delivery • Cancer treatment • Tissue engineering • Nano-vaccines	[26], [196], [198]–[200], [202]–[204]

6. Nanoparticles design and application in cancer research

Although the use of nanoparticles founds application in many biomedical sectors, cancer research remains one of the more studied scientific community fields. Cancer is the second leading cause of death globally (second only to cardiovascular diseases) due to the difficulty to detect, diagnose and treat. The possibility to project NPs depending on the biomedical aim permits to overcome of standard therapeutic drug administration limitation.

Regardless of the material used, NPs can be functionalized by conjugating various therapeutic agents like drugs (i.e. doxorubicin), nucleic acids (i.e. siRNA) or biological molecules (i.e. inhibitors) to obtain a drug and gene delivery (Figure 4). Furthermore, the introduction of stimuli-responsive linkers allows a controlled release mechanism depending, for example, on redox state, pH or temperature [208]–[210]. It is well-known that tumour cells present a lower pH and higher redox state (due to the higher glutathione concentration) compared to healthy cells, and, in particular, each type of cancer cell presents a specific profile so that it is possible to design the appropriate NP better.

Moreover, it is also possible to cover NPs with specific tumour-targeting molecules (i.e. antibodies, aptamers, small molecules) to selectively address tumour cells or tumour

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mass in both *in vitro* and *in vivo* systems: this permits to overcome of chemotherapy limitations, reducing the side effects on normal cells. Furthermore, nanoparticles can be simultaneously linked to a fluorescence probe allowing the bioimaging detection of cancer mass and therefore tumour diagnosis. At the same time, some NPs like quantum dots present autofluorescence acting as tags with excellent sensitivity [211]. This represents a considerable advantage in cancer treatment because it permits the detection of a tumour mass in the early-stage or individuates all the metastasis in the body.

Another very significant NPs tumour application is the photothermal therapy adopted with gold NPs as previously discussed. The light absorbed by AuNPs is converted to heat confined around the particles resulting in eliminating a targeted cancer tissue [212].

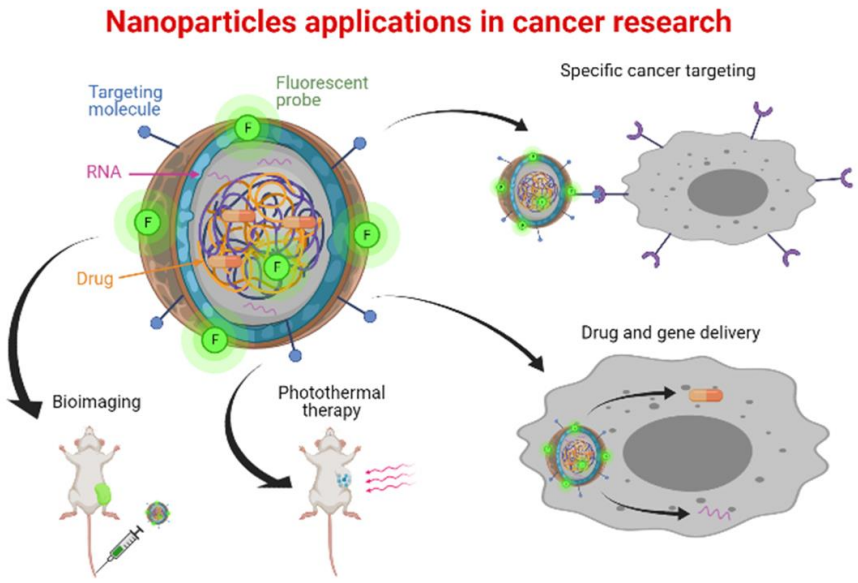

Figure 4. Nanoparticles (NPs) applications in cancer research. NPs can be functionalized by conjugating drugs or RNAs, fluorescent probes and/or targeting molecules for the appropriated cancer application: specific cancer targeting, drug and gene delivery, photothermal therapy and bioimaging.

7. Conclusions

With a particular focus on nanomedicine, nanotechnology is providing an entirely new concept and new approaches in vast fields of modern science and medicine. The small size of nanoparticles confers them unique properties because they are subject to physical laws in the middle between classical and quantum physics. In this context, the nanoparticles' project plays a key role because the material, size, shape, and functionalization need to be chosen and optimized to reach the desiderated aim, as suggested by this review. A NPs size-dependent cytotoxic effect was amply analysed as well as the influence of size in cellular uptake and cytotoxicity. On the other hand, the shape is deeply related to body distribution, blood lifespan, macrophage uptake, and membrane internalization.

Moreover, other features like biocompatibility, aggregation, and stability can be modulated by the synthesis processes and, especially, by the material adopted. Indeed, each type of NPs presents specific advantages and disadvantages (Table 1), which confer unique properties for specific biomedical applications. Furthermore, the possibility to conjugate them with a large number of different molecules permits to obtain a controlled

release mechanism (i.e., mediated by pH, temperature, redox state) and specific targeting, as adopted in cancer treatment to overcome the chemotherapy limitations[213]. In this manner, NPs can be employed as drug delivery systems (DDS) to act on malignant cells selectively, but also for diseases diagnosis, thanks to the capability to detect, for example, primary tumours, lymph nodes, and metastasis or to act as contrast agents in medical imaging techniques. Additionally, nanosystems are amply employed in tissue engineering and regenerative medicine to promote tissue differentiation thanks to the possibility of local delivery of bioactive molecules (i.e., growth factors). Finally, it is recently an object of interest by the scientific community to recruit nanotechnology in vaccine delivery (Table 1), as adopted for BNT162b2 mRNA Covid-19 Vaccine, consisting of lipid nanoparticle to deliver mRNA vaccine[214].

Taken together, these features make NPs the starting point for the future of nanomedicine, having a considerable impact on human health. The potential application sectors in which they can be involved are more than those reported in this review are. For example, these molecules could permit the development of personalized DDS by improving the patient's life quality or could be involved as nano-robots to make repairs at the cellular levels. Undoubtedly, intelligent multifunctional nanosystems will be the most promising candidates as vectors of biological molecules for a vast range of applications in nanomedicine.

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