Handbook of Functionalized Nanomaterials for Industrial Applications

Section 4 Functionalized nanomaterials for biomedical, pharmaceutical, agriculture and agri-food industry Section Functionalized nanomaterial and biology | (Bio)pharmaceuticals related processes using functionalized nanomaterials, nanotechnology.

CHAPTER 9

Biomedical Related Applications of Functionalized Nanomaterials

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Abstract

This book chapter provides an overview on the use of nanoparticles in the biomedical field. Nanomaterials have been a topic of research due to their unique properties, which have shown to be of crucial relevance towards the development of innovative technologies within the biomedical field. Nanoparticles (NPs) can be prepared from a wide diversity of materials and modified with a plethora of functional groups or ligands, allowing them to display specific properties and to be applied in a range of applications. In this book chapter, both the methods to functionalize nanoparticles and their applications in the biomedical field are overviewed. Metal-based, silica and carbon NPs, and their use in membranes, are described. Since drug delivery systems are the main application of functionalized nanoparticles, a specific section devoted to this topic is also presented.

Key Words

Nanoparticle; surface modification; drug delivery system; diagnosis; membrane preparation; carbon nanomaterials; silica-based nanoparticles; metal-based nanoparticles; nanotechnology

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Chapter starts here

18.1 Introduction

For many decades, nanomaterials have been a topic of research. This interest is due to their unique properties at the nanoscale, which make of nanoparticles (NPs) a promising tool for developing innovative bio-based technologies within the biomedical field. Accordingly, the number of scientific papers related to NPs in this field has been continuously increasing along the years (Figure 18.1), where NPs may be used in the design, process, action, delivery, and disposition of drugs. NPs can be prepared from a wide diversity of materials and modified with various functional groups or ligands, allowing them to display specific properties and to be applied in a plethora of applications.

Based on the exposed, the aim of this book chapter is to provide an overview on the use of NPs in the biomedical sector. Both developed methods to functionalize NPs and their applications are overviewed. This book chapter is useful to graduates, post-graduates, senior researchers, educators, and scientists working in biomedicine, pharmacy, biotechnology, nanotechnology and related areas.

'FIGURE 18.1 HERE'.

Figure 18.1 – Number of publication entries and total citations referring to keywords "functionalized nanoparticles and drugs" from 2006 to 2017 (search on the ISI Web of Knowledge, November 2018).

18. 2 Functionalized nanoparticles in (bio)pharmaceutical sector

Biopharmaceuticals are products derived from biological sources used as a therapeutic and medical solutions to treat diseases and pathological conditions, while pharmaceuticals can be considered the drugs produced from chemistry route [1]. According to the report by Mordor Intelligence, it is expected that the pharmaceutical market presents a compound annual growth rate (CAGR) of 8.5% from 2018 to 2023 reaching a market value of USD 341.16 billion in 2023 [2]. The growing of the biopharmaceuticals market results from the high efficacy and safety of these drugs, improving the quality of life and reducing the death rates in patients with chronic diseases

[2]. Although there are several studies focusing on the development of (bio)pharmaceuticals production processes (upstream and downstream processes) [1, 3, 4], an important challenge for (bio)pharmaceutical industry is to expand the use of these compounds. In this sense, Figure 18.2 summarizes the related applications of functionalized nanomaterials in biomedicinal and pharmaceutical areas. NPs and functionalized NPs have been used in fields such as drug- and gene-delivery, separation and purification of biological molecules and cells, biodetection of pathogens, detection of proteins, among others.

'FIGURE 18.2 HERE'

Figure 18.2 - Functionalized nanomaterials and their biomedical related applications. (Adapted from Robles-Garcia et al., 2016 [5]).

NPs have been used in different industrial applications [6], such as in separation and purification processes of bio-based therapeutics. For instance, Mesgari-Shadi *et al*. [7] used nanozeolite microspheres to purify scFv antibodies produced by *Escherichia coli* HB2151 cells, achieving a scFv purity of 90% with a purification yield of 60%. Magnetic NPs are other type of nanomaterials commonly used to extract/purify bioproducts, being this topic recently reviewed by Gadke *et al*. [8]. With a different goal, gold nanoparticles were functionalized with the papain enzyme to produce a heterogeneous biocatalyst, which has been used in bionalysis and biopharmaceutical analysis [9].

Amongst the several applications of functionalized materials in the biomedical field, one of the most relevant and investigated is their use as drug delivery systems, where NPs act as carriers and protect the specific drug from degradation and release it in the target tissue/cell, while improving the drug bioavailaibility [5]. It should be however remarked that the material used for the pharmaceuticals delivery has to be biocompatible and biodegradable, or at least totally eliminated from the body since it will have an intimate contact with biological systems and the immunogenic reaction has to be avoided [5, 10].

Several functionalized NPs have been as drug delivery systems. For example, carbon nanomaterials such as carbon nanotubes (CNT), carbon nanohorns (CNH) and graphene oxide (GO), and surface functionalized silica (silicon dioxide)- and silicon-based particles have been proposed for delivering pharmaceuticals through oral

administration [11]. More details on the preparation of these materials are given below. Carbon nanomaterials have been particularly investiagted for the delivery of anticancer drugs [12]. The major advantages of these systems comprise their easiness of fabrication, chemically inert properties and tailorable physicochemical properties [11, 13]. Metallic carrier systems have been also considered to deliver drugs. For example, Unamuno *et al*. [14] studied Fe and Zr-carboxylated Metal-Organic Frameworks (nanoMOFs) to encapsulate the aminoglycoside antibiotic Gentamicin, demonstrating that this system preserved the antibiotic characteristics under the intestinal conditions [14]. Other metal particles such as gold-NPs [15] and supramagnetic metal oxides (iron oxides: Fe_2O_3 or Fe_3O_4) [16] have o been studied for oral delivery applications.

Due to their great capacity for increasing photoelectric interactions at lower energy levels, functionalized gold-NPs were studied for radiosensitizing and imaging cancer cells. These materials are able to enhance the impacts of radiotherapy by increasing the energy deposition in tumor tissues [17]. Since drug delivery systems are the main application of functionalized NPs, we thus present a specific section devoted to this topic after the description of the NPs types and their synthesis.

18.3 Types and synthesis procedures of functionalized nanomaterials

18.3.1 Metal-based nanoparticles

Metal-based nanoparticles (metal-NPs) have attracted many scientists for over a century. This interest is due to their unique properties, which allow their wide applications in biomedical and pharmaceutical sciences, biotechnology and engineering. These metal-NPs can be used in magnetic separation, preconcentration of target analytes, targeted drug and gene delivery, and diagnostic imaging [18]. Nowadays, metal-NPs can be synthesized and modified with several chemical functional groups, allowing them to specifically bind to antibodies, ligands, drugs and other biomolecules of interest [18].

Metal-NPs are simply made through metals precursors and all stable metals can be used to synthesize nanoparticles [19]. However, some noble metal-NPs, such as silver and gold, have been attracting attention due to their unique properties and diversity of applications [20]. Although the most widely used nanoparticles are silver-NPs, due to their antimicrobial and antifungal properties, gold-NPs have also attracted an intensive interest since they can be easily functionalized with various targeting ligands [20]. In

pharmaceutical applications, it is highly desirable to functionalize the metal-NPs surface. It not only helps to maintain properties such as stability, adsorption characteristics, therapeutic efficacy and targeting ability, but it can also help in other challenges, such as in *in vivo* environment (such as reticulo-endothelial system) detection, adsorption of antibodies, cells, thiols and proteins, and cell uptake processes [21]. There are many methods involving surface functionalization of metal-NPs, such as PEGylation, thiol functionalization, Layer by Layer (LbL) assembly, coating with biomolecules, and silica coating [21].

PEGylation is the process of attaching metal-NPs surfaces with engrafted polymer chains, such as polyethylene glycol (PEG) [21]. This is a commonly applied method since it can customize the surface properties of metal-NPs allowing the targeting moieties to covalently bond to the free ends of tethered chains, making metal-NPs to specifically and firmly bind to receptors over the surface of the diseased cell [22,23]. After selection of the proper PEG, the next consideration is annealing it to the metal-NP surface. Both covalent and noncovalent approaches can be used. For solid NPs, such as gold, thiol binding is the classic approach where a sulfhydryl-capped PEG chain adheres to the gold surface [24].

Thiol functionalization involves the thiolate monolayers assembly on different bulk surfaces such as silver, gold or platinum [25]. The thiol monolayers are covalently linked to the surfaces by the reaction of the metal-NPs surfaces with the respective thiols and for some metals by the reaction with disulfides [25]. For gold-NPs surfaces, two different mechanisms for the thiol functionalization are applied [26, 27]. As the upper gold surface includes an oxide sublayer, its reduction by the thiol occurs and a gold-thiolate bond is established. On the other hand, hydrogen evolution by the gold-mediated reduction of the thiol protons could lead to the gold-thiolate bonding. The formation of the gold-thiolate bond in the presence of disulfide is a redox process, where the gold surface is oxidized by disulfide and the disulfide bond is cloven [25–27].

LbL method is the most widely used for metal-NPs functionalization due to the easy coating and good control over film thickness [21]. Alternate adsorption of cationic and/or anionic polyelectrolytes on the metal-NPs surfaces results in polyelectrolyte multilayers. Although most of the reported LbL films are driven by electrostatic interactions, other interactions such as hydrogen bonding are also used in LbL assembly [28]. Availability of various polyelectrolytes provides the option to vary the surface

charge of metal-NPs from very positive to negative that might play a key role in biological applications [29].

The metal-NPs functionalization with biomolecules is of crucial importance in developing biocompatible platforms with minimal toxicity for various (bio)pharmaceutical applications. Molecules such as folic acid, DNA, proteins, and oligonucleotides can be used in surface functionalization of metal-NPs [21]. For instance, there are three ways to functionalize gold-NPs with functional groups or biomolecules: (i) by binding with functional groups of self-assembled monolayer (SAMs), (ii) by direct deposition of gold colloid onto the electrode surface, and (iii) by co-modification of mixed gold colloid with other components in the composite electrode matrix [30]. Proteins or enzymes can readily be immobilized on colloid gold by dipping a protein solution onto the colloid gold modified electrode surface. The electrostatic interaction between the negatively charged citrate surface of colloidal gold and positively charged groups of the protein leads to the adsorption of protein onto the electrode surface. SAMs can provide a simple way to tailor surfaces with well-defined compositions, structures, and thickness, which can then be employed as specific functionalized surfaces for the immobilization of gold nanoparticles and enzymes [30]. Gold-NPs-modified electrode surfaces can be prepared by covalently binding gold-NPs with surface functional groups (-CN, $-NH_2$, or -SH) of SAMs modified solid surfaces [30]. Short-chain molecules, such as cysteamine (Cyst) and 3-mercaptopropionic acid (MPA), can be self-assembled on the gold disk electrode for further binding of gold-NPs.

Metal-NPs covered with silica have become increasingly important in the last decade for many promising catalytic and biomedical applications [31]. This type of material exhibits good water solubility, colloidal stability, as well as low level nonspecific binding with biological matrices and molecules. In the late 1960s, Stöber *et al.* developed the sol-gel chemistry of silicon alkoxides for growing monodisperse spherical silica nanoparticles in basic aqueous solutions containing different alcohols, such as methanol, ethanol, or isopropanol [32].

Based on the well-established Stöber method, gold colloids nanoparticles were developed by Liz-Marzán *et al*. [33]. The method includes the weak surface attachment of the bi-functional (3-aminopropyl)trimethoxysilane in aqueous solution. The $-NH_2$ groups are bound to the gold surface and $-Si(OEt)_3$ groups are extended outward for hydrolysis and condensation with sodium silicate to deposit a thin surface-protective silica layer, so as to be transferred into alcohols to form a stable water/alcohol solution of

gold-NPs [33]. Then, thicker silica shells can be grown on surface-stabilized gold-NPs by further hydrolysis/condensation of tetraethyl orthosilicate, a typical precursor of silicon alkoxides [33] (see Figure 18.3). By modifying the silica-coating with a variety of functional groups using silane and silane coupling agents, the intrinsic surface properties of silica-coated metal-NPs can be easily manipulated according to the intended application [21]. Prior to silica-coating, numerous surface-attachment strategies have been developed in aqueous solutions using bifunctional molecules by means of strong surface-coordination or electrostatic interaction onto metal-NPs for creating colloidally stable surface-protected NPs in alcoholic solutions. This surface-protected NPs interface needs to have reactive hydroxyl groups to facilitate the hydrolysis/condensation of tetraethyl orthosilicate [31].

'FIGURE 18.3 HERE'

Figure 18.3 - Scanning Electron Microscopy (SEM) of gold nanoparticles (core) covered with silica (shell) by the Stöber method.

18.3.2 Silica nanoparticles

Silica is a widely used material due to its excellent thermal and mechanical properties, being currently used in a wide variety of areas, such as paints and coatings, electrical and thermal insulation, moisture and flame retardants, catalysis, chromatography, cosmetics, pharmaceutical and food industry [34]. Moreover, there is a solid knowledge on silica and its derivatives regarding the preparation of silica colloids and nanoparticles of different sizes in the nanometric range [34]. The synthesis of silica is based on relatively simple methods that allow high purity samples with narrow size distributions to be obtained. Beyond these advantages, the raw materials low cost and the process of silica fabrication has boosted the use of silica NPS in several industrial applications [34]. Silica nanomaterials have been synthesized and functionalized in order to be applied in controlled release, purification and synthesis, coatings, catalysis, and sensing [35]. Due to the high tunability of silica NPs, they have been particularly engineered for drug delivery such as delivery of genes for gene therapy [35].

Silica NPs are usually obtained from alkoxysilanes through hydrolysis followed by condensation reactions involving oligomeric species, with tetraethoxysilane (TEOS) as one of the most used precursors. The most common method to prepare silica NPs, the so called Stöber method [32], allows the control of the particles dimensions down to spherical submicrometric silica particles by varying the reaction parameters. An example of the morphology of silica NPs is given in Figure 18.4. These particles have a narrow size distribution, with size ranging from tens to hundreds of nanometers [32]. This sol-gel method is based on the hydrolysis of tetra-alkyl silicate in a homogeneous alcoholic medium, using ammonia as a catalyst. Due to the existence of silanol (Si-OH) groups on the surface of the silica particles, their reactivity can be altered by thermal treatments and covalent bonds through these groups can be established [36].

'FIGURE 18.4 HERE'

Figure 18.4 - Scanning Electron Microscopy (SEM) of spherical silica particles obtained by the Stöber method.

With improved properties, mesoporous silica nanoparticles (MSN) have emerged in the last decades. These porous materials have high thermal and chemical stability, high hydrophilicity, enriched surface by silanol groups, easy surface modifications, high surface area, and tunable pore size and pore volume. These improved characteristics turn these materials suitable for drug delivery, transport of therapeutics and/or encapsulation of target molecules [37–40].

MSN can be synthesized by several methods, being one of them the sol-gel method. A common synthetic route to obtain MSN is a modified Stöber synthesis based on the use of templates that act as structure directing agents [41, 42]. The most usual are surfactants (such as cetyltrimethylammonium bromide (CTAB) and dodecyltrimethylammonium bromide (DTAB)) and/or micelle forming agents or polymers. The concentrations and compositions of silica sources, template-agents, temperature, and stirring conditions originate materials with different properties in terms of particle size, pore size, pore volume and shape [37]. The surfactant content constrains the particle morphology since it changes the hydrolysis of the alkoxide and the micellization of the surfactant [41, 43]. Vazquez *et al*. [41] studied the synthesis of several MSN that could be used as drug deliverable containers. These particles were produced by the sol-gel method with TEOS as the alkoxide precursor and CTAB as the surfactant. By

fixing the molar ratio of TEOS/EtOH to 1/20 and by varying the molar ratio of $H_2O/NH_3{\cdot}H_2O$/CTAB, the particle morphology changed from dispersed nanospheres to agglomerates [41]. No pore size changes were observed, and particles were obtained with pore diameters from 2.5-2.8 nm [41]. Although MSN size and shape have a large influence on the nanoparticle's behavior, the surface modification have an even more relevant impact.

One of the most common ways of obtaining functionalized silica materials is through reaction with alkoxy silanes. This type of functionalization allows not only to obtain functionalized materials, with different functional groups, but also to use these molecules as a bridge to connect to others. The silica surface can be chemically functionalized with silicon alkoxides, such as methyltriethoxysilane (Me-TES), 3-mercaptopropyltriethoxysilane (SH-PTES), 3-glycidoxypropyltriethoxysilane (Gly-PTES), 3-chloropropyltriethoxysilane (Cl-PTES), phenyltriethoxysilane (Ph-TES), 3-(2-(2-aminoethylamino)ethylamino)propyltrimethoxysilane ($NHNH_2$-PTMS) or amino propyl trimethoxysilane (NH_2-PTMS) [38, 42–45].

Regarding the functionalization of already synthetized silica materials, silica and the silane-coupling reagent are added to toluene and the mixture is heated and refluxed [46]. Depending on the size of the silica materials, the solid is collected by centrifugation or by filtration, and the precipitate washed with toluene and other solvents such as ethanol, water and methanol, and then dried at room temperature. Nevertheless, the composition of silica materials can be controlled by hydrolysis/condensation of the organosilane compounds or by co-condensation of silica precursors [46].

As mentioned above, in some cases, the functionalization with the alkoxi silane is an intermediate step, in which this molecule acts as a bridge for other molecules. MSN organo-functionalized with g-glycidoxypropyltrimethoxysilane (-Gly), phenyltriethoxysilane (-Ph) and 3-mercaptopropyltrimethoxysilane (-SH) were further functionalized with Protein A by its addition to a phosphate buffer (pH 7.4) dispersion with the previous prepared MSN [44]. An additional example is related to the preparation of SiO_2-SH/IDA-Ni^{2+}. In this case, after the synthesis of silica functionalized with thiol groups (SiO_2-SH) by a hydrothermal method, a second functionalization is performed with 3-glycidoxypropyltriethoxysilane-iminodiacetic acid (IDA), achieving SiO_2-SH/IDA, followed by a third step related to the chelation of Ni^{2+} [47]. (3-glycidyloxypropyl)trime-thoxysilane (GPTMS) was used as a bridge to link MSN and chitosan, loaded with doxorubicin hydrochloride (DOX) [48]. By an acid-catalyzed

amino-oxirane addition reaction, chitosan reacts with SiO_2-Gly [49]. Curcumin (CCM) amino-functionalized silica (CCM/SiO_2-NH_2) were prepared by ammonia-catalyzed hydrolysis of TEOS, in the presence of CCM, and then functionalized with 3-(aminopropyl)-triethoxysilane (APTES). This material was further functionalized with folate using water-soluble carbodiimides (EDC) and N-hydroxysuccinimide (NHS). The carboxyl groups of folic acid were activated with EDC in the presence of NHS and reacted with the amino groups of CCM/SiO_2−NH_2 by forming stable amide bonds, leading to CCM/SiO_2−FO [49].

Silica NPs can be also modified with enzyme molecules (glutamate dehydrogenase (GDH) and lactate dehydrogenase (LDH) [45]. According to the authors, after getting the amino functionalized silica with 3-(2-(2-aminoethylamino) ethylamino)propyltrimethoxysilane ($NHNH_2$-PTMS) the particles are treated with succinic anhydride in dimethylformamide solution, resulting in carboxylate modified particles. These nanoparticles were then treated with GDH or LDH in phosphate buffer solutions [45]. Anti-IgG was also immobilized on the surface of silica materials after the functionalization with methyltriethoxysilane (Me-TES), 3-mercaptopropyltriethoxysilane (SH-PTES), 3-glycidoxypropyltriethoxysilane (Gly-PTES), or 3-aminopropyltriethoxyilane (NH_2-PTES) [43].

Silica functionalization with ionic liquids (ILs) has been also reported, in which in an intermediate step the silica surface hydroxyl groups react with a functional alkoxi silane, namely 3-chloropropyltriethoxysilane [50]. SiO_2-Cl in then refluxed in toluene with the molecule that originates the cation of the IL: imidazole, 1-methylimidazole, and 2-ethyl-4-methylimidazole [50]. The obtained material is washed with toluene, ethanol, and methanol, and dried to obtain a supported IL silica material [51].

The LbL technique, as previously described, can be also used to functionalized MSN with polyelectrolytes (see Figure 18.5) given than polyelectrolytes are commonly used blocking materials in pH-responsive drug delivery. The number of layers of the polycation, i.e. polyallylamine hydrochloride (PAH), and of the polyanion, i.e. polystyrene sulfonate (PSS), onto the surface of MSN influence the release profiles of the target molecule [52].

'FIGURE 18.5 HERE'

Figure 18.5 – Scheme of Polyelectrolyte assembly on silica surface.

In summary, modified silica NPs have several applications in the biopharmaceutical and biomedical field, for which specific examples are given in Table 18.1.

Table 18.1 - Applications of modified silica nanoparticles in the biopharmaceutical and biomedical field.

Applications of modified silica nanoparticles	**Reference**
Stimuli-responsive drug delivery	[39]
Nucleic acid delivery	[53]
Drug delivery and biomedical applications	[54]
Molecular imaging	[55]
Biodistribution and toxicology Biomedical imaging	[56]
Cancer theranostics	[57]

18.3.3 Carbon nanomaterials

Carbon-based nanomaterials, including carbon nanotubes, nanohorns, fullerenes and graphene derivatives, have earned relevant attention in the scientific community due to their unique physico-chemical properties. These properties are highly promising in many biomedical-related fields. In particular, their low cytotoxicity, achieved when properly functionalized, along with the possibility to link multiple bioactive molecules, reinforces their potential in the biopharmaceutical field.

Graphene had become one of the most interesting focuses of research in the last decade. The single layer graphene possesses an extended honeycomb network - the basic building block of other important allotropes. Graphene can be stacked to form 3D graphite, rolled to form 1D carbon nanotubes (CNTs), wrapped to form 0D fullerenes, and shaped as conical nanotubes to form nanohorns, as depicted in Figure 18.6.

'FIGURE 18.6 HERE'

Figure 18.6. Examples of carbon allotropes used in biomedical applications.

Although all these carbon allotropes have been explored for biomedical applications, active research in this field is mostly devoted to the use of CNTs and graphene derivatives as drug delivery vehicles, biosensors, nanoprobes for biomedical imaging and, in certain circumstances, as nano-drugs by themselves [58–60].

As for any nanomaterial to be used in living organisms, water solubility and biocompatibility need to be achieved. Different carbon nanomaterials require different strategies of surface functionalization to make them soluble in an aqueous environment and compatible with cells and tissues. Regarding clinical applications, carbon-based materials confront with many challenges. Graphene and pristine CNTs are hydrophobic materials, thus easy to aggregate in aqueous medium including proteins, salts or other ions, which can lead to toxicity. This happens due to the screening electrostatic charges and non-specific interactions between charged carbon nanomaterials and proteins. Due to these reasons, carbon nanomaterials' surface normally requires chemical functionalization or modification to obtain the desired properties [61].

Surface functionalization of carbon nanomaterials may be carried out through covalent or noncovalent routes. Covalent functionalization involves chemical reactions and the formation of bonds at the surface of the carbon nanomaterials surface, while noncovalent functionalization exploits favorable interactions between the hydrophobic domains of an amphiphilic molecule and the surface of the nanomaterial.

Surface oxidation is the most common technique to functionalize carbon nanomaterials. Nitric acid is commonly used as oxidizing agent. During the thermal oxidation, oxygen groups such as carboxylic acids, phenols, anhydrides and lactones are formed at the surface of the carbon materials [62]. Further modification can be achieved by attaching hydrophilic polymers such as PEG to oxidized CNTs, yielding CNT-polymer conjugates stable in biological environments which have been used in both in vitro and in vivo applications [63]. Apart from PEG, there are also many other molecules that could be used for nanocarbons functionalization, namely folic acid, DNA, chitosan, poly(vinyl alcohol), polyethylenimine (PEI), sulfonic groups and polyacrylic acid [58].

Noncovalent surface functionalization occurs through supramolecular interactions between the pristine carbon nanomaterials and the coating molecules/polymers, which impart minimum structural damage and disturbance to the intrinsic properties of the functionalized materials. Carbon nanomaterials are known to noncovalently interact with various molecules through weak interactions, such as π–π stacking interactions, electrostatic interactions, hydrogen bonding, and van der Waals forces [64–66]. Such

noncovalent methods increase water miscibility, reducing their toxic effect. However, there are some disadvantages related to the noncovalent functionalization, namely the vulnerability to the external environment. Many biomolecules, polymers, and surfactants have been used for the noncovalent functionalization of carbon nanomaterials to obtain a better biocompatibility. For instance, interactions between proteins and CNTs may occur by π–π stacking between CNTs and aromatic residues (Trp, Phe, Tyr) of proteins, enhancing their adsorptivity and biocompatibility, rendering them as less toxic compared to pristine CNTs [67, 68]. CNT-protein bioconjugates have been applied in biosensor fabrication, drug delivery, and cancer therapy. Some of the proteins that have been immobilized onto CNTs through covalent linkages include chrymotrypsin, ferritin, fibrinogen, hemoglobin, and streptavidin. Several researchers have reported the covalent immobilization of proteins onto CNTs using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) as the crosslinking agent [62, 69]. CNTs functionalized with DNA have actually been shown to enhance stability. DNA can bind to single-walled CNTs, forming tight helices around them, or can form noncovalent conjugates with CNTs [70]. DNA-functionalized CNTs can be used as biological transporters and also as biosensors [70].

The hydrophobic interactions, π–π stacking and electrostatic interactions between carbon-based nanomaterials and drugs can be used for efficient drug/gene loading. Based on this concept, modified carbon nanomaterials are used to deliver drug/gene to improve its therapeutic effect and reduce its severe adverse effect [71]. CNTs and graphene-based materials possess high photo-thermal conversion coefficient, being suitable for photothermal therapy. In addition, photosensitizers can be loaded onto the surface of carbon-based materials allowing them for being used in photodynamic therapy (PDT) [72].

18.4 Immobilization of functionalized nanomaterials in membranes

The immobilization, deposition or encapsulation of functionalized nanomaterials in supports has many advantages, such as an easy material recovery from the reaction media, low leaching, enhanced mechanical strength and prolongated reusability. An ideal support should be stable during the application, offer a high specific surface area and a strong adherence for the material NPs, as well as a high affinity towards target substances (e.g., pharmaceuticals). The use of porous membranes offers also the scale-up of the

process in continuous operation and an easy regeneration procedure by simple cleaning with solvents (e.g., hot distilled water) [73, 74].

The successful application of membranes in biopharmaceutical-related processes depends on several membrane properties, in particular a desired pore size and a narrow pore size distribution, which control the molecular transport; a high porosity and low membrane thickness, which favor a high permeate flux; a certain mechanical strength; and sufficient thermal and chemical stability. Furthermore, biocompatibility and resistance to biofouling are utmost *in vivo* applications to avoid the immunological response and loss of functionality, respectively [75]. A membrane acts typically as a physical barrier separating two different phases, but it may also be applied to immobilize enzymes [62, 76–78], liposomes [79–81], microspheres [82, 83] and NPs [84–86]. Inorganic membranes provide high chemical resistance, but they are expensive, less available and with limited mass transfer. On the other hand, polymer membranes are cheaper and more suitable to be prepared with different shapes (flat-sheet, fibers, beads) and a wide variety of functionalities.

The immobilization of NPs in polymer membranes can be achieved by several techniques (Figure 18.7), which are divided in three main categories: (i) chemical methods, where covalent bonds are established between NPs and the polymer matrix, (ii) physical methods, where weaker and non-covalent interactions take place, and (iii) entrapment, where particles are retained or entrapped in the membrane pores [75]. The porous structure and the surface hydrophilicity of the membrane can be tuned by adding different amounts of these nanomaterials, as well as their dispersion, interaction and distribution in the polymer chains that can define the overall performance and stability. More typical polymers such as PEG, poly(lactic-co-glycolic acid) (PLGA), poly(vinyl-2-pyrrolidone) (PVP), polyvinylalcohol (PVA), cellulose, alginate and chitosan have been investigated, as well as other less used polymers such as poly(vinylidene fluoride) (PVDF), polypropylene (PP), polyamide (PA), polyamidoamine (PAMAM), polyacrylonitrile (PAN), polyethylene (PE), PEI and polyethersulfone (PES). These membranes were studied in drug- and gene-delivery, therapy of cancer or other diseases, detection of proteins, biosensing and immunoisolation [84, 86–91]. Some examples are described in Table 18.2.

‘FIGURE 18.7 HERE’

Figure 18.7 – Scheme of the different techniques to immobilize nanoparticles in membranes.

Table 18.2 – Nanoparticle and polymers used in biopharmaceutical and biomedical processes.

Nanoparticle type	**Polymer type**	**Application**	**Ref.**
Nano-MnO_2	Chitosan	Immunosensor for CEA*	[84]
Nano-CeO_2	PLGA-PEG	Cerebral ischemic therapy	[86]
CNT / graphene	Chitosan	Detection of EBNA-1	[87]
Gold	PEG	Delivery of anticancer drug	[88]
Graphene oxide	PEG-PEI	Gene delivery	[89]
Platinum	PLGA-PEG	Delivery of cisplatin	[90]
SiO_2	PAMAM	Drug delivery	[91]

*CEA: carcinoembryonic antigen; EBNA-1: Epstein Barr virus nuclear antigen 1.

Regarding the chemical method, nanomaterials can be linked by covalent bindings to specific functional groups (e.g., –COOH, –CHO, –OH, –CN and $–NH_2$), which are created in the membrane by different pre-treatments. Some of these functional groups include epoxies, anhydrides, aldehydes, carboxylic, amides and amines. The chemical methods of preparation generally allow a strong and stable immobilization of NPs, which is usually carried out by immersion of the membrane in the corresponding solution containing the NPs or even, by filtrating this solution through the membrane [74]. In certain cases, an external energy source like UV irradiation can be used to start the binding reaction [92]. In general, NPs covalently bonded to membranes possess good resistance to variations in pH, ionic strength, temperature, good reusability and low leaching.

In the physical method, NPs are linked to the membrane by different non-covalent interactions, such as Van der Waals, H-bonding, and hydrophobic-hydrophilic or electrostatic interactions. In general, these methods lead to weaker interactions than those stablished by chemical methods and, consequently, a larger leaching is expected. Adsorption is one of the most simple and common mechanisms used to non-covalently

bind NPs to a membrane [74, 93]. The surface chemistry of the nanomaterial plays an important role in this aspect, because NPs with different point of zero charge (pH_{PZC}) lead to more intense interactions in the membrane due to the formation of electrostatic interactions. Furthermore, the charge of the membrane can be negatively (e.g. carboxyl groups) or positively (e.g. protonated amino groups) charged depending on the isoelectric point of the polymer and the pH of the media.

In the entrapment category, NPs are not bound but entrapped or retained in membranes. This approach does not require any modification of the surface chemistry of both NPs and membranes, and the immobilization can be achieved following two different strategies: (i) NPs are incorporated into the membrane during the fabrication step and (ii) a solution containing NPs is filtered through the membrane with the NPs being retained in the pores [75]. The first method possesses the advantage of a low leaching, although the activity of the resulting membrane should be lower because some NPs cannot be accessible to target substances. On the contrary, the second approach allows to obtain membranes with a higher activity but higher leaching.

18.5 Functionalized nanoparticles as drug delivery systems

Amongst the several applications of NPs in the biopharmaceutical or biomedical field, their use as drug delivery systems has been one of the most explored, and therefore is here discussed in more detail. The increasing need for more efficient and less invasive methods to treat diseases is stimulating the development of modified NPs to be applied in drug delivery systems (DDS). In fact, in terms of health care market, nanotechnology for developing DDS is evaluated to be around $300 billion [94]. The main challenge in the development of DDS using NPs is to achieve a perfect biological activity, with high stability while being able to maintain the drug levels in the body and, at the same time, with minimized side effects [95]. In the pharmaceutical sector, this type of systems is considered as a nanopharmaceutical or as nanomedicine [96]. The NPs used can be from either organic or inorganic materials, with their sizes between 1 and 100 nm [97].

Drug administration routes can be oral, parenteral, transdermal, inhalational, subcutaneous injection, among others [98]. Accordingly, drugs must be loaded into NPs without leakage or catabolism by enzymes to recognize and access the specific target tissue/cell [98]. The functionalization of NPs by targeting ligands (drugs, biomolecules and other chemical moieties) able to identify a specific biological target is indeed

possible, promoting the drug delivery to a specific type of cells [96]. Modified NPs usually offer better transport properties and pharmacokinetic profiles and can penetrate deeper into tissues through fine capillaries and epithelial lining, resulting in a more efficient delivery of therapeutic agents to target sites [99].

Comparing to traditional pharmaceuticals, formulations and administration routes, DDS carrying the active pharmaceutical ingredient (API) offer notable advantages, including: (i) improved delivery of drugs that are poorly soluble in water; (ii) delivery of a high dose of the therapeutic agent; (iii) improved protection of a drug from harsh environments; (iv) decrease of dosing regularity; (v) controlled and precise release of drug; and (vi) prevention of side effects if a proper DDS is used [95, 96]. However, some disadvantages can be also found, such as material's toxicity, products degradation products, drug rapid release and high cost [95]. All these advantages and disadvantages depend on the material type and functionalization. Thus, there is an urgent need on the search of improved (bio)materials to be used as DDS, and that can provide a more efficient loading and controlled release of APIs [96].

Recently, the design of surface modification for advanced DDS has allowed new treatment strategies [11]. There are a number of carriers, including organic based, inorganic based, or a hybrid combination of the organic and inorganic compounds that can be used to produce a DDS. Inorganic nanoplatforms include metallic nanostructures, silica nanoparticles, and quantum dots, whilst organic nanocarriers includes polymeric, lipid-based (e.g., liposomes and nanoemulsions), dendrimers and carbon-based materials. Hybrid combination can be exemplified by colloidal gold encapsulated in liposomes or superparamagnetic iron oxide particles encapsulated in polymeric nanoparticles [100].

Regarding the optimal ligand design, surface properties must be controlled for a precise material three-dimensional structure and chemical composition of specific functional groups that can interact with the API. The most used method of nanoparticles modification is though the attachment of PEG, minimizing the risks of opsonization and the immunological barriers (phagocytic) of NPs [20]. Hydrophilic coatings, such as PEG, can also reduce interactions with plasma proteins [24] and reduce agglomeration of the nanoparticles, avoiding a higher cytotoxicity [101, 102]. Other types of ligands described in Table 18.3.

Examples of most common functionalized nanoparticles as drug carriers and their applications are present in Table 18.3. For more information about the incorporation of nanoparticles into drug delivery applications, current databases such as the Nanomaterial

Registry (https://www.nanomaterialregistry.com/) and CaNanoLab (https://cananolab.nci.nih.gov/) can be easily accessed and are recommended. A general scheme on the properties and characteristics of modified nanoparticles as drug delivery systems is presented in Figure 18.8.

Table 18.3 - Examples of functionalized nanoparticles and their applications as drug delivery systems.

Material	**Functionalization**	**Application**	**Reference**
Chitosan	DNA	Peanut allergy vaccine	[103]
Dextran	N, N′-carbonyldiimidazole	Inflammatory	[104]
Magnetite	Anthracendion derivative mitoxantrone	Tumor angiogenesis	[105]
Magnetite	Poly(ethylene glycol)	Breast cancer	[106]
Silica	2-devinyl-2-(1-hexyloxyethyl) pyropheophorbide	Cancer	[107]
Mesoporous Silica	Folic acid*; mannose; glycerol-derived polyol-based silanes, orthosilicic acid, sodium metasilicate, tetraethyl orthosilicate (TEOS); tetramethoxysilane (TMOS); tetrakis (2-hydroxyethyl) orthosilicate.	Cancer	[108]
Gold	Amino acids and peptides	Gene delivery vector	[109]
Gold	Amine	Prostate carcinoma	[110]
Gold	Peptide	Adjuvants for vaccine delivery	[111]
CNT**	Adsorption of phospholipid with poly(ethylene glycol) (PL-PEG 2000) chains and terminal amine or maleimide	Intracellular delivery of siRNA	[112]

	groups (PL-PEG-NH_2 or PL-PEG-maleimide		
CNT	Polyethylenimine-grafted	Delivery of DNA	[113]
Graphene	Peptide-silica coated	Glioma therapy	[114]

*Review article: some examples of surface ligand are given; **CNT: carbon nanotubes.

'FIGURE 18.8 HERE'

Figure 18.8 - General scheme highlighting the properties, characteristics and way of action of modified nanoparticles to be used as drug delivery systems.

Conclusions and future perspectives

NPs represent a bridge from materials to the (bio)pharmaceutical and medicinal fields, where they have a remarkable role e.g. as therapeutic carriers and diagnostic tools. Several materials have been investigated, together with a range of functionalization methods and functional groups. In this book chapter we briefly described the several materials used, type of functionalization and preparation, and the related biomedical applications of these NPs. The design of nanostructures by controlling their surface properties is the main strategy to achieve improved response of each type of application. We briefly described the synthesis and functionalization of metal-based, silica, and carbon NPs that have been mainly used in diagnosis, biosensing and bioimaging, and as drug delivery systems. The work that has been conducted in this field so far has provided a better understanding of the NPs relevance and has brought significant contributions to the biomedical field. However, there is still the need of finding materials with improved efficacy and low cytotoxicity, where bio-based materials and bio-inspired functional groups may play a significant role. Stimuli-responsive NPs, responding to stimuli such as temperature, pH and light, aiming at improving drug release and diagnosis performance must be also deeper investigated.

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