Hybrid Multifunctional Nanomaterials for Diagnostic and Therapeutic Applications

Nefeli Lagopati, Efstathios P. Efstathopoulos, Dimitris Veroutis, Hector Katifelis, George Theocharous, Pavlos Pantelis, Konstantinos Evangelou, Vassilis G. Gorgoulis, and Maria Gazouli

N. Lagopati · D. Veroutis
Molecular Carcinogenesis Group, Department of Histology-Embryology, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece

Biomedical Research Foundation of the Academy of Athens, Athens, Greece

E. P. Efstathopoulos
Department of Radiology, Medical School, National and Kapodistrian University of Athens, Athens, Greece

H. Katifelis
Department of Biology, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece

G. Theocharous · P. Pantelis · K. Evangelou
Molecular Carcinogenesis Group, Department of Histology-Embryology, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece

V. G. Gorgoulis
Molecular Carcinogenesis Group, Department of Histology-Embryology, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece

Biomedical Research Foundation of the Academy of Athens, Athens, Greece

Division of Cancer Sciences, Faculty of Biology, Medicine and Health, Manchester Academic Health Science Centre, Manchester Cancer Research Centre, NIHR Manchester Biomedical Research Centre, University of Manchester, Manchester, UK

Center for New Biotechnologies and Precision Medicine, Medical School, National and Kapodistrian University of Athens, Athens, Greece

Faculty of Health and Medical Sciences, University of Surrey, Surrey, UK

M. Gazouli (✉)
Department of Radiology, Medical School, National and Kapodistrian University of Athens, Athens, Greece

Department of Biology, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece
e-mail: mgazouli@med.uoa.gr

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1 Introduction

The development of nanoscience and molecular biology, in the last decades, inevitably led to spectacular progress in the bio-nano interface. This scientific evolution is also obvious in material science. Thus, great effort has been devoted to the exploration of the possible clinical applications of the innovative nanotechnology achievements [1, 2], particularly in the detection, diagnosis, and treatment of various diseases [3].

A variety of nanomaterials with interesting properties is now available, and a continuously increasing number of new nanomaterials is reported, with sizes that range from a few to several hundred nanometers [4]. Some of the nanoscale materials have been proven promising in biomedical applications, for a wide range of purposes, such as for drug delivery, cancer treatment, and diagnosis [5], since they allow high pharmaceutical loadings, controlled size, charge, crystallinity [6–8], release manipulation [9], biocompatibility improvement, and precise targeting and accumulation in the area of interest [10]. Thus, these materials present numerous opportunities, improving particularly the current anticancer approach and minimizing the undesirable side effects of the conventional diagnosis and treatment.

A hybridism in nanomaterials synthesis is consequently observed. Hence, the previous well-known categorization of two classes of nanoparticles, the organic and the inorganic ones, has enriched with a third category, the so-called hybrid nanomaterials [11]. Hybrid nanomaterials are designed with both inorganic and organic components. Various strategies can be applied to prepare hybrid nanomaterials. Moreover, many combinations of different materials can be chosen to develop hybrid complexes [12]. For instance, gold nanoparticles can be mixed with natural polysaccharides, or metal oxides with polymers, leading to advanced composites with specific properties. Actually, researchers often choose to mimic complex natural materials, while sometimes they create artificial materials with hybrid composition and structure [13].

Recently, hybrid materials have attracted significant attention, since not only they retain the beneficial properties of all their ingredients, but also, they show additional synergistic performance, such as target specificity or biodegradability, improving the outcome of many biomedical procedures [14]. Therefore, it is reasonable that these nanomaterials are exploited in various applications, particularly in nanomedicine.

Hybrid nanomaterials can be utilized in diagnostics, as imaging and contrast agents, enabling precise visualization at a molecular level, exploiting the potential of a various novel or multimodal imaging techniques, and reducing any adverse effect in healthy tissues. Active targeting enhancements have been incorporated into the nanoparticles to create multifunctional formulations [5]. These materials allow the improvement of the implementation of well-known effective imaging techniques, such as MRI (magnetic resonance imaging), PET (positron emission tomography), CT (computed tomography), etc. [15, 16].

Hybrid nanomaterials can also be used in therapeutic applications and modalities, particularly in anticancer therapy, such as photodynamic and photothermal therapy, hyperthermia and radiotherapy, neutron capture therapy, magnetic therapeutic approaches, drug delivery, and gene therapies [17].

Furthermore, hybrid multifunctional nanomaterials can be incorporated with several components that can be used for simultaneous diagnostics and therapeutics (theranostics), allowing real-time monitoring of an efficient treatment process, leading to more personalized healthcare systems [18]. The new field of theranostics is referred to as a treatment strategy that monitors the response to treatment, increasing the efficacy of a drug and permitting the concurrent detection of the target area throughout the treatment process [19]. Limitations and safety issues are still under consideration before the clinical routine finally incorporates nanotheranostics.

Thus, the synthesis of advanced hybrid nanomaterials for targeted and on-demand theranostics can be considered a state-of-the-art topic in nanomedicine, and for this reason, these applications will be discussed in this chapter.

2 Nanomaterials

Engineered nanostructured materials are among the most impressive materials and unveil distinctive chemical, physical, and/or biological characteristics. Various nanostructured materials (NSMs) have been developed, in the last decades, so the need for their classification was early presented [4]. Several attempts of categorization have been reported. The most systematic classification was achieved by Pokropivny and Skorokhod [20] that was based mainly on the number of dimensions of a nanomaterial that are not considered on nanoscale. Thus, according to this scheme, there are zero-dimensional (0-D) (e.g., nanoparticles, nanocapsules nanospheres, composite nanoparticles, etc.), one-dimensional (1-D) (e.g., nanotubes, nanowires, etc.), two-dimensional (2-D) (e.g., nanoplates, nanobelts, graphene, etc.), and three-dimensional (3-D) nanomaterials (3-D electrodes, fullerene, etc.) (Fig. 1).

The group of nanoparticles is a very important class of nanomaterials, possessing specific properties that differentiate them from their bulk materials. Among the most characteristic properties, it is worth mentioning their controlled particle size, stability, bioactivity, bioavailability, and the fact that they include large active surfaces and easily controllable surface chemistry that allows binding to small molecular drugs, imaging labels, and several ligands [21, 22] (e.g., antibodies, peptides, and nucleic acids). Their interesting characteristics make them the materials of choice for various applications, particularly in biomedicine. Their small size allows for exclusive intracellular and extracellular interactions, such as extravasation via endothelial cells and increased permeability and retention in tumor tissues. Due to their capacity to enter cells, tissues, and organs than macro particles so, conquer the poor bio-accessibility and high toxicity of present pharmaceutics, their potential for medication delivery can be considered as incredible [23].

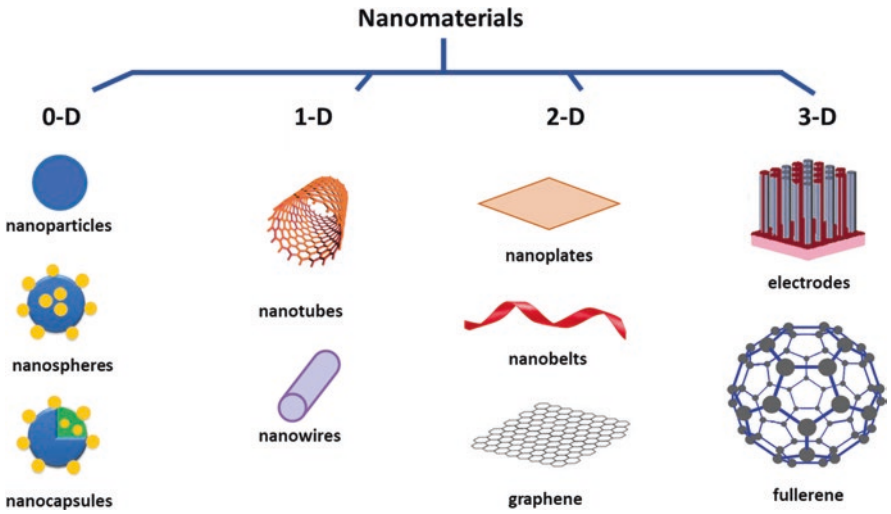

Fig. 1 The common classification of the nanomaterials is mainly based on the number of dimensions of a nanomaterial that are not considered in nanoscale

Thus, focusing on the category of 0-D nanomaterials and particularly on nanoparticles, two main classes can be traditionally detected: the inorganic (e.g., metallic and semiconductor nanoparticles, quantum dots, magnetic nanoparticles, etc.) and the organic nanoparticles (e.g., liposomes, dendrimers, micelles, etc.) [24]. The inorganic nanoparticles typically are biocompatible and less cytotoxic than organic ones. They provide novel electrical and optical characteristics which can be adjusted during assembling [4]. Gold, silver, iron oxide, silica, and titanium dioxide are among the characteristic nanoparticles of this group. The organic nanoparticles are commonly used as nanocarriers, loading several types of pharmaceuticals. There is also one more class, that of hybrid nanoparticles which is a mixture of nanoparticles from the two previously mentioned categories (Fig. 2).

2.1 Hybrid Nanomaterials

The progressing demand for innovative materials with tailored physicochemical properties has led to the development of hybrid materials with remarkable properties and multifunctional nature [25]. Hybrid nanostructured materials (HNMs) are formed by two or more components of nanoscale dimensions. HNMs are also defined as a mixture of organic and inorganic compounds. These materials can be either homogeneous or heterogeneous systems including domains of each organic or inorganic component that range from a few angstroms to a few tens of nanometers [26]. The nature, structure, and contents of the organic and inorganic components of a hybrid nanomaterial affect its properties and determine its behavior.

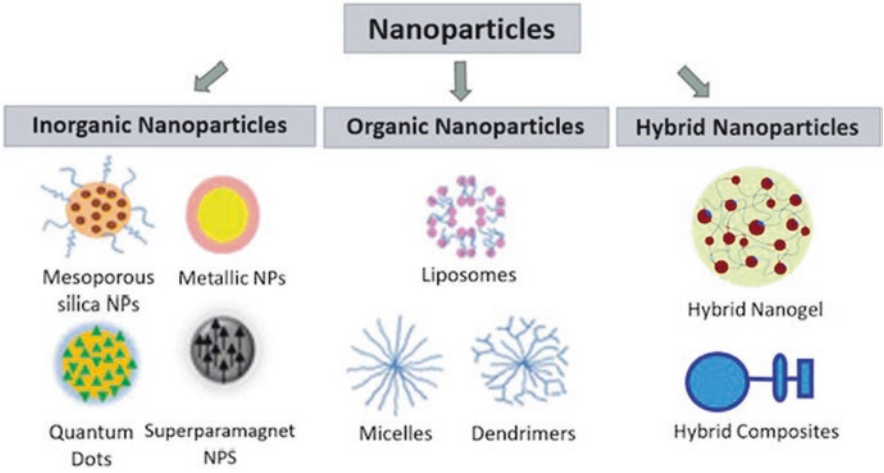

Fig. 2 The main categories of nanoparticles are three, the inorganic (e.g., metallic nanoparticles, quantum dots, magnetic nanoparticles, etc.), the organic nanoparticles (e.g., liposomes, dendrimers, micelles, etc.), and the hybrid nanoparticles (a mixture of organic and inorganic components)

Various studies suggest several strategies for the preparation of hybrid nanomaterials. Moreover, many combinations of different materials can be chosen to develop hybrid ones [12]. Metal oxides are commonly mixed with natural polysaccharides or polymers. Also, liposomes can encapsulate many types of nanoparticles. The combination of different nanomaterials leads to the development of advanced composites with specific properties [4, 21]. Recently, based on the physicochemical properties of hybrid materials, they have attracted significant attention, since not only they retain the beneficial intrinsic properties of all their individual ingredients, but also, they show additional synergistic performance [25]. Hence, the properties of the HNMs can be tuned by modifying their structure and morphology leading to advanced materials with improved performance characteristics, such as target specificity or biodegradability, electron conductivity, high thermal stability, mechanical strength, etc. improving the outcome of many biomedical procedures [14, 27]. The wide spectrum of their accessible properties allows the exploitation in significantly diverse fields (microelectronics, optics, energy, environment, catalysis, etc.) [28]. It is reasonable that hybridism in material science is considered as a burgeoning and highly interdisciplinary research field. Consequently, it is logical that these nanomaterials can be exploited in various biomedical applications, in the frame of nanomedicine.

2.2 Hybrid Multifunctional Nanomaterials

Mono-functional nanoparticles typically provide a single function, while multifunctional nanoparticles can participate in a series of different processes. The functionality of a nanoparticle can be modulated by several strategies, such as surface modification, to fit some specific and targeted applications [29]. Stabilization of the nanoparticles in a solution for the development of a controlled growth system; incorporation of functional groups at the surface for enhanced performance or additional capabilities; improvement of the solubilization in various solvents to extend the application spectrum; changes in optical, spectroscopic, mechanical, chemical, optical, and magnetic properties; and modification of the toxicity are among the main goals of a functionality modification [24, 29]. Thus, multifunctional nanomaterials have gradually attracted scientific interest due to their ability to combine numerous properties [29]. Multifunctional nanomaterials are smart and highly functional materials which gather all the desirable characteristics of their components and sometimes possess some modified properties, quite different from those of the individual conventional materials [30]. Thus, multifunctionality gives them the ability to perform various goals such as co-delivery of contrast agents, targeted treatment, and surface modification in order to attach different ligands.

Great achievements are reported in biomedicine, regarding diagnosis, treatment, and prevention. However, worldwide, the highly prevalent diseases (cardiovascular diseases, cancer disease, neurodegenerative diseases, etc.) are still leading death causes or quality of life reduction factors [31]. Therefore, the development of new nanotechnological tools for relevant application in biomedicine is still an ongoing research goal addressed by different methodological approaches. In this context, the specific targeting of disease-related pathways and molecules, mostly proteins, is a promising strategy [32].

Hybrid nanomaterials can be utilized in diagnostics, as imaging and contrast agents, enabling accurate visualization at a molecular level, and exploiting the potential of several innovative or multimodal imaging techniques. In parallel, these materials can reduce any adverse effects on healthy tissues [33]. The main advantage is that they can allow the improvement of the implementation of well-known effective imaging techniques. Hybrid nanomaterials can also be used in therapeutic approaches, or palliative treatment, particularly in anticancer therapy [17]. Hybrid nanomaterials can also be used for the prevention of various diseases through their use in nanovaccines or point-of-care applications, such as rapid diagnostic tests and lab-on-chip approaches [34]. Furthermore, hybrid nanomaterials are used in tissue engineering for the development of scaffolds. Hybrid multifunctional nanomaterials can be used for simultaneous diagnostics and therapeutics (theranostics), allowing real-time monitoring of an efficient treatment process, leading to more personalized healthcare systems [18]. Hence, there is a great variety of applications of hybrid multifunctional nanomaterials in biomedicine (Fig. 3).

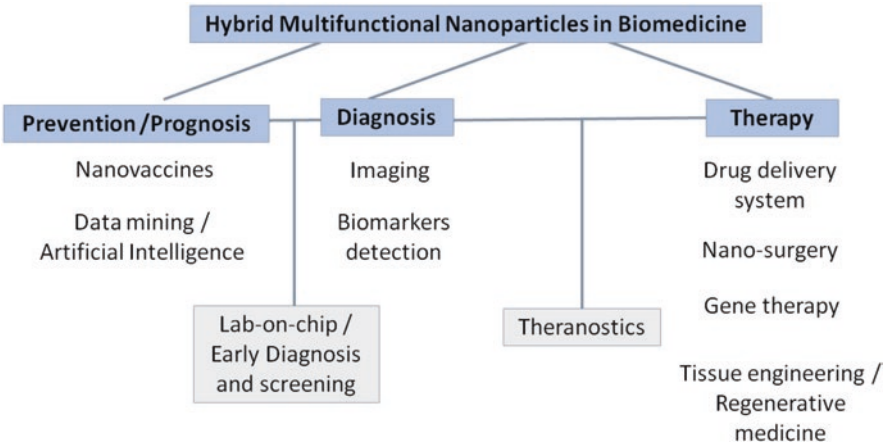

Fig. 3 The applications of hybrid multifunctional nanomaterials in biomedicine

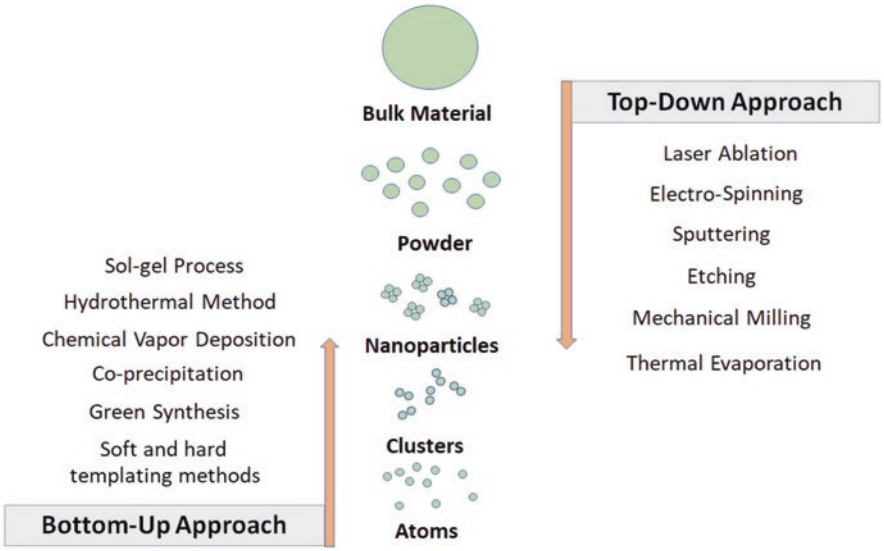

Fig. 4 Top-down and bottom-up synthesis methods for nanomaterials synthesis

3 Synthesis of Nanomaterials

Top-down and bottom-up approaches are used for the synthesis process of nanomaterials [35]. Figure 4 shows the most common methods of synthesis.

3.1 Top-Down Methods

Applying top-down methods, bulk materials can be transformed into small nanoparticles. These techniques are quite simple to use, but are not efficient for applications with advanced requirements, regarding difficult geometric shapes and very small sizes of nanoparticles [4]. Laser ablation, electrospinning, sputtering, etching, mechanical milling, and thermal evaporation are among the most common top-down methods of synthesis [4, 35].

3.1.1 Laser Ablation

Laser ablation is a straightforward process of nanoparticle synthesis from various solvents [36]. A pulsed laser is used to remove molecules from a surface, creating micro- and nanostructures. Ablation occurs when the surface area absorbs enough energy to melt or evaporate [37]. This method has numerous applications in ceramics, glasses, metals, as well as polymers.

3.1.2 Electrospinning

This is a very simple method, generally used for nanofiber production. Coaxial electrospinning is among the most famous techniques. The required equipment includes two coaxial capillaries including viscous liquids. It is also common to use a viscous liquid as the shell and a non-viscous liquid as the core, if the producing material is core-shell structured [38].

3.1.3 Sputtering

During sputtering, high-energy particles, such as plasma or gas, target solid surfaces. The sputtering gas is added to an evacuated chamber, and a high voltage is applied, and free electrons-gas collisions lead to the production of gas ions. The positively charged ions continuously hit, due to the acceleration in the electric field of the cathode. This fact results in the ejection of atoms from the target area. Thin films are usually produced through this method [39].

3.1.4 Etching (Lithography)

Two main types of lithography are distinguished, masked and maskless lithography. Masked lithography applies a mask or template, and nanopatterns are transferred over a surface area. Photolithography, nanoimprint lithography, and soft lithography are among the most famous processes. Maskless lithography-free nanopatterns are

created without the use of a template. Scanning probe lithography, electron beam lithography, and focused ion beam lithography are the most common methods of maskless lithography [40].

3.1.5 Mechanical Milling

Mechanical milling allows the production of nanoparticles by attrition. Kinetic energy from the grinding medium is transferred to the material that is reduced. Metal alloys and various nanoparticles can be produced via this method [41].

3.1.6 Thermal Evaporation

Thermal evaporation is an endothermic method. During this process, heat causes chemical breakdown. Stable monodisperse suspensions, several inorganic nanoparticles, and thin films are often produced through this method [42].

3.2 Bottom-Up Methods

The bottom-up approach is based on the exact opposite fundamentals, compared to the top-down approach. Atoms and molecules are used as structural units, and through the growth and self-assembly of them, chemical development of nanomaterials is achievable. Hence, it is considered as a constructive approach [43]. Sol-gel process, hydrothermal method, chemical vapor deposition, co-precipitation, and green synthesis soft and hard templating methods are among the most famous bottom-up approaches.

3.2.1 Sol-Gel Process

Sol-gel method is a simple wet chemical technique and, for this reason, is widely applied. The term sol-gel is derived from the combination of sol and gel. Sol is a colloidal solution that is produced when solid particles are suspended in a liquid medium. Gel is a solid macromolecule which dissolves in a liquid. During this method, the liquid precursor is transformed into a sol [44]. The sol is finally converted into a jelly network structure that is called gel. Actually, sol-gel method is a five-step procedure. Hydrolysis, polycondensation, aging, drying, and calcination are the aforementioned steps. A great variety of nanomaterials, including thin films, nanoparticles, glasses, and ceramics, can be produced through this method.

3.2.2 Hydrothermal Method

The hydrothermal process is a well-known and extensively used method to produce nanomaterials. Nanostructured materials are attained through a heterogeneous reaction carried out in an aqueous medium at high pressure and temperature around the critical point in a sealed vessel. Nanowires, nanorods, nanosheets, and nanospheres are usually produced hydrothermally [38].

3.2.3 Chemical Vapor Deposition

Carbon-based nanomaterials are commonly developed via chemical vapor deposition (CVD). A thin film is created on the heated substrate surface as a result of a chemical reaction of vapor-phase precursor compounds. At high temperatures, the decomposition of the gas leads to the release of carbon atoms. These carbon atoms can recombine, forming carbon nanotubes on the substrate. Thus, the production of one- or two-dimensional nanomaterials is achievable through CVD [45].

3.2.4 Co-precipitation

Co-precipitation is a wet chemical technique, which is considered as basic and is widely used. The method refers to the use of a precipitation reaction to consistently compose two or more cations in a homogeneous solution. Then, this solution is combined directly or dropwise with another solution which contains dissolved precipitation agents. To obtain nanostructured material with desired crystal structures and morphologies, post-treatment such as annealing, sintering, or calcination is also applied [46].

3.2.5 Green Synthesis

The biogenic synthesis (green synthesis) of nanomaterials requires the use of non-toxic chemical substances (solvents, reducing and stabilizing agents, etc.) and generally utilizes plants and microorganisms. It is an eco-friendly approach with several applications [47].

3.2.6 Soft and Hard Templating Methods

Nanoporous materials are typically produced via soft and hard template methods. These methods are generally easy and allow a great range of morphologies and sizes. Soft templates like block copolymers, flexible organic molecules, and anionic, cationic, and non-ionic surfactants are used in soft templating methods. Interactions between the precursors and the templates are based on electrostatic forces,

hydrogen bonding, and van der Waals forces. Nano-casting or hard templating method uses solid templates, whose pores are filled with precursor molecules [38].

3.3 Synthesis of Hybrid Multifunctional Nanomaterials

The design and development of advanced multifunctional hybrid nanomaterials is a challenging procedure. The synthesis methods that are commonly applied are already mentioned. Additional methods to produce liposomes and dendrimers and generally several nanocarriers and drug vehicles are also needed to be mentioned as well as some methods improving the functionality, such as chemical surface modification and doping and standard methods for conjugation between the separate parts of the hybrid nanomaterials [24].

3.3.1 Liposomes Preparation and Drug Loading

The common methods that are used to prepare liposomes involve the following steps: drying lipids obtained from organic solvents, dispersing the lipids in aquatic solutions, purifying, and analyzing the produced material. Furthermore, as far as drug loading to liposomes, two approaches are distinguished, the active and the passive one [48].

3.3.2 Dendrimers Preparation and Drug Loading

Dendrimers lie at the interface between the polymer and molecular chemistry. The controlled synthesis based on specific steps is related to molecular chemistry procedures, and the structure which is consisted of monomers is associated with polymer chemistry techniques. Dendrimers are commonly prepared through a divergent or a convergent method. A multifunctional core molecule reacts with monomers including reactive groups and gradually leads to the first-generation dendrimer which is enriched with more monomers. The interactions between dendrimers and drugs can be categorized into two categories, physical and chemical bonding [49].

3.3.3 Functionalization of Nanoparticles and Conjugation Strategies

To modify the nanoparticles to achieve different properties, a variety of ligands can be incorporated into them, allowing them to be used in drug delivery systems, diagnosis, treatment, and sensoring [50].

Green fluorescent protein (GFP) has been electrostatically complexed with nanoparticles in many studies, for the accurate detection of healthy and cancer cells [51]. Other studies give an effort to functionalize nanoparticles with ligands

exhibiting differential affinity toward proteins. Thus, various cell surface molecules are employed for their identification [52]. Also, several studies focus on the modification of the surface charge, the hydrophobicity to optimize their performance in sensors.

Moreover, coating with polymers (e.g., polyethylene glycol (PEG)) can facilitate passive targeting of tumor areas exploiting the enhanced permeability and retention effect (EPR effect). Furthermore, coating with biomolecules leads to specific attributes, such as improved efficacy of drug delivery and minimal toxicity [50].

Various conjugation techniques are applied to produce hybrid multifunctional materials. Arginine-glycine-aspartate (RGD) and folate (FA) can be used as the functional targeted ligands for the conjugation of polydopamine (PDA) functionalized nanoparticles [53]. Biotin-streptavidin binding is also a widely used technique to conjugate two or more parts of a hybrid material [54].

3.3.4 Surface Modification

The modification of the surface of nanoparticles, to change physical, chemical, or biological characteristics, is called surface modification. It is usually applied to solid materials. Reactivity, biocompatibility, surface charge, hydrophilicity, roughness, and photocatalytic properties are among the characteristic that is commonly improved through surface modification [55].

3.3.5 Doping, Dye Sensitization, Coupling

The electrical, optical, and biological properties of the nanoparticles can be modified through the doping process. Light-sensitive dyes are used in dye sensitization methods to increase the light absorption of the nano-photocatalysts. Coupling with metals leads to the improvement of the semiconductivity, due to the enhanced charge separation and the increased light absorption [56].

3.4 Characterization of Hybrid Multifunctional Nanomaterials

Various characterization techniques are used to study the physical and chemical properties as well as the morphology of the synthesized nanomaterials. Hybrid multifunctional nanomaterials are typically characterized through the same techniques that are going to be presented concisely.

Dynamic Light Scattering (DLS)
Dynamic light scattering is utilized to estimate the size (hydrodynamic radius) and the zeta potential of the produced nanoparticles. Particles' size can influence the

medication discharge. Particles with a small size typically provide a bigger surface area. Zeta potential can give a sense of the surface charge [57].

X-Ray Diffraction (XRD) Analysis
The crystallinity and the crystallite size can be estimated through the XRD method [58].

Raman Spectroscopy
Raman spectroscopy can clarify the complexity of the structure of the nanomaterials, detecting different crystal forms [59].

Fourier-Transform Infrared (FT-IR) Spectroscopy
FT-IR spectroscopy is used to obtain an infrared spectrum of absorption or emission of a solid, liquid, or gas identifying the composition of the produced material, compared to standard databases [60].

Nuclear Magnetic Resonance Spectroscopy
NMR solid-state spectra can confirm the data obtained by FTIR analysis, providing further information on the structure of the material network and its condensation rate [44].

Ultraviolet-to-Visible (UV-Vis) Spectroscopy
UV-Vis spectrometer allows diffuse reflectance measurements for the determination of the band gap (Eg). It is also used for the quantitative determination of different analytes in a compound [61].

X-Ray Photoelectron (XPS) Spectroscopy
XPS can identify the elements existing within a material (elemental composition) or are covering its surface, as well as their chemical state, and the electronic structure and density of the electronic states in the material [62].

Scanning Electron Microscopy (SEM)
SEM allows morphological observation with direct perception. This technique is tedious, costly, and frequently needs reciprocal insights about estimating dispersion, but it is considered among the most reliable methods of characterization [63].

Transmission Electron Microscopy (TEM)
The transmission electron microscope is mainly utilized for recognizable proof of the morphology of the produced nanoparticles [64].

Atomic Force Microscopy (AFM)
AFM images are used to determine the surface topography and roughness profiles of nanoparticles [65].

Surface Hydrophobicity
Surface hydrophobicity is determined by numerous techniques along with hydrophobic interplay chromatography, biphasic partitioning, and adsorption of probes [66].

Drug Release
Drug release assays are used to investigate the drug release mechanism. Drug loading assays are widely used [67].

Stability
The conventional methods to check the stability of the produced nanomaterials include TEM, DLS, UV-Vis spectroscopy, and zeta potentiometer [68].

4 Hybrid Nanomaterials for Diagnostic Applications and Population Screening

Nanoscale materials are designated to allow the implementation of highly effective imaging techniques. The majority of the imaging applications include both inorganic materials, such as quantum dots, gold nanoparticles, iron oxides, etc., and organic ones, like dendrimers, liposomes, micelles, and polymeric nanohydrogels. A significant increase in the use of nanomaterials in bioassays and biosensors has been seen in the last decades [24]. New strategies for surface functionalization to create versatile recognition agents allow the development of innovative biodiagnostic assays and sensors, allowing precise and robust detection of a wide range of compounds. Hybrid stimuli-responsive nanomaterials can be used to transduce molecular interactions into biomedical signals [69]. These signals are recorded by standard equipment or optical observation. Several biosensors can provide reliable and fast monitoring with high sensitivity and specificity. Sometimes it is crucial to detect even incredibly low levels of a marker for a particular disease. Thus, the optimization of the materials that are used is of crucial importance for early diagnosis and population screening [70, 71].

Gold nanoparticles have been thoroughly studied for bioassay applications and particularly for biosensors and molecular diagnostics. Their optical properties as well as the well-established synthesis methods allow their wide use. Gold nanoparticles can form stable suspensions in aquatic solutions. Hence, they allow simple conjunction with biomolecules [72]. In combination with various synthetic materials such as fluorescence dyes, oligonucleotides, polymers, and quantum dots, graphene oxides can form gold-based hybrid nanomaterials providing multifunctionality in molecular detection. Due to the localized surface plasmon resonance (LSPR) phenomenon, which is observed in gold nanoparticles suspensions, the development of a large number of colorimetric bioassays, exploiting the color shift, detected when the aggregation or redispersion of the nanoparticles is induced by the presence of an analyte [73]. Various bioassays based on gold nanoparticles have been utilized. One of the first attempts was testing cerebrospinal fluid from patients with syphilis. In pathogenic samples, gold nanoparticles aggregated, while they remained dispersed in normal samples. Also, antibody-functionalized gold nanoparticles have found extensive commercial applications. The pregnancy test and SARS-CoV-2 rapid antigen test are representative examples of their wide use. DNA-conjugated

gold nanoparticles are used for colorimetric detection of non-nucleotide analytes (metal ions, growth factors, narcotics, etc.). Real-time screening of endonuclease inhibitors is allowed through the use of DNA-crosslinked gold nanoparticles that can be disassembled by endonucleases, cleaving the double-stranded DNA in a site-specific manner [72, 74]. Functional peptide-gold-based hybrid nanoparticles have also been proposed as promising candidates in the field of molecular imaging (biosensing/diagnostics and cell targeting/imaging) [75].

Magnetic nanoparticles, quantum dots (QDs), and plasmonic noble metal nanoparticles are among the most common nanoparticles that are used in biomedical applications intended for diagnostics, due to their unique physical and chemical characteristics, their small size, and their large surface to volume ratio [29]. These groups of nanoparticles are utilized in various applications in vivo and in vitro. In cases of non-transparent, colored, or turbid biological solutions (blood, serum, etc.), gold nanoparticles are not suitable. However, magnetic nanoparticles, composed of iron oxides, metallic iron, and copper, are advantageous. Their response is detected through modalities that are based on magnetic resonance [76].

QDs are crystalline semiconductors with sizes ranging between 2 and 10 nm in diameter. They are a great alternative to organic dyes, due to their optical properties (broad-spectrum, narrow emission profile) and their chemical stability. PbS, CdSe, and InP are among the most common combinations for QDs [77]. The combination of magnetic nanoparticles and QDs improves the sensitivity in the detection of proteins and nucleic acids even at very low levels (bio-barcode assays), avoiding the conventional drawbacks, such as photobleaching, broad bands, etc. that are observed in other bioassays using fluorescent dyes [29].

Recent progress in the production of polypeptide-based hybrid nanomaterials that transduce molecular interactions, exploiting their magnetic and optical properties, leads to their utilization in bioanalytical applications, instead of the conventional biomolecular receptors, such as antibodies [78].

Also, nanomaterials that are constructed through metal-ligand coordination bonds are commonly used in diagnostic applications. Nanoscale metal-organic frameworks (NMOFs) and nanoscale coordination polymers (NCPs) are among those that are widely used as imaging and contrast agents. Complementary enhanced techniques might enable the multiple functionalities of those materials to be used also in therapeutics [1].

4.1 Imaging Modalities

Precise diagnosis of tumors requires detailed image information. Conventional imaging cannot provide comprehensive data. Nanomedicine has received extensive attention to cover this gap. A single injection of a nanostructures contrast agent might provide complementary information. The imaging modalities that have been studied for the possible incorporation of nanopharmaceuticals include positron emission tomography (PET), single-photon emission computed tomography

(SPECT), computed tomography (CT), and magnetic resonance imaging (MRI), optical imaging, and ultrasound imaging [79].

4.1.1 Positron Emission Tomography (PET) Multimodalities Based on Nanomedicine

Whole-body imaging is allowed through PET scans, routinely. PET is utilized in nuclear medicine for various organ imaging, using β^+-emitting radionuclides such as ^{11}C, ^{13}N, ^{15}O, ^{18}F, ^{64}Cu, ^{68}Ga, ^{82}Rb, and ^{124}I. PET sensitivity is higher than conventional, single-photon nuclear imaging [80]. Nanoparticles might improve the biodistribution, targeting, accumulation, and contrast capabilities of the conventional radiopharmaceuticals, acting as amplifiers. Nanocarriers can contribute to the maximization of the efficacy of the radiopharmaceutical in each injection. Typically, five radiolabeling strategies are applied (Fig. 5), chelator-mediated labeling, specific trapping, ion-exchange method, hot and cold precursor labeling, and activation via proton beam.

PEGylated liposome-based ^{18}F [81], solid lipid nanoparticles-based ^{64}Cu [82], polyaspartic acid (PASP)-coated iron oxide (IO)-^{64}Cu, ^{64}Cu-CuInS/ZnS nanoparticles, and [^{64}Cu]CuInS/ZnS QDs are among the studied hybrid nanomaterials that are used in PET modalities, offering bifunctional imaging probes, to perform several diagnostic functions simultaneously. Particularly, iron oxide nanoparticles (IONPs) can be used in tandem for both MRI and PET or hybrid, synergistic multimodal systems (PET/MRI). Silica (SiO_2)-based core-shell nanoparticles have been studied in patients with melanoma. ^{124}I-Cy5 dye(organic fluorophore)-PEG-SiO_2 have also been tried for PET imaging for the detection of integrin-expressing lesions [83]. Amorphous dense silica nanoparticles have also served as general substrates for chelator-free radiolabeling of ^{22}Na,^{68}Ga, and ^{64}Cu [84]. In nuclear medicine, both single-walled (SWNTs) and multi-walled (MWNTs) carbon nanotubes have been studied for in vivo use in PET or SPECT, using ^{125}I, [85], ^{111}I [86], and ^{99m}Tc [87] offering rapid clearance from blood circulation.

4.1.2 Single-Photon Emission Computed Tomography (SPECT) Multimodalities Based on Nanomedicine

SPECT is routinely utilized in nuclear medicine for various organ imaging [80]. It commonly uses γ-emitters, such as ^{99m}Tc, ^{123}I, ^{67}Ga, ^{111}In, and ^{201}Tl [88]. SPECT can be used for monitoring biodistribution, pharmacokinetics, and target site accumulation because of its penetration depth and sensitivity [79]. SPECT is often used in parallel with CT or MRI. Nanocarriers have been used in SPECT imaging, forming nano-probes. Liposomes conjugated with ^{99m}Tc [89], ^{111}In-radiolabeled pluronic nanocarriers [90], Cy5-Gd_2O_3 cores within a polysiloxane shell [91], ^{99m}Tc-Annexin V-gold nanoparticles for SPECT/CT imaging of atherosclerosis plaques [92], ^{99m}Tc-bevacizumab-loaded human serum albumin pegylated nanoparticles [93],

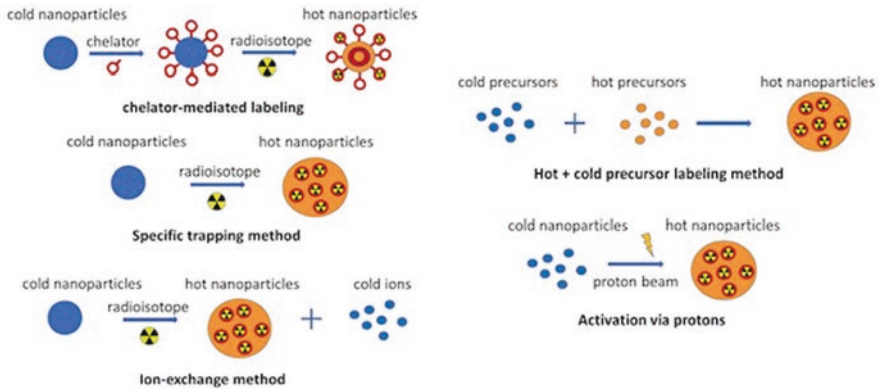

Fig. 5 The common strategies for radiolabeling nanomaterials

Gd-nanopartices labeled with ^{111}In [94], ^{99m}Tc-labeled multifunctional polyethyleneimine-entrapped gold nanoparticles for SPECT/CT imaging [95], 99mTc- folated-γ-glutamic acid, and 99mTc-chitosan [96, 97] are among the promising attempts in molecular imaging based on SPECT.

4.1.3 Computed Tomography (CT) Multimodalities Based on Nanomedicine

Computed tomography is one of the most common X-ray tomographic techniques. Generally, CT provides high-contrast images due to the inherent distinctions in X-ray absorption and attenuation by different organs and tissues of the body. Soft tissues with similar texture cannot be imaged clearly, and for this reason, several contrast agents are used (iodine, platinum, gold, tantalum, bismuth, etc.) [79]. High Z-nanoparticles have been studied for their performance as contrast agents for CT, providing extended circulation lifetime and accumulation in the regions of interest. Gold nanoparticles are among the most famous nanostructures for CT and hybrid modalities (PET/CT, CT/MRI, etc.) due to their high X-ray attenuation coefficient and their biocompatibility. Bismuth sulfide nanoparticles coated with bovine serum albumin for multimodal imaging and liposome-coated metallic core nanoparticles are among the common agent choices for CT imaging [98].

4.1.4 Magnetic Resonance Imaging (MRI) Multimodalities Based on Nanomedicine

MRI is an imaging method, based on nuclear magnetic resonance and provides details images with high contrast, and spatial resolution for this reason, and is widely used in clinical diagnosis. Early diagnosis of tiny tumors is still a challenge even for this advanced technique. Magnetic nanoparticles might be used to enhance

MRI detection accuracy. Manganese (Mn^{2+}), iron (Fe^{3+}), and gadolinium (Gd^{3+}) are among those ions that are used as contrast agents. Superparamagnetic nanostructured iron oxide has been used in MRI due to its effect on the reticuloendothelial system and its biocompatibility [79]. Gd-embedded iron oxide nanoparticles acquire T1 (positive) and T2 (negative) contrast agents to enhance the contrast in the regions of interest, improving the diagnostic results that are obtained. Metal-chelating lipids can conjugate to Gd^{3+}, $^{64}Cu^{2+}$, or $^{111}In^{3+}$. Liposomes can be rendered T1 MRI active by the conjugation with the bilayer of Gd (Gd–DTPA). Superparamagnetic liposomes, the so-called magnetoliposomes, can be used as T2 contrast agents. Thus, various nanoparticles and complexes have been studied as MRI contrast agents, and fortunately several of them have been approved for clinical use [15].

4.1.5 Optical Imaging Multimodalities Based on Nanomedicine

Optical imaging gathers various advantages and, for this reason, is commonly used. Its simplicity, and the fact that it allows multiple marker detection and observation of a wide range of tissues as well as subcellular structures, makes optical imaging an integral tool in clinical routine [99]. Biological experiments, histopathology, fluorescence-guided surgeries, and endoscopy are some of the main applications of optical imaging.

Nanomaterials enhance optical imaging, due to their properties. Quantum dots have been regarded as innovative optical biomedical probes. Magnetic nanoparticles conjugated with quantum dots are also an interesting combination for optical imaging [77]. Poly(lactic-co-glycolic acid) (PLGA) is applied as a template to bind quantum dots and iron oxide for multifunctional optical imaging, since the quantum dots can enable optical imaging, while iron oxide is used for magnetic targeting, allowing optical imaging in parallel with MRI [100]. Upconverting nanoparticles have also been considered as promising agents for optical imaging since they emit higher-energy visible light when excited by NIR light [101]. Also, if upconverting nanoparticles conjugate to Gd^{3+} and ^{18}F can also be used for MRI and PET and if it binds to lanthanides, then it becomes a CT contrast agent.

4.1.6 Ultrasound (US) Imaging Multimodalities Based on Nanomedicine

The US is a widely used technique that is based on the difference in the ultrasound impedance through tissues and allows real-time and safe imaging. It lacks sensitivity, resolution, and penetration capability [79]. Nano-emulsions (liquid-liquid, gas-liquid), as well as solid nanoparticles, can enhance the contrast of a US image. PLGA has been used to encapsulate nanoparticles for US imaging or hybrid modalities (US/MRI). The core-shell choice seems also efficient. The shell, consisting of surfactants, polymers, or proteins, allows stability and durability, whereas the core (usually SF_6 or C_3F_8) determines solubility as well as acoustic properties [102]. Focused ultrasound (FUS)-mediated drug delivery, using thermosensitive

liposomes, is another promising technique [103]. Thus, nanosized ultrasound contrast agents have gained great scientific interest.

5 Hybrid Nanomaterials for Therapeutic Applications

Nanoparticles can deliver single or multiple therapeutic agents to tumor tissues and thus improve cancer treatment efficacy. The design and development of those nanomaterials focus on the improvement of the therapeutic processes [100]. Photodynamic therapy, photothermal therapy, immunotherapy, gene therapy, drug delivery systems, and applications related to regenerative medicine and tissue engineering are the main therapeutic fields that have incorporated nanotechnological achievements [104].

5.1 Therapeutic Applications

5.1.1 Photodynamic Therapy

Photodynamic therapy (PDT) is also known as photoradiation therapy, phototherapy, or photochemotherapy [105]. PDT is widely used in dermatology, treating precancerous cells, sun-damaged skin, acne, rosacea, onychomycosis, as well as other inflammatory disorders and cutaneous infections. It is also used in various neoplastic diseases, such as squamous cell carcinoma, actinic keratoses, basal cell carcinoma, and cutaneous T cell lymphoma [106].

A photosensitizer, a light source, and oxygen are the three required components of a PDT (Fig. 6). The photosensitizer localizes to the target tissue and is activated upon irradiation with light in the presence of oxygen [107]. Then, the photosensitizer changes from the "ground state" into the "excited state," and returning to the initial condition, an amount of energy is released, generating reactive oxygen species (ROS). ROS can oxidize essential cellular components, causing apoptosis or necrosis [108]. The phototoxic effects typically occur through intracellular localization of the photosensitizer, which consequently triggers surrounding immunologic effects, including the production of interleukin 1-beta, interleukin 2, tumor necrosis factor-alpha, granulocyte colony-stimulating factor, and others [105].

Many types of photosensitizers are commercially available, allowing topical, oral, or intravenous administration. Porphyrin is one of the common photosensitizing agents, leading abnormal cells to apoptosis [109]. Nanotechnology-based drug delivery systems can improve PDT, providing accurate targeting of the region of interest and minimizing the accumulation in healthy tissues [110]. Encapsulation of photosensitizers in nanomaterials, such as poly(lactic-co-glycolic acid (PLGA) or PEG-b-poly(aspartate) (PEG-b-PAsp), has been studied. Also, lanthanide ion-doped upconverting nanoparticles (UCNPs) have received attention as a means of

controlling the limitations of conventional PDT [111]. Furthermore, pH-sensitive smart photodynamic nanomaterials, which consisted of self-assembled photosensitizers grafted to pH-responsive polymeric ligands (PLLs) and UCNPs, demonstrated a great killing effect in the target area. Cyanoacrylic nanospheres (~100–200 nm) or nanocapsules (15–400 nm) have been synthesized developing an oil-in-water emulsion incorporating phthalocyanine or naphthalocyanine [112]. Biopolyesters, natural biocompatible polymers (e.g., poly(hydroxyalkanoates) (PHAs)), and synthetic polymers (e.g., poly(orthoesters), PLGA, etc.) are usually used for micelles preparation, acting as nanocarriers [113]. PEGylated PLGA nanoparticles have been shown to have enhanced circulation time, remaining in the blood for an extended period. The famous liposomal Visudyne was a great choice in the 2000s for patients suffering from age-related macular degeneration or subfoveal choroidal neovascularization secondary to pathological myopia [114]. Dendrimer-based nanoparticles, natural macromolecule-based nanoparticles (chitosan, gelatin, etc.), magnetic nanoparticles, and fullerenes are among the common nanomaterials that are used for PDT. Moreover, silica, titanium dioxide, and zinc oxide are used as photosensitizers due to their photocatalytic potential. Gold nanoparticles, CdS quantum dots, and hybrid porphyrin-decorated quantum dots have been thoroughly studied [115]. Polymeric nanovesicle poly(ethylene glycol)-block-poly(D,L-lactic acid) [PEG−PDLLA] loaded with doxorubicin and lipophilic PS hematoporphyrin monomethyl ether (HMME) allowed combined PDT and chemotherapy in HepG2 human hepatocellular carcinoma cells [116]. Hence, hybrid nanomaterials can surely contribute to the improvement of the conventional PDT.

5.1.2 Photothermal Therapy

Photothermal therapy (PTT) refers to efforts to use electromagnetic radiation (NIR near-infrared) for the treatment of various diseases. The heat-shock response that is induced by hyperthermia (≤43 °C) is significant and can kill cancer cells since they

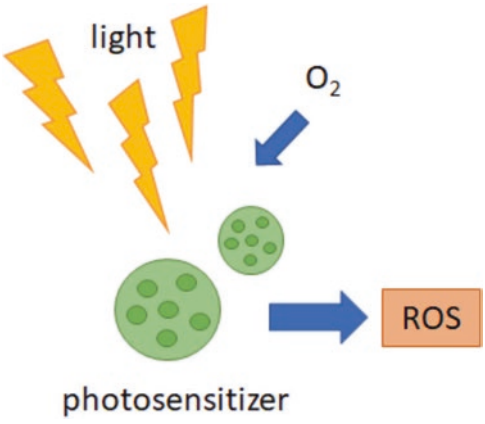

Fig. 6 Schematic representation of photodynamic therapy

are more vulnerable to hyperthermal damage than normal ones [117]. Nanoparticle-mediated hyperthermia can actively target the tumor, enhancing the tumor-to-normal tissue nanoparticle accumulation ratio. Gold nanorods, gold nanoshells, metallic ultrasmall nanoparticles, graphene and graphene oxide, iron oxide, and conjugated polymers (e.g., polyaniline (PANI), polypyrrole (PPy), polythiophene (PTh), polydopamine (PDA)) are among the available choices for the development of hybrid nanosystems for PTT [26].

5.1.3 Immunotherapy

Nanoparticles have served as vehicles, transferring immunostimulants that induce the generation of endogenous cancer antigens, eliciting an adaptive immune response. Hybrid nanosystems improve the antitumor immune responses (e.g., T- and B-cell response) compared to conventional antigens and adjuvants [118]. Gold nanoparticle-based vaccines seem to stimulate CD8+ T-cell responses through antigen delivery to lymph nodes [119]. Other studies focus on the parallel synergistic action of immunotherapy and magnetic hyperthermia, applying iron nanoparticles conjugated with anti-cytotoxic T-lymphocyte antigen-4 (anti-CTLA4) checkpoint inhibitor to enhance the therapeutic effect [120]. Encouragingly, nanotherapeutic strategies can transform cold into hot tumors, in tandem with PDT. Nanosized metal-organic frameworks (MOFs) allow this approach. Tumor hypoxia can suppress antitumor immunity, promoting tumor development and recurrence. Alleviating hypoxia might lead to the improvement of the efficacy of PDT-driven immunotherapy. Hemoglobin, catalase (CAT), manganese dioxide nanoparticles, and gold nanocages are among the common choices to develop efficient hybrid systems for cancer immunotherapy, based on nanomedicine [104]. pH-responsive copolymer (PDPA) conjugated to PDPA, and PD-1-PD-L1 interaction inhibitor (siRNA), is a nanosystem that has been studied with encouraging results. GSH-responsive heterodimers, mesoporous silica nanoparticles, micelles, and liposomes can be used as parts of hybrid nanocomplexes for immunotherapy [120].

5.1.4 Gene Editing/Gene Therapy

Editing technology of the genome is based on the general fundamental of induction of controlled double-strand breaks and the modifications, replacement, or insertion of genes with desired characteristics in the selected sequence. Clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated (Cas) proteins, as a part of the bacterial adaptive immune system, are considered an efficient gene-editing tool [121]. The CRISPR/Cas9 system can contribute to the knock-out of disease-related genes and edit genetic defects in abnormal cells. Nano-based CRISPR/Cas9 using cationic arginine gold nanoparticles was proven to increase the efficiency of the whole system to be delivered in the targeted area [122]. Several

similar attempts show the significant positive effect of nanotechnology in biomedicine.

5.1.5 Drug Delivery Systems

The development of delivery systems that can provide therapeutic agents to a targeted area is a field of research and development in nanomedicine [123]. Nano-based drug delivery systems focus on improving properties like solubility, immunogenicity release profiles, diffusivity, and bioavailability of the conventional delivery systems. Hence, smart hybrid drug delivery systems allow spatial-temporal release control, convenient administration routes [124], low toxicity, extended drug life cycle, and better biodistribution. In this way, they can maximize the efficacy of a drug in the region of interest, minimizing the side effects. Various bionanomaterials can be used as nanocarriers in drug delivery systems, such as chitosan, alginate, xanthan gum, cellulose, liposomes, polymeric micelles, and dendrimers. Inorganic nanoparticles (iron oxide and silica nanoparticles, etc.), nanocrystals, metallic nanoparticles (silver, gold, etc.), quantum dots, protein and polysaccharides nanoparticles, natural extracts, oligonucleotides, peptides, pharmaceuticals, and radioisotopes are among the vast variety of the available candidate for the development of hybrid nano-based drug delivery systems [24].

5.1.6 Regenerative Medicine/Tissue Engineering

Nanocomposite materials can be utilized in biomedical applications related to scaffolds for tissue engineering, medical devices, vascular stents, and dental materials. The development of nanocomposite scaffolds can minimize the limitations of the conventional polymer scaffolds, mimicking the physical and chemical properties of normal tissues [125]. Silicate-based, carbon-based, and metal/metal oxide nanoparticles can be combined with synthetic or natural polymers, creating advanced materials with great properties, allowing better interaction between cell and matrix, controlled migration, cell growth and proliferation, and cell differentiation. Artificial vascular stents coated with surface-modified $Mg(OH)_2$ nanoparticles and heart valves, hip and knee joints, and dental implants are those applications that have incorporated nanotechnology, in the last decades with significantly better performance than the conventional biomaterials.

6 Hybrid Nanomaterials for Health Prevention

Nanovaccines and sensors for rapid screening of the general population for a wide range of diseases are the two main aspects of the applications of nanomedicine for the needs of health prevention.

Vaccination is perhaps the most effective tool that we can use to control the disperse of infections, and nowadays it is more reasonable than ever before, due to the pandemic of COVID-19 [126]. Nanoparticle (NP)-based carriers, such as micro/nanoemulsions, liposomes, micelles, dendrimers, virosomes, and nanogels, offer improved efficacy compared to the traditional vaccine adjuvants. Nanovaccines allow targeted delivery, stimulation of the body's innate immunity, and strong T-cell response [127, 128].

Biosensors, as is already mentioned, offer accurate and rapid detection of a wide range of diseases at the very early stages. Breath analysis is also among those promising tools for detecting important biomarkers for various disease types [129].

7 Hybrid Multifunctional Nanomaterials for Theranostics

Theranostics is a promising field that combines therapeutic and diagnostic applications, by exploiting the properties of multifunctional systems and devices. Nanomedicine can improve the field of theranostics since it allows precise molecular imaging and targeted and individualized treatment [130]. Cytotoxicity, immunotoxicity, and genotoxicity issues are still among the limitations that need to be improved before nanotheranostics become a part of the everyday clinical routine [5]. Thus, functionalized nanomaterials have been highly investigated as promising platforms for disease diagnosis and therapy or palliative treatment (Fig. 7).

The commonly applied imaging modalities of the theranostic procedures include magnetic resonance imaging (MRI), computed tomography (CT), optical imaging (fluorescence and bioluminescence), and radionuclide imaging (PET and SPECT), while the widely used therapeutic approaches include photodynamic therapy, photothermal therapy, and gene therapy [19]. Various organic and inorganic nanoparticles can be used in theranostic systems (e.g., proteins, antibodies, polymers, lipids, dendrimers, gold, and iron oxides). The size of the whole system does not exceed 150 nm. High stability and homogeneity are the main characteristics of the theranostics and also their ability to be further functionalized for direct binding and optimized efficacy, as well as the controlled, slow, and often stimuli-responsive release on the target area. Hybrid multifunctional nanomaterials are very promising for the development of advanced theranostic systems [5, 24, 131].

8 Conclusion and Future Perspectives

The evolution in the field of nanomedicine has led to the development of hybrid multifunctional nanomaterials that gather the benefits of all their components, providing also optimized characteristics, regarding their performance in biomedical procedures. Hybrid nanomaterials are designed and synthesized to include both inorganic and organic components. These materials are utilized in diagnostics, as

imaging and contrast agents, enabling precise visualization at a molecular level, exploiting the potential of various imaging techniques, and reducing any side effect in healthy tissues. Moreover, they are used in therapeutics, particularly in anticancer therapy, such as photodynamic and photothermal therapy, hyperthermia and radiotherapy, drug delivery, and gene therapies. Furthermore, hybrid nanomaterials can be incorporated with several components that can be used for simultaneous diagnostics and therapeutics (theranostics), and thus they can be considered as multifunctional nanomaterials. It is expected that in the near nanomedicine will attract scientific interest and if some of the present limitations can be controlled, then it will be part of the clinical routine.

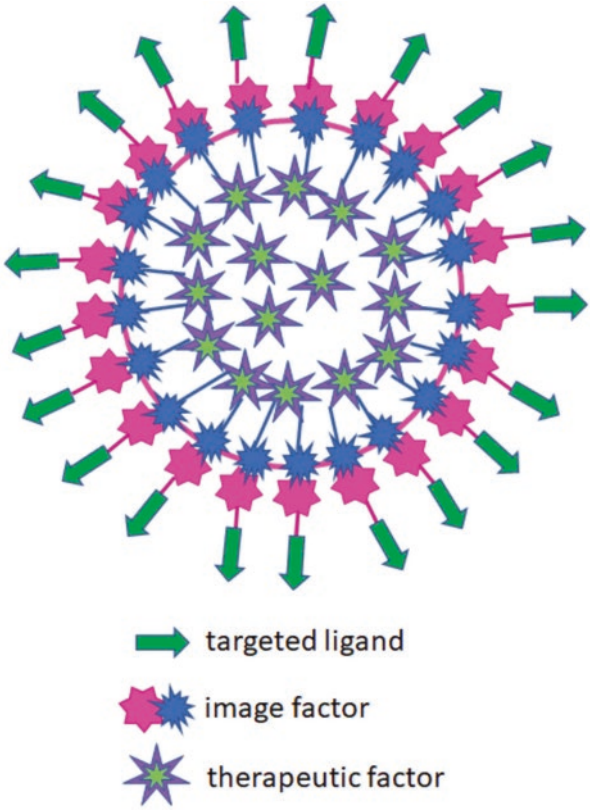

Fig. 7 Schematic representation of a typical theranostic system

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