Nanostructures for imaging, medical diagnostics and therapy

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1. Introduction

The application of nanotechnology for diagnostic or therapeutic application is called nanomedicine (Jain & Stylianopoulos 2010). Nanomedicine is an interdisciplinary field in which biology, medicine, chemistry, physics and other disciplines are brought together in order to develop nanomaterials suitable for biomedical application (Wicki et al. 2015). Nanoparticle size (in the range from a few nanometers to a few hundred nanometers) allows them to interact with biological entities in a fundamentally different manner than non-nanostructured materials (Albanese et al. 2012). Some of those size-dependent properties derive from the surface to volume ratio being much higher than for non-nanostructures materials. These properties can be exploited to design nanostructures that can be used to diagnose or treat different pathologies. Some examples would be the unique thermal, electrical, magnetic and optical properties that present different types of nanoparticles and that greatly differ from the properties of non-nanostructured materials with similar chemical composition (Chen et al. 2016).

The design of nanoparticles is essential to allow their correct function for biomedical application. Amongst the most important parameters to consider are nanoparticle size, chemical composition and surface characteristics (Chen et al. 2016). For example, nanoparticle size is critical for *in vivo* imaging as well as for therapeutic application of nanoparticles. As it will be explained later in this chapter, nanoparticles tend to accumulate in tumor tissue, due to an enhanced permeation and retention (EPR). In that context, if the particles are too big, they will not be able to reach the diseased tissue and, therefore, they will not achieve

their function. On the other hand, if the nanoparticles are too small (less than 10 nm), they will be excreted in the urine, potentially preventing accumulation in the desired target, which would lead to, again, the material not achieving its function. For *in vitro* diagnostics, the size of nanoparticles is also a fundamental parameter, since it often determines the optical properties of the nanoparticle suspension (for example, in plasmonic gold nanoparticles), and a modification in size will lead to changes in the measured response after exposure to a sample containing the analyte.

This chapter is divided in three parts. In the first one, some of the most important types of nanoparticles proposed for biomedical application will be classified based on their chemical composition. Then, the rationale for their use in medicine will be explored in two different applications, each with their needs and particularities: diagnostics (*in vitro* diagnostics and *in vivo* imaging) and therapy (or the combination of diagnostic and therapeutic nanoparticles: theranostics) (**Figure 1**).

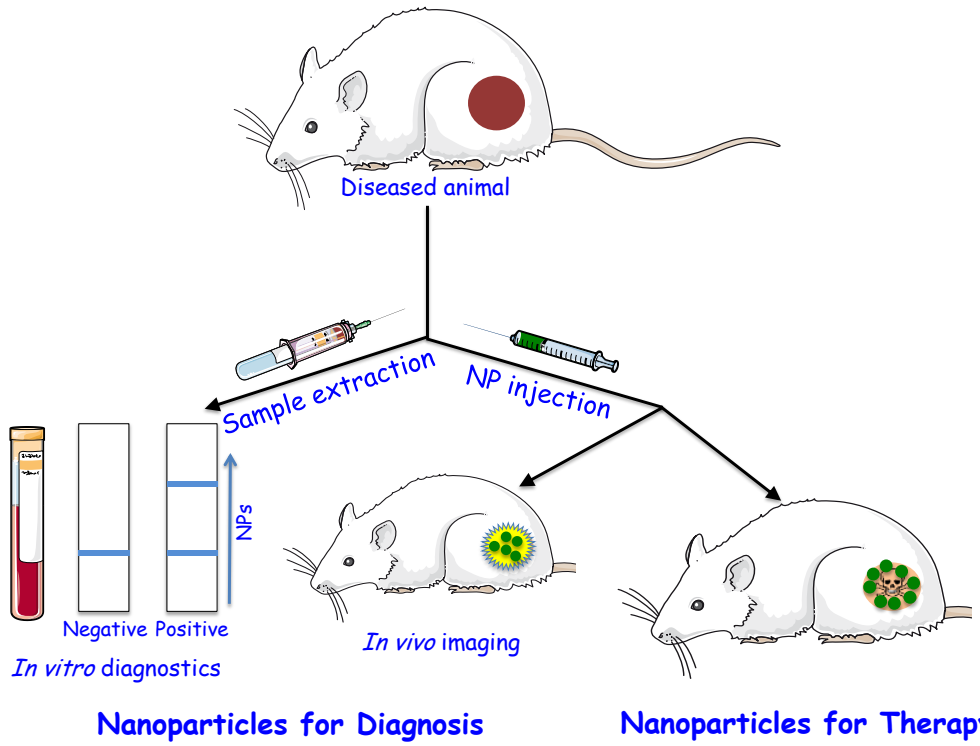

Figure 1. Representation of the three applications of nanostructures in medicine that will be described in this chapter: *In vitro* diagnostics, *in vivo* imaging and therapy.

2. Types of nanoparticles for nanomedicine.

A wide variety of nanoparticle types have been proposed for nanomedicine, some of the most important ones are going to be highlighted in this chapter. These types of nanoparticles will be classified based on their chemical composition in organic or inorganic nanoparticles (**Figure 2**). However, nowadays a very high percentage of the nanoparticles under evaluation are actually hybrid nanoparticles, joining organic and inorganic structures to yield multifunctional materials. These materials will be treated inside the section regarding the organic or inorganic core nanoparticle used to obtain the hybrid.

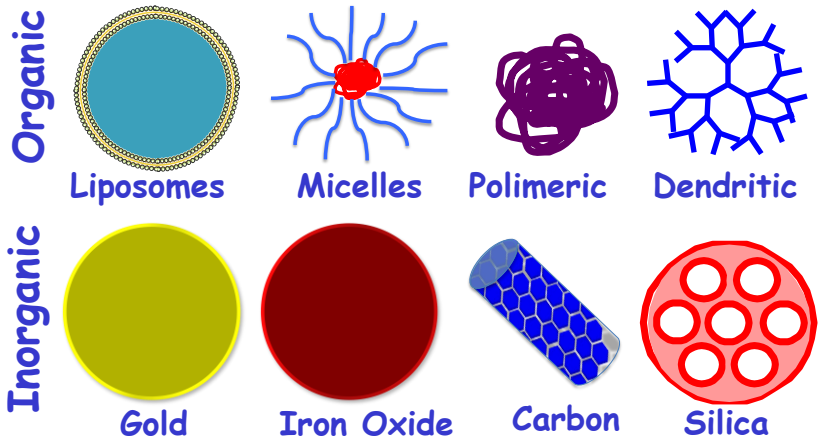

Figure 2. Schematic representation of some of the most important types of nanoparticles used for nanomedicine, divided by their chemical composition.

2.1 Organic nanoparticles

2.1.1 Liposomes and lipid nanoparticles

Liposomes are one of the most widely evaluated types of nanoparticles for biomedical application, and they have already reached clinical use (Bozzuto & Molinari 2015). Liposomes consist of amphiphilic lipids arranged in vesicles with an aqueous cavity surrounded by one or more lipid bilayers.

Liposomes can be prepared by different methods, being the most common ones reverse phase evaporation or vesicle extrusion (Bozzuto & Molinari 2015). They can be produced with natural or synthetic lipids and their chemical composition, size, lipid bilayer structure and surface charge (amongst other parameters) will determine the formulation characteristics, from the stability to the drug release

behavior. For example, the stability of liposomes can be increased by the incorporation of cholesterol in their structure, which induces a denser packing of the hydrophobic chains inside the lipid bilayer. On the other hand, drug release from unilamellar liposomes is usually faster than from multilamellar ones, since the drug has to diffuse through more lipid bilayers in the second case. Liposomes can load hydrophilic molecules in the inner aqueous compartment (as is usually the case in unilamellar liposomes) or hydrophobic ones, in that case inserted in the lipid bilayer (mostly used in multilamellar liposomes). The combination of liposomes with other types of nanoparticles can yield multifunctional systems that allow simultaneous therapy and diagnosis (theranostic materials), for example, by conjugating the liposomes with nanoparticles with imaging capabilities, like quantum dots (optical imaging) or superparamagnetic iron oxide nanoparticles (magnetic resonance imaging). All of these characteristics, together with their high biocompatibility, easy preparation with tunable sizes and high loading capacity make them the most successful nanostructured drug delivery system to date (Bozzuto & Molinari 2015). However, they present a rather low physicochemical stability compared to other nanoparticles. To increase nanoparticle stability, solid lipid nanoparticles can be obtained by using high-melting lipids. These lipid nanoparticles can be stabilized in aqueous suspension by adding surfactants or other functionalities (Bozzuto & Molinari 2015).

2.1.2 Micelles and solid polymeric nanoparticles

Polymeric micelles are also amongst the most studied nanocarriers. They are colloidal nanoparticles with a size around 5-100 nm, constituted by an amphiphilic block copolymer that self-assembles in aqueous medium (Oerlemans et al. 2010). At low concentrations, amphiphilic molecules are dissolved in the aqueous medium, but with increasing concentration, once the critical micellar concentration (CMC) is reached, the amphiphiles self-assemble, forming a hydrophobic core with a hydrophilic shell stabilizing the particles by hydrogen bonding with the surrounding water. The CMC of polymeric micelles is usually very low, which allows the presence of micelles at almost any dilution (Oerlemans et al. 2010). The polymer composition determines the micelle characteristics, like the drug molecules that can be loaded or the stability and

biodistribution of the formulation. For example, the most common hydrophilic block is a chain of polyethyleneglycol (PEG), because it can stabilize the micelles and prevent the rapid uptake by the Reticuloendothelial system (RES) after injection. The polymer composition can also be modified to provide different functionalities to the material. A targeting moiety can also be included in the hydrophilic shell, as well as different components that provide imaging capabilities (like superparamagnetic iron oxide nanoparticles) to obtain theranostic nanodevices. Micelles can efficiently carry high amounts of hydrophobic drugs within their core, preventing the use of toxic adjuvants that would be necessary to administer the free hydrophobic drug. However, they have poor stability and cannot carry high amounts of hydrophilic molecules.

Other polymeric nanoparticles can also be prepared from natural or synthetic, biodegradable or non-biodegradable polymers (Kumari et al. 2010). Amongst them, biodegradable nanoparticles are preferred, due to a better safety profile. These particles have higher stability and the vast amount of polymers available make them a very versatile choice to develop particles for nanomedicine. Some of the most common methods to obtain polymeric nanoparticles are emulsification-diffusion, solvent emulsion-evaporation, nanoprecipitation, interfacial deposition and inotropic gelation (for example, in the case of polycations). They can load different types of drugs depending on the chemical nature of the chosen polymer; however, the loading efficiency is usually lower than with other types of nanocarriers. The most commonly biodegradable polymers used to develop nanoparticles for nanomedicine are: Poly-D,L-lactide-co-glycolide (PLGA), Polylactic acid (PLA), Poly-ε-caprolactone (PCL), chitosan and gelatin (Kumari et al. 2010). Protein nanoparticles (like those made of albumin) have also attracted great attention, because of the high biocompatibility and biodegradability behavior, and their high chemical versatility due to the large amount of different free functional groups throughout the biopolymer structure.

2.1.3 Dendritic nanoparticles

Dendrimers are hyperbranched polymers that can be obtained with different functional groups. They can be prepared in a highly defined way (with a

polydispersity index close to 1) (Khandare et al. 2012). Some of the most used dendritic molecules are polyamidoamine (PAMAM), poly(propylene imine) and polyglycerol. Branched polymers present several advantages for biomedical application, for example, they have shown a longer blood circulation half-life than linear polymers with similar molecular weight and chemistry (Khandare et al. 2012). The obtention of polycationic dendrimers allows gene therapy application (due to the interaction with negatively-charged nucleic acid molecules). An important disadvantage of these types of materials might be the significant cytotoxicity found for highly charged polycations.

2.2 Inorganic nanoparticles:

2.2.1 Metal-based nanoparticles

A wide variety of metallic nanoparticles can be obtained for biomedical application. The synthesis of these nanoparticles is in general very tunable and allows the preparation of monodisperse nanoparticles that can be easily modified afterwards (Boisselier & Astruc 2009). **Gold nanoparticles** in particular have been extensively studied for diagnostics and therapy. Gold nanoparticles ranging from 3 to more than 120 nm present a phenomenon called surface plasmon resonance, which allows their use for photodiagnostics and photothermal therapy. Moreover, not only spherical nanoparticles can be synthesized, but gold nanorods with different aspect ratios (ratio of length between long and short axis of the nanoparticle) can be obtained, with profound impact in the properties of those colloidal nanoparticles. **Silver nanoparticles** with plasmonic behavior can also be obtained. One of the most promising applications of silver nanoparticles is in antibacterial nanodevices, due to the bactericidal effect of the ion Ag^+ (Boisselier & Astruc 2009).

Fluorescent semiconductor nanocrystals **(Quantum dots,** QDs) are inorganic fluorophores with very small diameter (2-10 nm) composed of a are single crystal of a semiconductor (like CdS, PbSe) or core/shell structures of two semiconductors (like CdSe/ZnS) (Azzazy & Mansour 2009; Michalet 2005). Compared to organic dyes, quantum dots have a broad-range excitation spectrum and a very narrow emission band, and their photostability is much higher. They have been studied very extensively for *in vitro* diagnostics and *in*

vivo imaging. Near-Infrared (NIR)-emitting QDs hold great promise for *in vivo* imaging, since NIR light is known to penetrate more in living tissue and shows much better signal-to-noise ratio (due to less autofluorescence in that range), however, most NIR-emitting QDs currently known are significantly less stable than visible-light emitting QDs. An important concern with QDs is regarding their potential toxicity *in vivo* after the metal ions they are composed of are released from the material. For this reason, heavy metal-free quantum dots are gathering great interest for their biomedical use (Yaghini et al. 2016).

Nanoparticles for photon **upconversion** can also be prepared from rare earth ions (for example Er^{3+} or Yb^{3+}) dispersed in a dielectric lattice (Azzazy & Mansour 2009). These kinds of particles can be excited by two or more low-energy photons (usually in the NIR range) and emit one higher energy photon (generally in the ultraviolet or visible region). Therefore, they present many advantages for biomedical use, as they can be excited deep inside the organism (by highly-penetrating NIR light) and then emit UV or visible light to elicit the desired biological effect. They present very high photostability and low toxicity. Many formulations have been developed by joining upconversion nanoparticles with other particles or functionalities that can respond to the emission from these particles after NIR irradiation.

Superparamagnetic nanoparticles (like maghemite, γ-Fe_2O_3, and magnetite, Fe_3O_4, nanoparticles) present very interesting properties for their use in biomedicine (Banerjee et al. 2010). When these types of nanoparticles have less than 50 nm in diameter, the whole nanoparticle acts as a single magnetic domain, showing superparamagnetic behavior. Without an applied magnetic field, the particles do no present macroscopic magnetization, what eliminates the possibility of agglomeration of the nanoparticles due to their magnetic behavior, which is of outmost importance if the nanoparticles are to be injected in a living organism. Superparamagnetic iron oxide nanoparticles have been extensively studied as *in vivo* imaging tools (Through magnetic resonance imaging, for example) and therapeutic agents, due to their capacity to induce a rise in the local temperature when exposed to an alternating magnetic field. These types of nanoparticles can also be induced to accumulate in the diseased area by the application of a magnetic field that can slow down nanoparticle flow at the desired site, easing extravasation. While these types of

nanoparticles are well tolerated and generally considered to be non-toxic, some toxicity may be derived from the generation of Reactive Oxygen Species (ROS) from iron oxide nanoparticles *via* the Fenton reaction (Baeza 2014).

2.2.2 Carbon-based nanoparticles

Different carbon-based materials have been proposed for biomedical application. Fullerenes and carbon nanotubes are hollow structures that consist of one or several graphene-like sheets shaped as a sphere (fullerene) or a cylinder (carbon nanotube) (Ji et al. 2010). The structure of sp^2-bonded carbon atoms confers graphene, carbon nanotubes and fullerenes unique electrical properties. Graphene oxide has also been proposed for drug delivery and gene transfection (Shen et al. 2012). Mesoporous carbon nanoparticles and luminescent carbon dots have also been proposed for biomedical application. In order to give stable suspensions in aqueous environment, these carbon-based materials have to be modified with hydrophilic moieties on their surface. They have a high physicochemical stability and their size and morphology can be finely tuned. The particular electrical properties of carbon nanotubes can be exploited in the design of biosensors. However, issues regarding their toxicity may hinder their translation to the clinical setting for *in vivo* application. The safety profile of carbon-based nanoparticles is a matter of discussion, as it appears that particle parameters such as size and surface properties can affect the toxicological characteristics of the material (Zhang et al. 2014).

2.2.3 Silica nanoparticles

Silicon oxide (silica) nanoparticles have been thoroughly studied as model nanoparticles for a wide variety of applications. Silica is not cytotoxic and its excellent physicochemical stability allows multiple functionalization strategies. Mesoporous Silica Nanomaterials have attracted a lot of interest in the context of drug delivery, since their very high surface area enables them to load high amounts of drugs within their pores (Mamaeva et al. 2013; Vallet-Regi et al. 2001). The textural parameters of the nanoparticles, such as surface area and pore size can be finely tuned depending on the needed characteristics for a particular application. Surface functionalization to ensure nanoparticle suspension stability is needed to allow these nanoparticles to achieve their

function *in vivo*. Mesoporous Silica Nanoparticles for therapeutic and diagnostic applications have been developed, in most cases by obtaining hybrid nanoparticles by modifying the inner or outer structure of the silica particles with other nanoparticles or molecules that provide the desired function to the material (Mamaeva et al. 2013; Li et al. 2012). Also, Mesoporous Silica is known to undergo dissolution under physiological conditions, giving rise to non-toxic products that can be excreted from the organism. A potential drawback of these materials might be their high rigidity, which may obstruct their penetrability in solid tissues.

3. Nanoparticles for diagnostics

A promising approach in nanomedicine is the use of nanoparticles to develop diagnostic tools. Nanoparticles can be used for *in vitro* detection of pathologically relevant analytes. They can also be used as imaging agents for *in vivo* imaging.

3.1 *In vitro* diagnostics

The size of nanoparticles (typically bellow 100 nm) is in the same range as that of many biomolecules (enzymes, antibodies, other proteins). This fact allows an intimate interaction between both structures, which can be seized to develop *in vitro* setups that enable the detection of a wide variety of analytes. The high surface to volume ratio of nanoparticles allows the interaction of a large number of biomolecules with a single nanoparticle, potentially enabling detection of the analyte with very high sensitivity (Chen et al. 2016). Besides the potential implications of these strategies in the evolution and growth of analytical chemistry, the highly sensitive detection of pathologically relevant analytes in biological fluids could dramatically improve the diagnosis of several diseases, potentially improving the prognosis of many patients thanks to an early diagnosis (Azzazy & Mansour 2009). Amongst the plethora of nanoparticles that have been developed for *in vitro* diagnostics, we will only review a few of them in order to illustrate some of the most common strategies.

Inorganic nanoparticles are of particular interest for *in vitro* diagnostics, especially gold nanoparticles, quantum dots and superparamagnetic

nanoparticles (Azzazy & Mansour 2009). Different strategies developed for *in vitro* diagnostics can be divided by the parameter measured as well as by the types of nanoparticles that are used for that detection.

3.1.1 Assays based on fluorescent nanoparticles

A wide variety of methods can be used to develop fluorescent nanoparticles that act as sensors for different molecules (Chen et al. 2016). For example, an organic fluorophore can be introduced in the nanoparticles, either as part of the structure or loaded within them. An archetype of this type of systems are the so called "probes encapsulated by biologically localized embedding" or PEBBLEs. These systems have been adapted to be able to detect changes in pH, oxygen concentration and temperature, among others, by means of a change in fluorescence after exposure to the particular stimulus (Azzazy & Mansour 2009).

QDs have also been very thoroughly evaluated for this application, since they present much higher photostability and a much narrower emission band than organic dyes. Also, one of their main disadvantages for other biomedical applications, the potential toxicity due to heavy metals in their structure, is not relevant in this application, since the nanoparticles would never enter in contact with the organism of the patient. Sensors based on QDs have been developed to measure pH, temperature and different analytes (Azzazy & Mansour 2009). For example, a sensor to detect maltose was developed by grafting a maltose-binding protein to the surface of QDs (Medintz et al. 2003). Then, a dark quencher conjugated with a cyclodextrin was used to occupy the maltose-binding site of the protein, eliminating the fluorescence of the QDs by Fluorescence Resonance Energy Transfer (FRET). Then, after addition of the analyte, maltose displaced the dark quencher from the protein, allowing the recovery of nanoparticle fluorescence, enabling a straightforward measurement of maltose concentration. Similar concepts have been evaluated to determine the concentration of specific proteases and other molecules (Azzazy & Mansour 2009).

Upconversion nanoparticles are also promising for *in vitro* diagnostics because the excitation of the sample can be performed with NIR light, thus avoiding

many potential interferences from molecules in the biological fluids that might be found when exciting the sample in the ultraviolet of visible range (where background signal is commonly found). Then, the emission in the visible part of the spectrum could be easily measured to determine the result of the test. Also, the excitation and emission spectra of upconversion nanoparticles are relatively independent of their environment, making them a good choice for detection of analytes in biological fluids that are often very complex. Upconversion nanoparticles have been used to develop detection methods for temperature, oxygen, nucelic acids, antigens and many other biomolecules (Azzazy & Mansour 2009).

3.1.2 Assays based on plasmonic nanoparticles

Plasmonic nanoparticles have been very extensively evaluated as the main component to develop *in vitro* diagnostic systems (mainly based on gold nanoparticles)(Chen et al. 2016). Localized Surface Plasmon Resonance (LSPR) is a phenomenon due to collective electron charge oscillations in the surface of some metallic (for example, gold) nanoparticles when they are excited by light. Since it is a surface phenomenon, any change in the surface of the nanoparticle (or its size, since it is a highly size-dependent phenomenon) will induce a change in the LSPR and, therefore, in the optical properties of the nanoparticle suspension, which can be then detected by a colorimetric method. The surface of the plasmonic nanoparticles can be modified to include a recognition molecule for the analyte of interest. After the interaction with the desired molecule, a modification on the surface due to the interaction with the recognition ligand will lead to a change in the color of the sample, which can be measured and analyzed to give a concentration of the analyte present in the medium. An excellent example is the home pregnancy test, which is a colorimetric assay for the detection of human gonadotropic hormone, and is based on gold nanoparticles (Azzazy & Mansour 2009). Gold nanosensors have also been developed to determine biomolecules due to nanoparticle aggregation when the analyte is present in the sample. The change in nanoparticle size and shape due to aggregation induces a change in the LSPR peak of a magnitude such that it is usually detectable by the naked eye. For example, systems for detecting DNA in the medium by decorating gold

nanoparticles with complementary single strand DNA, inducing aggregation and a sharp change in the LSPR peak (Azzazy & Mansour 2009; Chen et al. 2016).

The interaction of plasmonic nanoparticles with fluorophores can also be utilized to develop different types of nanosensors. When a florescent molecule is in close proximity to the surface of a plasmonic nanoparticle, there is a dipole-induced quenching of the fluorophore. After being removed from the surface, the molecule will recover its fluorescence, which can be detected in the sample. A system based on this effect was developed to detect proteins in the sample, due to the specific displacement of a fluorescent polymer from the surface of gold nanoparticles caused by the analyte (You et al. 2007; Chen et al. 2016).

Another type of sensors for *in vitro* diagnostics are those based on Surface Enhanced Raman Scattering (SERS), which can provide very high sensitivity and also giving information about the conformation of the analyte. For this reason, they are under extensive evaluation for immunoassays, amongst other applications (Chen et al. 2016).

3.1.3 Assays based on superparamagnetic nanoparticles.

A wide variety of superparamagnetic nanoparticles (especially iron oxide nanoparticles) have been developed for *in vitro* detection (Azzazy & Mansour 2009). Upon exposure to an external magnetic field, superparamagnetic nanoparticles can be employed to capture different analytes that are bound to them (by interactions with recognition ligands decorating their surface), separating them from the sample medium. An example is an immunoassay detection method for C-reactive protein (CRP) based on this kind of nanoparticles (Kriz et al. 2006). A monoclonal antibody for CRP was grafted to the nanoparticle surface. A policlonal anti-CRP antibody was also conjugated to silica microparticles (to ease sedimentation). The increase in magnetic permeability of the sediment correlated with the amount of CRP in the sample (Azzazy & Mansour 2009).

Superparamagnetic nanoparticles can also be used to separate pathogenic cells from healthy ones by decorating the nanoparticles with antibodies for specific markers of the pathogenic cells, which can then be separated from the

rest of the sample by applying an external magnetic field or with the use of a magnetic needle (Azzazy & Mansour 2009; Bryant et al. 2007).

3.1.4 Assays based on electric properties of nanostructures

The particular electric properties of different nanoparticles (like carbon nanotubes) can also be exploited to develop *in vitro* nanosensors. The high surface area of single wall carbon nanotubes (SWNTs), up to 1600 m^2/g allows grafting a vast amount of antibodies on their surface. The very efficient electric conductivity of SWNTs along their longitudinal axis (often referred to as ballistic electron conduction) enables the development of nanosensors with the desired performance. Such methods have allowed, for example, the development of a Prostate Specific Antigen (PSA) detection kit with better performance than commercial immunodetection assays, with a detection limit of 4 pg/mL (Ji et al. 2010; Rusling et al. 2009).

While all of the different strategies that have been briefly collected here are very exciting and promising for their application in clinical diagnostics, several problems are still to be addressed before the majority of them will reach the clinical setting. One of the most important ones is getting the nanoparticle-based diagnostic assays to work properly in real complex biological fluids like those that are found in the clinical setting.

3.2 *In vivo* imaging

Another promising field for nanotechnology application in medicine, and still in the diagnostic context, is the use of nanoparticles as imaging agents *in vivo*. Due to several characteristics of diseased tissues, like tumors, nanoparticles can be preferentially located in those pathological locations. If the nanoparticle is designed in order to be detected generating some kind of image, then the clinician can take advantage of that selective accumulation of nanoparticles to provide a diagnosis or a prognosis of the pathological situation of a particular patient, or can be used to assess the evolution of the pathology throughout the treatment or the success of a surgical intervention.

There are several means by which nanoparticles can be used to generate a diagnostic image *in vivo*, depending on the physical phenomenon in which

those nanoparticles will be involved. They can be divided in optical imaging, magnetic resonance imaging (MRI), radioisotope imaging and X-Ray computed tomography (CT) imaging. In the last years, the preparation of nanoparticles that enable the use of different imaging modalities simultaneously has been extensively evaluated. This multimodality can allow a single nanoparticle formulation to combine the advantages of the different techniques. Again, Inorganic nanoparticles will be of outmost importance in this context.

3.2.1 Optical imaging

Optical imaging methods are based on the difference in the optical properties of the contrast nanoparticle and the background signal from the surrounding tissue. Most of the current optical imaging nanodevices are based on fluorescent nanoparticles. Fluorescent nanoparticles (like QDs of dye-doped silica nanoparticles) present several advantages over traditional organic dyes, such as improved photostability (with greatly diminished photobleaching effect) and the capability to accumulate in the desired tissues by targeting strategies. The development of highly efficient NIR-emitting nanoparticles is a rising strategy in this context. QDs are specially promising due to their narrow emission spectrum in a finely tunable region (and heavy-metal free QDs would be highly desirable due to toxicity issues related to nanoparticles containing heavy-metal).

Photoacoustic imaging is another strategy for optical bioimaging. It is based on the generation of an acoustic wave as a consequence of heat generated by light absorption by the contrast nanoparticles (Chen et al. 2016). It presents several advantages, since it combines the higher contrast of optical imaging with the higher penetration and spatial resolution of ultrasound imaging. One example of this strategy is the development of indocyanine green-loaded nanoparticles to provide photoaccoustic imaging *in vivo* (Witte et al. 2008).

Upconversion imaging can be performed with upconversion nanoparticles, which show a significantly better safety profile than QDs. The excitation of these nanoparticles is generally in the NIR region, allowing the excitation of nanoparticles located deep inside the body. Besides, the imaging background can be diminished by using short-pass filters, since the emitted light is

significantly shifted from the excitation source (Chen et al. 2016). Upconversion nanoparticles in which both the excitation and the emission wavelengths are in the Infrared region seem very promising for this application, since they allow the imaging of deep tissues and organs with a low background noise. An example of this kind of particles was developed with $NaYF_4$ nanocrystals doped with Yb^{3+} and Tm^{3+}, showing an excitation wavelength of 975 nm and an emission one of 802 nm (Nyk et al. 2008).

3.2.2 Magnetic Resonance Imaging

There are two modalities of MRI: T_1 (relying on spin-lattice relaxation of protons in the organ/tissue) and T_2 (dependent on spin-spin relaxation of protons in the organ/tissue). It creates images with good spatial resolution, but contrast agents are often needed to improve the sensitivity of the technique. These contrast agents are magnetically active species that can be divided in T_1 or T_2 agents depending on which of the modalities provides a better contrast with that particular contrast agent.

The most common T_1 contrast agents are species containing Gd (III) as the magnetic agent, chelated with either diethylamine pentaacetic acid (DTPA) or tetraazacyclododecane tetraacetic acid (DOTA)(Chen et al. 2016). These are the most widely used MRI contrast agents in the clinical setting, even though there is some concern about the toxicity of Gd (III) ions that can be released. Modifying the surface of nanocarriers with DTPA or DOTA in order to chelate Gd (III) is a very common and successful way to provide MRI capabilities to virtually any type of nanodevice that could be used simultaneously with therapeutic purpose. This kind of strategies allow for real-time monitoring of the therapy. Gd (III) ions can also be incorporated into an inorganic matrix of different types of nanoparticles (like upconversion nanoparticles) to provide MRI contrast ability. The use of nanoparticles capable of providing different imaging capabilities is promising, since they would allow taking advantage of the benefits of the different imaging modalities with a single formulation (Chen et al. 2016).

Superparamagnetic iron oxide (Fe_3O_4) nanoparticles can be used as negative T_2 contrast agents (since they reduce the spin-spin relaxation of the proton and

therefore, give a dark contrast). They have been used in clinical MRI and their lack of toxicity is one of their biggest advantages. However, the contrast that these types of particles provide is rather low, and has to be improved in order to be more extensively used (Chen et al. 2016).

Another option would be using nanoparticles to produce magnetic resonance imaging using spin transitions of the nucleus of other species, like ^{19}F. This isotope can be introduced in the structure of different nanoparticles in a fairly easy manner, allowing MRI with increased sensitivity and very low background noise, due to the lack of ^{19}F in the organism. This promising strategy would only need minor modification in existing MRI devices to allow their use for ^{19}F detection (Chen et al. 2016).

3.2.3 Radioisotope imaging

The incorporation of radioactive isotopes in different types of nanoparticles allows their use for *in vivo* imaging. The radioactive isotopes to be used are chosen to emit low energy species, so that there will not be any radiotoxicity derived from their use for bioimaging. The most common modalities of radioisotope imaging are positron emission tomography (PET) and single photon emission computed tomography (SPECT)

In PET imaging, a radioisotope (like ^{124}I or ^{64}Cu) emits γ rays that after detection, are used to generate a three dimensional image. Nanoparticles allow a large number of radioisotope labeling, providing a great sensitivity and reducing the amount of contrast agent needed to perform the imaging. The most common ways to obtain nanoparticles for PET imaging are the use of chelators in the surface of the nanoparticles and radiolabeling by ion exchange within the nanoparticle matrix. SPECT imaging is based on a similar rationale, and suitable radioisotopes (like ^{125}I) can also be included in a nanoparticle structure to evaluate nanoparticle distribution with whole-body *in vivo* SPECT imaging (Chen et al. 2016).

3.2.4 X-ray Computed Tomography

CT imaging is based on using several X-Ray scans to produce a tomographic image after being processed by a computer program. CT contrast is very high

for hard tissues, but it is not sufficient when the objective is to image soft tissues, where a contrast agent is needed. Atoms with large atomic number provide high contrast in X-ray images, and are therefore used for CT imaging. For this reason, gold nanoparticles with different shapes have been extensively studied for CT imaging. Some of the main reasons are, besides the high contrast in X-ray, their easy synthetic procedures, biocompatibility and the capacity to finely tune their optical properties in order to combine CT imaging with other optical modalities. Other inorganic nanoparticles, like upconversion nanoparticles containing Yb have also been studied for this application (and also in combination with optical imaging modalities) (Chen et al. 2016; Liu et al. 2012).

4. Nanoparticles for therapy

Nanoparticles can be designed for therapeutic application based on two different (but compatible) approaches: either the nanoparticle itself is the therapeutic agent that will exert the desired function (material-based therapy) or the nanoparticle is used as a carrier for a therapeutic molecule (constituting a nano-Drug Delivery System, nano-DDS). Nano-DDS can overcome many of the problems related to traditional drugs for the treatment of several diseases. They are particularly useful when dealing with toxic drugs of hydrophobic ones that are difficult to administer in a stable formulation. Therefore, nanomedicine can improve the bioavailability, and increase the target specificity while decreasing the systemic toxicity of a wide variety of drugs. The inclusion of drugs inside a nanocarrier can also protect the drug from degradation that might take place when exposed to the physiological environment (such as enzymatic degradation) (Chen et al. 2016). This can potentially allow the use of drugs that would otherwise be unable to reach the clinic, either due to poor solubility, systemic toxicity of lack of chemical stability.

Multifunctional nanosystems can be obtained by adding therapeutic capabilities to the *in vivo* diagnostic nanoparticles already discussed, developing theranostic nanodevices. Nanoparticles can be used for a wide variety of pathological conditions, including infection, osteoporosis, gene therapy, cancer

treatment and others (Chen et al. 2016). However, most of the research has been focused on the use of nanoparticles for cancer treatment.

4.1 Cancer nanomedicine

The most important reason why the vast majority of nanomedicine research has been focused on cancer is what has been called the Enhanced Permeation and Retention (EPR) effect (**Figure 3**). By that name, Maeda defined in the 1980s the preferential accumulation in tumor tissues of macromolecules and nanosized structures (Matsumura & Maeda 1986). This effect is possible due to the fast and chaotic growth of most solid tumors. During that indiscriminate growth, the tumor cells are capable of inducing the formation of blood vessels (in a process called angiogenesis) in order to receive enough nutrients and remove waste products from their metabolism. However, and in contrast to the formation of healthy blood vessels, this angiogenesis is fast and disorganized, leading to imperfect blood vessels, leaving pores in the walls of capillaries that are bigger than those in healthy tissues and organs. The presence of those pores or fenestrations allows the extravasation of large macromolecules and nanoparticles to the diseased site, what would not be possible in a healthy tissue (enhanced permeation). Besides that, solid tumors are generally very compact structures with a high interstitial pressure. Under those conditions, the lymphatic vessels present in the tissue will be blocked, preventing the drainage of extravasated particles (enhanced retention). The discovery of this phenomenon in the 1980s lead to the proposal of using nanoparticles to treat tumors, since the enhanced accumulation would allow the delivery of higher doses of antitumor drugs in the tumors, potentially reducing the dose of the drug and, therefore, reducing side effects without compromising the efficacy of the treatment. The EPR effect became then the main justification for the development of nanomedicine, and would provide what would be known in the field as a “passive targeting”.

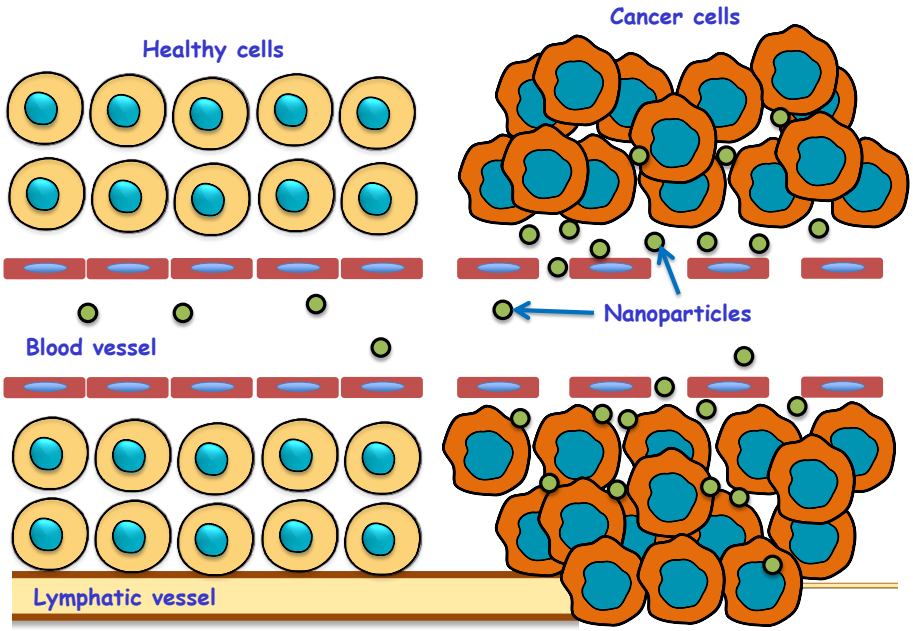

Figure 3. Schematic representation of the enhanced permeation and retention (EPR) effect.

Nanoparticle accumulation in the tumor area by the EPR effect relies on the circulation time of the nanoparticles in the bloodstream. After intravenous injection of the nanoparticles, a series of steps must take place to allow the EPR effect to take place. First, the nanoparticles have to be stable in suspension while circulating in the blood. Otherwise, nanoparticle aggregation will lead to the obstruction of blood vessels, potentially compromising the patient´s life. Then, the nanoparticles have to remain in the bloodstream long enough to extravasate to the tumor in a sufficient amount to elicit the therapeutic effect. One key aspect to consider regarding nanoparticle circulation time is the opsonization of the nanoparticles (Wicki et al. 2015). Opsonization is a process by which a pathogen or foreign body is surrounded by a type of proteins called opsonins. Opsonins act as a label for phagocitic cells to ingest the labeled structure in order to destroy it. When nanoparticles without any surface modification are put in contact with the blood, they are covered by a mixture of different proteins (forming what is called the protein corona). The formation of this protein corona can accelerate very dramatically the clearance of the nanoparticles from systemic circulation due to the presence of opsonins in its composition. One of the most employed strategies to slow down the formation of the protein corona consists on modifying their surface with highly

hydrophilic moieties that will hinder protein adsorption on the nanoparticle surface. The most common molecule to achieve this is polyethyleneglicol (PEG), and the process of coating a nanoparticle with PEG has been called PEGylation.

PEGylation has been shown to significantly increase circulation time of nanoparticles and, therefore, their accumulation in tumors (Wicki et al. 2015). However, the protein corona will eventually form (although more slowly), and the nanoparticles that have not yet reached the diseased site will be removed from the circulation by the organs of the reticuloendothelial system (RES, mainly liver and spleen). It has been recently estimated that the amount of nanoparticles that arrive to the tumor tissue is in average less than 1% of the injected dose (Wilhelm et al. 2016). Even though that number seems low, it is greatly superior to the percentage of free drug that would reach the tumor site without the nanocarrier (Lammers et al. 2016). Moreover, several strategies can be applied to further improve nanoparticle accumulation in tumors, like injecting angiotensin II (a vasoconstrictor) to increase the systemic blood pressure and facilitate nanoparticle leakage to tumors (Maeda et al. 2013), normalizing tumor vasculature to diminish interstitial pressure within the tumor (Chauhan et al. 2012) or destabilizing the blood vessels in the tumor by hyperthermia or ultrasound to increase their permeability (O'Neill et al. 2009).

4.1.1 Active targeting of nanoparticles

In order to improve the efficacy of nanoparticles for therapy, a second strategy of nanoparticle targeting was then developed, the one known as "active targeting". Active targeting is based on decorating the nanoparticle surface with molecules that would induce selective internalization in tumor cells, and decreasing the amount of particles that would reach healthy cells. When the release of the cytotoxic agent takes place inside the tumor cell, the high local concentration of the drug would increase its efficacy, lowering even more the required dose of the highly toxic antitumor drug. The most studied types of molecules used for active targeting are either nutrients (for which the tumor cells overexpress receptors, due to their high demand to allow their rapid growth) or antibodies (for specific antigens present in the tumor cell membrane).

These targeting molecules can be small molecules (like folic acid (Stella et al. 2000) or biotin (Yang et al. 2009)) or macromolecules (like the protein transferrin) (Greish et al. 2014). Another step of nanoparticle targeting can take place after cellular uptake of the nanodevice. In this case, the surface of the nanoparticles is also decorated with moieties that can drive nanoparticle accumulation in a particular subcellular structure or organelle. For example, nanoparticles presenting triphenyl phosphonium moieties on their surface are known to target the mitochondria, and are often used to carry drugs that act at the mitochondria, increasing the therapeutic efficacy of the nanodevice.

It is worth noting that, since active targeting strategies involve the interaction of nanoparticle with a receptor in the target cell membrane, this strategy relies on passive accumulation first, which would bring the particles close enough to the cells so that active targeting can take place. Once the nanoparticle has reached the tumor environment and has interacted with the tumor cell receptors, another consideration must be taken. If the interaction with the cell receptor is very efficient in inducing nanoparticle uptake, a paradoxical effect can appear. The first line of cells in the tumor will internalize all of the nanoparticles, preventing their penetration to deeper areas. This might hinder the therapeutic efficacy of the nanoparticles, since most of the tumor will not be exposed to them. In order to try to solve this issue, hierarchical targeting strategies are being developed (Wang et al. 2016). In these strategies, the targeting moiety is hidden until the nanoparticles are already distributed in the tumor. Then, the presence of an internal or external stimulus will induce the exposure of the targeting moiety, allowing particle uptake by the tumor cells. These kinds of strategies would also be useful to increase the circulation time of those targeted nanoparticles, since the presence of targeting moieties on the surface of PEGylated nanoparticles has been shown to facilitate their removal from the bloodstream by the RES (Wang et al. 2016). If the targeting ligand is hidden until after the nanoparticles have accumulated in the tumor, the nanoparticles will be able to remain in the circulation for longer periods of time. After extravasation to the tumor tissue, PEGylated nanoparticles are poorly internalized in tumor cells (due to what has been called the "PEGylation dilemma") (Hatakeyama et al. 2013). However, with these hierarchical targeting strategies, the exposure of the targeting

molecule will allow their successful internalization in tumor cells, increasing their therapeutic efficacy (Wang et al. 2016).

Besides this problem, other considerations must be taken into account. First, nanoparticle diffusion in tumor tissues is hindered by the high interstitial pressure. Therefore, the size of the nanoparticles will be a key factor in determining whether the particles can reach deeper areas of the diseased tissue. To attack this problem, size-changing materials are being developed to allow for a deeper penetration once the nanoparticles have reached the tumor (Wang et al. 2016). Besides, strategies directed to reducing interstitial pressure are also interesting approaches with potential to obtain very positive results (like using collagenase prior to nanoparticle injections) (Goodman et al. 2007).

All the strategies we have discussed so far depend on the EPR effect to induce selective accumulation of the nanoparticles in the tumor microenvironment. However, it is well established that the EPR effect presents a very high variability, even amongst tumors of the same type, and it changes greatly when discussing different tumor types (Greish et al. 2014). Two possibilities arise from this perspective: either developing methods that would allow for a selection of patients that are most likely to respond to nanoparticle-mediated therapy (by determining whether their particular tumor presents a strong EPR effect or not), or developing new strategies to induce nanoparticle accumulation in tumors that do not rely on the EPR effect (Greish et al. 2014; Hu et al. 2010).

4.1.2 Material-based therapeutic approaches

Several approaches have been studied to induce therapeutic effects by using nanoparticles without relying on drug release, because the nanomaterial itself is responsible for the desired response. These effects are often used in combination with drug release from the same nanoparticles, looking for a synergistic effect between them. Some of the most important ones will be briefly mentioned here.

Photodynamic therapy (PDT): PDT is a type of therapy involving two components: light and a molecule called photosensitizer. None of them are toxic individually, but they are capable of inducing cell death when they are

combined. Photosensitizers (generally porphyrin molecules) can be excited by light (at different wavelengths depending on the particular photosensitizer) into a triplet state. This excited photosensitizer in its triplet state can transfer its energy to oxygen in the medium, producing highly reactive singlet oxygen (Chen et al. 2016). The short half-live of singlet oxygen implies that only the cells very close to the photosensitizer will be affected by the generation of those reactive oxygen species (ROS). Since photosensitizers share many shortcomings with other drugs regarding their poor solubility and difficulty to get high concentrations in the tumor tissue, the use of nanoparticles to transport them to the desired area of the body has emerged as a very powerful tool to increase the efficacy of PDT (Chen et al. 2016). As PDT will happen independently of whether the photosensitizer is free in solution or inside a nanoparticle, the sensitizer can be included in the formulation without having to be released to induce the desired effects.

Photothermal Therapy (PTT): In PTT, the nanoparticle itself is responsible for the biological effects of the therapy. PTT is mainly based on the plasmonic absorption of metallic nanoparticles. Metallic nanostructures can be designed to absorb Near-Infrared (NIR) or Infrared (IR) light, generating heat as a consequence. If the nanoparticles are embedded inside a tumor mass, the increase in the temperature will eventually cause cell death by necrosis (cancer cells are more vulnerable to hyperthermia than healthy cells, in a temperature range 40-43ºC)(Chen et al. 2016; Hildebrandt 2002). Gold nanorods are amongst the most studied nanoparticles for PTT, since they can generate heat very efficiently when exposed to NIR light, while also having an easy and finely tunable synthesis. Another type of nanoparticles that is under extensive evaluation for PTT is graphene oxide, which has been shown to generate heat when exposed to 800 nm lasers. Also, incorporating NIR dyes or porphyrins in the structures of different nanoparticles not only allows their use for imaging, but also the generation of PTT (obtaining theranostic nanodevices) (Chen et al. 2016).

Magnetic hyperthermia: Besides the possibility to increase accummulation of superparamagnetic nanoparticles in tumors by the application of an external magnetic field (magnetic targeting), these nanoparticles can also generate heat

when exposed to an alternating magnetic field (Chen et al. 2016). In the same way as for PTT, the local heating caused by magnetic hyperthermia can cause the death of cancer cells without the need for any drug molecules to be released from the material. The possibility of obtaining theranostic nanodevices for magnetic hyperthermia is also exciting, since superparamagnetic iron oxide nanoparticles that can be employed to induce it can also be used to provide MRI capabilities.

4.1.3 Nano-Drug Delivery Systems

Most of the work that has been developed about the therapeutic application of nanoparticles involves using them as DDS, so that they can improve the pharmacokinetic parameters of different active molecules, getting them to the site of action. Once the nanoparticles are located in the tumor area, nanoparticles carrying cytotoxic drugs will have to release them so that they can perform their action. The nanoparticles acting as nano-DDS can be designed to present a controlled release of the drugs they contain. Nanoparticles can be prepared to provide prolonged release of the drug, for example by introducing the active molecule in a biodegradable matrix (PLGA nanoparticles are a typical example of this strategy) (Chen et al. 2016). As the nanoparticle degrades, the drug is released to the medium, and carries out its function. However, since anticancer drugs are highly toxic, it would be very interesting to develop a nanocarrier that can release such drug only in the diseased site, without any drug loss during transport. In that context, as plethora of stimuli-responsive materials have been evaluated (most of them *in vitro*, although many *in vivo* evaluations have also been performed). In these materials, drug release is hindered by some component in the formulation that can respond to differences in its environment, inducing a change in the formulation that will lead to drug release. The different stimuli that can be employed to this end can be divided in internal and external stimuli (**Figure 4**).

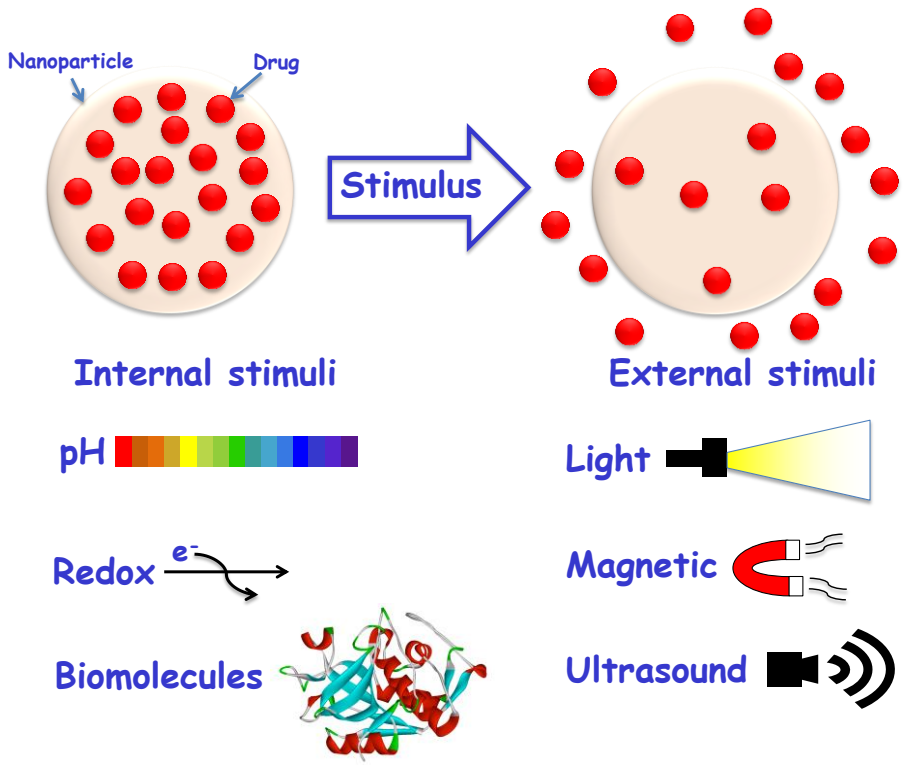

Figure 4. Concept of stimuli-responsive drug release. Different stimuli reported in the literature divided in internal or external stimuli.

Internal stimuli are differences in tumor tissues or tumor cells that are not present in the healthy counterparts (or are much less intense). Therefore, once the nanoparticle is in the tumor, the presence of such stimulus will induce some change that will provoke drug release. Typical examples of internal stimuli in cancer therapy are pH (tumors possess a lower pH than healthy tissues, and the pH in some intracellular compartments, like lysosomes, is also much lower than in the extracellular medium) (Liu et al. 2010), redox potential (which also presents significant changes in the intracellular compartment compared to extracellular media) (Z.-Y. Li et al. 2015), and the presence of different proteins or signaling molecules (like Matrix MetaloProteases, MMPs) (Singh et al. 2011). For example, pH-responsive mesoporous silica nanoparticles can be obtained by grafting gold nanoparticles acting as gatekeeper through a pH-labile acetal group (Liu et al. 2010). Once the nanoparticles are exposed to an acidic pH, the acetal linker is cleaved and the cargo inside the nanoparticle pores can be released. Another example are redox-responsive polymeric nanoparticles that can be obtained by the introduction of disulfide bonds inside the polymer

structure (Song et al. 2011). Once in the reducing intracellular environment, the disulfide bonds are broken and the nanoparticle releases its cargo. An advantage of internal stimuli is that once the nanoparticle is administered, no further action is needed from the clinician. This would in theory ease the translation of these strategies. However, a general piece of criticism towards these stimuli is that most of the differential characteristics between healthy and tumor tissues are not as exacerbated in reality as it is supposed in most research articles dealing with them, what would hamper the correct behavior of the material in real clinical situations.

On the other hand, external stimuli have also been extensively evaluated. External stimuli are not present in the organism and they are not a consequence of the pathology. They are exogenous agents that the clinician might apply to the diseased site after the administration of the nanocarrier and would then interact with the formulation, inducing drug release. Examples of external stimuli that have been evaluated are light (Martínez-Carmona et al. 2015), magnetic field (Guisasola et al. 2015) or ultrasound (Grüll & Langereis 2012; Schroeder et al. 2009; Li et al. 2016; Paris et al. 2015). For example, light-responsive liposomes can be obtained by taking advantage of the same effect as the one used for photodynamic therapy. A photosensitizer can be included in the formulation, as well as an unsaturated lipid. Once the formulation is exposed to light, ROS are generated, inducing the peroxidation of the unsaturated lipid component of the lipid bilayer (Carter et al. 2014). The lipid bilayer permeability is therefore modified, inducing drug release. As an example of magnetic-responsive drug release, superparamagnetic nanoparticles embedded in a mesoporous silica matrix can be employed to induce an increase in the local temperature when exposed to an alternating magnetic field, what can then induce a conformational change in a temperature-responsive polymeric gate, allowing cargo release from the material (Guisasola et al. 2015). An advantage of these kinds of stimuli is that, since they are not present in the organism, designing formulations that are sensitive to them will provide a high selectivity in the material response. On the other hand, there are several concerns about their use, like the poor penetration of the stimulus in the

organism (for example, with light), difficulty to focus the stimulus in the tumor or toxicity associated to the stimulus alone.

4.2 Nanomedicine for other applications

4.2.1 Infection: Nanoparticles can be used to deliver different antimicrobial drugs to treat different infectious diseases, by developing the so-called "nanoantibiotics" (Huh & Kwon 2011). Nanoantibiotics can be developed by using nanoparticles with inherent antimicrobial activities (like Ag nanoparticles, fullerenes or chitosan nanoparticles) or by acting as drug delivery vehicles that can release antimicrobial drugs (Huh & Kwon 2011). Antibiotic-loaded mesoporous Silica Nanoparticles have been proposed for intracellular pathogens like *Francisella tularensis* (Z. Li et al. 2015). The main rationale for this work was the fact that *F. tularensis* is found in macrophages of the infected host. Macrophages are professional phagocytes that tend to engulf large amounts of nanoparticles after they are injected in the bloodstream. Once nanoparticles have undergone uptake by them, the antibiotic moxifloxacin was released, killing the bacteria inside the macrophages and showing promising *in vivo* results for the treatment of Lethal Pneumonic Tularemia (Z. Li et al. 2015). Antimicrobial nanomedicine has gained great attention due to antibiotic resistance. Nanoantibiotics can be used to deal with this problem either by taking advantage of different mechanisms of action (less likely to induce resistance, like using silver or other metal nanoparticles), by better targeting the diseased site or by employing combinations of different drugs in a single formulation (combination therapy).

4.2.2 Osteoporosis: Osteoporosis is nowadays one of the main reasons of morbidity in the aging population (Luhmann et al. 2012). Nanoparticles have been proposed for the treatment of osteoporosis in order to try to improve the efficacy of anabolic (increasing bone production) and anti-resorptive (decreasing bone elimination) therapy (Luhmann et al. 2012). This could happen mainly by increasing the local concentration of the drugs of interest in the diseased site. For that reason, different strategies have been developed to improve bone targeting of nanomedicines, mainly by decorating the nanoparticle surface with different bisphosphonates (that present a strong

interaction with calcium present in the inorganic phase of bone, hydroxyapatite) or with the collagen binding domain of different proteins (targeting therefore collagen, the main organic component of bone) (Luhmann et al. 2012). Once located in the diseased bone, the nanoparticles can be used to release different therapeutic agents, like Parathyroid Hormone (PTH) or Bone Morphogenetic Proteins (BMPs), which are growth factors that have been extensively studied for bone regenerative therapy (Luhmann et al. 2012).

4.2.3 Gene Therapy: A great number of diseases are caused by a malfunction of one or several genes, and gene therapy implies a modification in the structure or expression of such genes to treat the pathologies that arise from that malfunction. The main barriers that gene vectors encounter to allow effective gene therapy are: prevention of degradation of the nucleic acid (DNA or RNA), efficient uptake by the target cells, release of the nucleic acid in the cytoplasm and outside of the endolysosomal system, entering the nucleus and producing sufficient gene expression (Chen et al. 2016). The use of a targeted nanocarrier that can protect the nucleic acid during transport and can release it in the proper location can therefore overcome most of those concerns. Especially appealing is the strategy of delivering small interference RNA (siRNA) to modify the expression of different genes of interest. The most common way of introducing nucleic acids in a nanoformulation is through the electrostatic interaction between the negatively charged nucleic acid (DNA or RNA) and a positively charged nanostructure, generally by using polycationic polymers. Among all the available polycations, the ones that are most widely used are polyethyleneimine (PEI, either linear or branched), PAMAM and chitosan (Chen et al. 2016). Besides polymeric nanoparticles composed of these kinds of polycations, liposomes with positively charged lipids are also very widely used for this application. The use of nanoparticles with high positive charges presents another advantage: lysosomal escape capacity. Nanoparticles with protonable groups under acidic conditions are capable of inducing lysosomal escape *via* the Proton Sponge Effect. Once the nanoparticles are inside the lysosomes after endocytosis, at the lysosomal low pH, the protonable groups acquire a high positive charge, which would tend to be neutralized by the entrance of chloride anions. These osmotically active ions enter the

lysosomes accompanied by water, making the lysosome swell until it bursts, releasing the nanoparticles in the cytoplasm (Sahay et al. 2010). The nanoparticles can also be modified by endosomolytic agents to induce lysosomal escape of the nucleic acid-carrying nanoparticles (Chen et al. 2016).

5. Conclusion

Nanomedicine is a multidisciplinary field aimed to develop diagnostic and therapeutic tools based on nanoparticles with different chemical compositions. The selection of a particular nanoparticle type and their further modifications are selected based on the particular application of interest.

The great versatility of nanotechnology enables the design and preparation of highly sensitive and specific diagnostic assays that could allow early detection of different pathologies, hopefully improving the prognosis of the patients.

Nanoparticles can also be used *in vivo* for the diagnosis and treatment of several diseases (sometimes at the same time, by developing theranostic nanoparticles). With the help of nanoparticles, more sensitive imaging contrast agents can be obtained for their use with different imaging techniques. Recently, great attention has been attracted by nanoparticles that can act as contrast agents for several imaging techniques simultaneously. At the same time, nanoparticles can constitute powerful tools for the treatment of those same diseases, either by their own intrinsic characteristics, or by using them as carriers of different drugs. It is worth noting that, for each particular application, the pathological characteristics of the diseased tissues and cells have to be taken into account.

The evolution of the field of nanomedicine in the last decades holds great promise, and their use could revolutionize the practice of medicine in a wide variety of clinical situations. However, before that can happen, all of the problems of current strategies have to be carefully addressed by the nanomedicine scientific community.

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