Peer-reviewed version available at *Nanomaterials* **2019**, *9*, 1365; doi:10.3390/nano9101365

Review

Interactions of Nanoparticles and biosystems: microenvironment of nanoparticles and biomolecules in nanomedicine.

Carlota Auría-Soro[1,2], Tabata Nesma[1], Pablo Juanes-Velasco[1], Alicia Landeira-Viñuela[1], Helena Fidalgo-Gomez[1], Vanessa Acebes-Fernandez[1], Rafael Gongora[1], María Jesus Almendral Parra[2], Raúl Manzano-Roman[3]* & Manuel Fuentes[1,3]*

[1] Department of Medicine and General Cytometry Service-Nucleus, Cancer Research Centre (IBMCC/CSIC/USAL/IBSAL), 37007, Salamanca, Spain.

[2] Department of Analytical Chemistry, Nutrition and Food Science, Faculty of Chemistry, University of Salamanca, 37008 Salamanca, Spain.

[3] Proteomics Unit. Cancer Research Centre (IBMCC/CSIC/USAL/IBSAL), Salamanca, Spain.

* Corresponding authors

* Correspondence: mfuentes@usal.es (M.F); Tel.: 923294811 (Ph.D., Department of Medicine and General Cytometry Service-Nucleus, Cancer Research Centre (IBMCC/CSIC/USAL/IBSAL). Avda. Universidad de Coimbra S/N, 37007 Salamanca, Spain.); rmanzano@usal.es (RM-R), PhD. Proteomics Unit. Cancer Research Centre (IBMCC/CSIC/USAL/IBSAL), Salamanca, Spain.

Abstract: Nanotechnology is a multidisciplinary science covering matters involving nanoscale level that is being developed for a great variety of applications. Nanomedicine is one of these attractive and challenging uses focused on the employment of nanomaterials in medical applications such as drug delivery. However, the uses of these nanometric systems requires specific parameters to establish the possible advantages and disadvantages in specific applications. This review presents the fundamental factors of nanoparticles and it´s microenvironment that must be considered to make an appropriate design for medical applications: (i) Interactions between nanoparticles and their biological environment, (ii) the interaction mechanisms, (iii) and the physicochemical properties of nanoparticles. On the other hand, the repercussions of the control, alteration and modification of these parameters in the final applications. Additionally, we here briefly report the implications of nanoparticles in nanomedicine and provide perspectives for some particular applications which still are challenged.

Keywords: Nanoparticles, interactions, protein corona, nanomedicine, biomolecules.

1. Introduction

One definition of nanotechnology is the statement by the US National Science and Technology Council [1], which states: "The essence of nanotechnology is the ability to work at the molecular level, atom by atom, to create large structures with fundamentally new molecular organization. The aim is to exploit these properties by gaining control of structures and devices at atomic, molecular, and supramolecular levels and to learn to efficiently manufacture and use these devices." Other authors describe nanotechnology [2] as the combinatorial study and integration of scientific technological advances and medical engineering at the nanoscale level [3]. All these definitions cover the design and manipulation of nanomaterials. Therefore, nanomaterials are defined as materials with at least one dimension smaller than 100 nanometers that enhance physical, chemical and biological properties of the original material [4].

Over the last few years, nanotechnology and related disciplines have undergone an exponential growth in applications such as nanomedicine, energy and electronics, environment and materials

because of the unique properties of nanomaterials [5]. Nanomedicine includes the development of nanoparticles (among other nanocomponents and devices) for molecular diagnostics, treatment and prevention of human diseases thanks to their compatibility with biomolecules [6].

Nowadays, nanoparticles are an impacting innovation showing an increasingly presence in many scientific design and developments [7]. In order to understand their current and potential applications in biomedicine, it is necessary to collect information about their disadvantages, such as the cytotoxic effects in living organisms, because they may limit their clinic use [8]. But also highlight their important advantages which may them an ideal approach for biomedical applications, such as their intrinsic ability to enter human body through inhalation, skin and digestive routes depending on their physicochemical properties and they can access vital organs through the blood flow [9]. Some key factors that must be taken into consideration are: (i) The interaction of nanoparticles with their ecosystem, mainly with other nanomaterials and biomolecules. Some studies show the possibility of using AgNPs as antibacterial agents thanks to be highly toxic against human pathogenic bacteria. In this sense, Singh T. et al. have demonstrated the use of endophytic fungi Alternaria sp. to synthetize AgNPs [10]. (ii) Physicochemical properties and the design of the nanoparticle such as particle size, shape, dispersity, surface charge and protein corona effects. Protein corona is complex plasma proteins layer around the nanoparticles that takes place after systemic administration, when nanoparticles are exposed to physiological fluids, mostly blood.

The adsorption of dozens of proteins with varying identities and quantities on nanoparticles can modify their physicochemical identity and influences the physiological response such as cellular uptake, targeting, circulation lifetime in the blood and toxicity [11]. Dutta P. H. et al. synthesized and characterized two types on nanoparticles, AgNPs and AuNPs, in order to design an antimalarial potential nanomaterial. The size, shape and surface morphology of the bio-synthesized nanoparticles were taken into consideration to optimize them. Finally, they showed that AgNPs had insignificant and lower cytotoxicity against human cervical cancer cell line (HeLa) and rat skeletal muscle cell line (L6) than AuNPs. [12]. And (iii) As well as interacting biocompounds (biomolecules, cells, proximal fluids) that favour a physical, chemical and mechanical relevant process [1,5]. Mirkin showed that siRNA-based gold nanoparticles inhibit its enzymatic degradation and facilitates it´s uptake by Hela cells [13.]

Despite of their potential advantages and promising applications, there are still many problems associated to the entrance of the nanoparticle in a physiological environment, which may be justified with different intrinsic characteristics of the nanoparticles. As the nanoparticles are embedded in human proximal fluids, inside cells, culture media among others; then, there are multiple conditions and a huge variety of biomolecules which could interact with the nanoparticles and with other biomolecules. Because of these inherent interactions, the nanoparticles might have a heterogeneous morphology which is also correlated with the resulting immuno-biocompatibility of these nanomaterials.

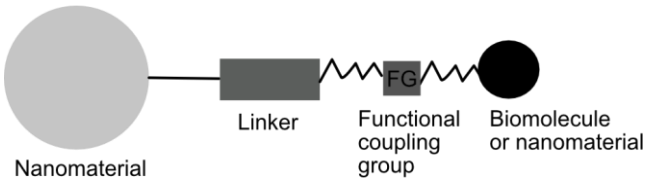

Figure 1. Schematic representation of method to couple nanoparticles and biomolecules or other nanoparticles.

Here, this mini-review is focused on global interactions of nanoparticles and biomolecules in biological environments which play a critical role in the final applications in biomedicine.

2. Nanoparticle-cell dynamics.

In a general way, nanoparticles enter the cells through different internalization mechanism (Figure 2), accumulate in targeted organs and later are eliminated.

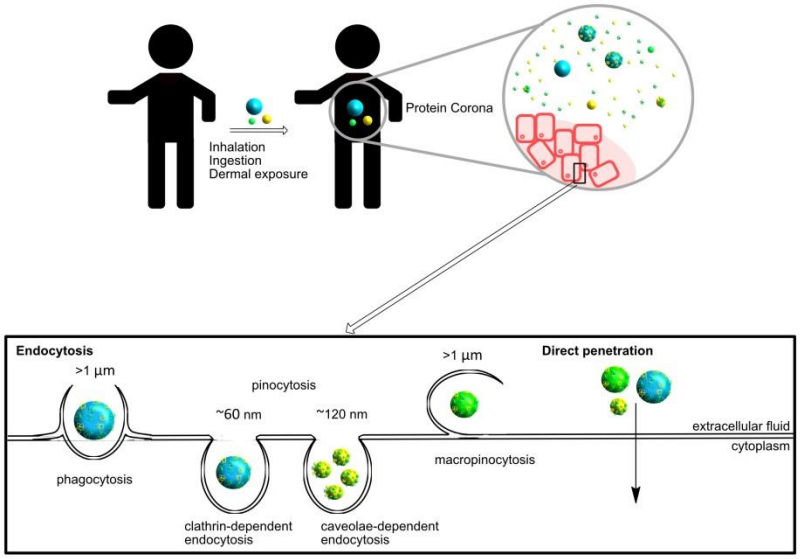

Figure 2. Schematic representation of different ways of nanoparticles to enter in human body and inside cells.

The small size of the nanoparticles allows them to enter the human body by inhalation, ingestion or through the skin. Once in the extracellular fluid they are conjugated with biomolecules presented in the media, which allows them to internalize in the cells through different mechanisms (phagocytosis, micropinocytosis, clathrin-dependent endocytosis, caveolae-dependent endocytosis or by direct penetration).

2.1. Cellular Internalization

The nanomedicines based on nanoparticles must cross the cell membrane to produce an effect inside the cells [14]. Then, nanoparticles rely on several and different mechanisms for cellular entry because commonly cell membranes are impermeable to diffusion by most of the nanoparticles (Figure 4). Hence, the cellular mechanism is very relevant on the nanomedicine application which is being desirable the suitable mechanism for internalization avoiding the non-specific internalization mechanisms because these ones are involved in toxicity and biocompatibility [15].

As it is expected, the size of nanoconjugate directly affects the internalization process; in fact, several studies report that nanoparticle size in the range 10-100nm have higher efficiency for cellular uptake, on the other hand, small ones implies a high energy cost to the cells [16]. Commonly, nanoparticles larger than 100 nm are internalized by specialized phagocytic cells (such as macrophages, dendritic cells).

Moreover, the optimal size for internalization inside the cells is strongly linked to the nanoparticle´s surface chemistry. In general, Van der Waals or electrostatic forces are critical in the interaction of the biomolecules and cells with the nanoparticles. In fact, several studies show correlation between zeta potential and endocytosis/exocytosis mechanisms [17]. Then, specific cellular internalization could be leaded by employing affinity ligands in order to increase internalization of the particles by specific interactions in comparison with nonspecific interactions (i.e. Hydrophobic). In this sense, antibody-coated nanoparticles present an internalization potential in targeted cells 4-8 folds higher than the positively or negatively charged nanoparticles without the affinity component [18]. Besides the use of antibodies for targeting delivery, non-specific interactions through chemical moieties are always present and influencing targets affinities so must be always take into consideration.

Protein adsorption also depends on the shape of nanoparticles and consequently affects the cellular uptake. It seems that spherical and highly homogeneous nanoparticle conjugates have better cellular uptake than amorphous and non-geometrically symmetric nanoconjugates [19]. Moreover, several authors claim that shape could be employed to prevent non-specific cellular internalization in the targeted cells [17].

2.2. Tumor Accumulation

Tumor tissues have a preferential accumulation of nanoparticles in comparison with the normal ones [20]; mainly because the vessels around the tumoral tissue have a higher permeability (than the normal vessels) and tumors have impaired lymphatic drainage which leads to retention of the permeated nanoparticles [21, 22]. In general, this effect is called enhanced permeation and retention (EPR) [23].

Regarding tumor accumulation, the tumors are densely packed with cells and extracellular matrix, then as it is expected the nanoparticles size plays an important role in the diffusion inside the tumor. Hence, the accumulation within the tumors could be modulated by the physical dimensions and surface chemistry of the nanoparticles. In general, diffusion and nanoparticle size are inversely correlated [24]. Then, nanoparticles with small size can diffuse freely across tumoral tissue and can present a widespread distribution within normal tissues; however, also because of their small size these small nanoparticles can easily and quickly clear out. By other way, when the nanoparticles are applied as imaging agents, the size is not as relevant as for therapeutics applications because only with nanoparticles on the periphery of the tumor helps to distinguish normal and pathological tissues.

As it is previously discussed, the biomolecules adsorption onto the nanoparticles surface is directly related with the opsonization and clearance capacity, thus related with blood concentration of the nanoparticles along time.

2.3. Elimination

In general, nanoparticles are eliminated from human body by renal and hepatobiliary routes. As it is necessary for clinical approval, the nanoparticles might be eliminated from the body in a reasonable timeframe. Then, the drug conjugated nanoparticles must be designed to avoid quick clearance and long period of body maintenance.

As it is expected, the elimination of nanoparticles is also influenced by the surface chemistry, shape and size. For example, surface chemistry is quite critical in the clearance efficiency even for small nanoparticles and PEG coating promotes more efficient hepatobiliary clearance [25]. Another point is the size, which is very important in secretion efficiency because the hydrodynamic size of the nanoparticles has a strongest influence on the renal clearance, where the glomerular pores are a physical barrier [21, 26].

3. Nanoparticle Interactions

Although nanoparticles in biological systems are surrounded by large quantities of biomolecules, depending on the different factors that characterize the biological environment and the nanoparticle, the nanoparticles themselves can generate interaction with each other. Multifunctional nanoparticles as nanomedicines (see Figure 3) are embedding in human proximal fluids, inside cells and culture media among others [27]. That implies a huge variety of different microenvironments with additional challenges for the design and development of nanoparticles suitable to be functional in all kind of conditions. However, depending on the conditions of the medium, pH [28], ionic strength, oxygen levels, organic matter, etc., nanoparticles can present different forms or stages, such as ionized [29], forming aggregates or combined into complex aggregates or even interacting with other nanomaterials [30]. In addition, this is especially relevant because it may be the origin of a heterogeneous morphology which might be also correlated with a lack of immunobiocompatibility of these nanomaterials [31].

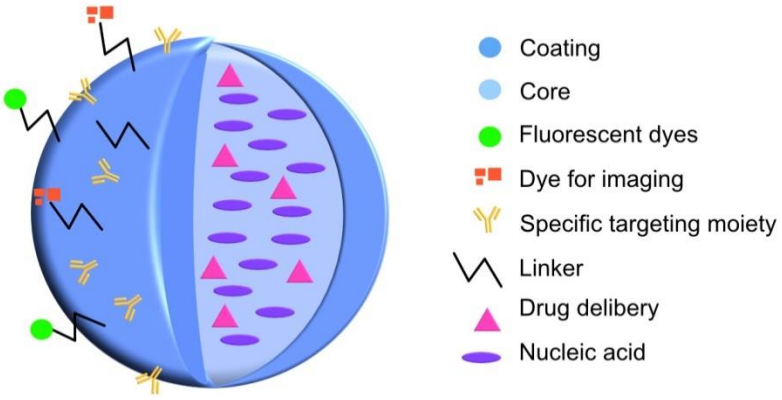

Figure 3. Schematic representation of a multifunctional nanoparticle for biology applications.

Nanoparticle aggregation and agglomeration have been recognized to affect cellular uptake and even potential toxicity based on the nanoparticle composition and the cell type [32]. Aggregation and agglomeration effects are often used in nanotechnology but both terms are commonly mistaken. On the one hand, aggregation indicates strongly bonded or fused particles [33] and it occurs when the van der Waals attractive forces between particles are greater than the electrostatic repulsive forces produced by the nanostructure surface [32]. On the other hand, agglomeration indicates more weakly bonded particles [33] and it does not require a definite pattern, shape, size. Pellegrino F. et at. studied the influence of agglomeration and aggregation on the optical properties of TiO_2 nanoparticles in order to demonstrate that this effect can lead to an incorrect assessment of the photoactivity [34]. Zook M. J. et al. [35] developed a bottom-up-based method to produce controllably, reproducible and stable nanoparticle agglomerates states in an aqueous medium. Finally, they used this method to show how silver nanoparticle agglomeration affects nanoparticle's hemolytic activity.

The main factors that will determine the type of interaction that takes place between nanoparticles are: the complementarity between nanomaterials, the distance between them and the geometry [36]. In addition, it is also essential to know what the main interactions drivers are in a nanoparticle assembly. For example, Van der Waals forces to form nanocrystal superlattice membranes, electrostatic interactions to obtain colloidal dimers, magnetic interactions where iron oxide nanoparticles coated with azobenzene-terminated catechol ligands can self-assemble by UV-light-induced, or even molecular force [36].

An example that demonstrates the importance of the complementarity between the materials and the influence of the forces used in such interaction is the one discussed by Pileni and co-workers. They stress the difference of using octanoic and dodecanoic acids as organic ligands in magnemite nanoparticles in absence (only with dipolar forces between the magnetic nanoparticles) and presence of Van der Waals interactions, when the distance is small [37,38].

By other hand, interaction between molecules on surfaces is highly dependent of surface functionalization. That implies the presence of reactive chemical moieties, being homo- or hetero-functional depending if there is only one chemical group onto the surface or co-exist different chemical reactive groups [39]. Due to their composition and structure, the surface may or not allow different types of interactions. Thus, for example circulatory cells are covered by a lipid bilayer with proteins and polysaccharides that, depending on the groups that the nanoparticle possesses, will favor one or another interaction mechanism [40]. Another example is manifested in proteins affected by their molecular weight, charge (there is greater adsorption near the isoelectric pH where repulsion of charges between different adsorbed molecules is minimized) or its stability (which influences the number of binding points [41]. A soft protein layer, which has a low structural stability, has a greater number of active centers through which it can interact); besides affecting physicochemical factors of the surface (i.e., humectability). The surface ratio of hydrophobicity/hydrophilic influences the

protein degree of reactivity and/or it´s adsorption properties. Another remarkable feature is the size, those with a size comparable to that of the nanoparticle will be more easily adsorbed.

Finally, it is not only necessary to consider the concentration or size of NPs, but also the species and quantity of resulting products from chemical interactions between nanoparticles.

3.1. Interaction mechanisms between nanoparticles and biomolecules

Regarding the biomolecules, there is a wide-open variety of different biomolecules which could interact directly onto the nanoparticles surface or through other biomolecules that are coating the nanoparticles surface (Figure 2). These layers of coating biomolecules are directly related with the type of organism, biological fluid, cells etc., among the physicochemical conditions of the media and nanoparticle surface, nature and structure of biomolecules.

According to the literature, most of the relevant interacting biomolecules to the nanoparticle surfaces are proteins and nucleic acids [42]. Proteins have many different binding sites (as amino acidic key structures and/or post-translational modifications) onto nanoparticles surfaces through specific or non-specific adsorption [41,43]; in addition, the proteins are critical on the immune-biocompatibility of the nanomaterials. Nucleic acids are another biomolecule of interest, which have many different applications as a consequence of its physicochemical stability, mechanical rigidity, easy accessibility and its high specificity of base pairing, result in a suitable receptor easily to design for molecular nanoconstruction [44].

Regarding interactions with biomolecules of the human organism, two factors must be considered in the description of the interaction [21]. First one is that nanoparticles in biological systems are surrounded by multiple potentially interacting biomolecules that may modify and saturate their surface. Therefore, these modified nanoparticles are the ones that actually interact with the biomolecules later on. The second factor is that nanoparticles enter the human body through multiple pathways, depending on which ones can influence the force of the interaction. For example, nanoparticles interact strongly with the pulmonary system (proteins and phospholipids) by inhalation.

Two immobilization mechanisms have been studied through interaction with different types of biomolecules [43]: by simple absorption or by chemical linkages. (i) Immobilization biomolecules on nanoparticles through adsorption is a very usable method between nanoparticles and enzymes because it takes place through non-covalent forces (hydrogen bonding, ionic interactions and Van der Waal forces), mainly through negatively charged phosphate groups and hydrophobic moieties, and it does not disturb the initial structure of the enzyme or its active site. (ii) Immobilization through chemical linkages consists of the biomolecule immobilization on a biocompatible matrix, such as within phospholipid bilayer, that it does not interact with the biological activity and native structure of biomolecule.

We also find two other types of mechanisms [45] to interact nanoparticles with cells: ligand-receptor interaction and chemical conjugation. An example of this first adhesion method is the functionalization of the surface of nanoparticles with a receptor, such as streptavidin-biotin, its non-covalent interaction results in greater bond strength, provides resistance to pH variations, temperature and denaturants. In addition, they together have a greater affinity for binding to cells. Chemical conjugation simply consists of the coupling of functional groups (such as thiol groups) to the surface of the nanoparticle, which favor subsequent binding to the cell and which, in turn, reduce the toxicity of this interaction. A disadvantage of this method of interaction is that, in terms of biomedical applications, the covalent binding of the drug to the nanoparticle restricts its efficient release, limiting its effectiveness.

3.2. Nanoparticle design: influence on interaction mechanisms

Interactions between different systems and nanoparticles depend on the changes that nanoparticles undergo in a concrete environment, for example the generation of a coating protein corona once plasma proteins are adsorbed on its surface, so it is a must to study the nanoparticle states and characteristics prior to interaction assays [46].

Many nanoparticle-based investigations focus on issues affecting nanoparticle characteristics and subsequently their impact on cellular internalization and biodistribution. Centi J. et al. [47] and Tatini J. et al. [48] talk about the interest of gold nanorods (GNRs) in biomedical field. GNRs are gold nanoparticles that are elongated along one direction with characteristic optical properties which depends on the particle size and shape [49]. They are attracting in biomedical optics because of their special and intense absorption band near infrared light (650-1000 nm). Other important features of GNRs include their coating which is crucial for biological applications, i.e. conjugated with polyethylene glycol (PEG), also their shape and size is critical to modulate cellular penetration, intracellular localization and biodistribution. GNRs may become coated with proteins which may modify their conformation causing a loss of their biological activity. i.e. BSA is chosen as protein target to investigate nanoparticle-PEG exposition to biological fluids, because of his importance as the most abundant protein in blood which can transport metal compounds. Tatini J. et al article proposes CA-125 as the molecular target cancer antigen to model in vitro some of the most critical issues that arise from the interactions between GNRs and bloodstream with an analytical approach.

The physicochemical properties of nanoparticles (Figure 4) represent their identity and depend on the synthetic moieties incorporated [50] among all including size, shape, surface, coating and morphology, surface charge, solubility, chemical composition and crystalline structure and, finally, the agglomeration status. These properties will also play a characteristic role in mechanisms such cellular biocompatibility studies.

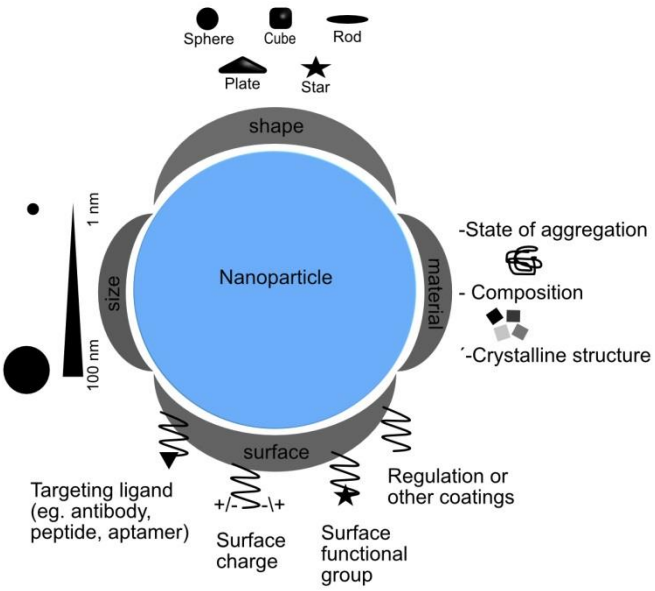

Figure 4. Schematic illustration of the main physicochemical properties of nanoparticles governing interaction mechanisms in biological systems.

3.2.1. Size

Size plays an important role in interacting with the biological system and many biological mechanisms such as endocytosis, cellular uptake and particle processing efficiency in the endocytic path depend on it [51]. The ion release rate, the smaller the size, the faster the release rate and the more interaction with cell membrane too [52]. In general, size-dependent toxicity of nanoparticles and therefore on their ability to enter in human system. As the particle size decreases, the surface/volume ratio increases, so, their contact surface will increase, making penetration into the body easier and increasing their toxic effect [52]. Nanoparticles sizes of less than 50 nm through intravenous injection connect to all tissues faster and exert stronger toxic effects [53].

The size of nanoparticles indicates their distribution *in vino*, or pharmaceutical behaviour [54], and their impact most direct on physiological activity. Nanoparticles sizes larger than 1μm cannot easily enter the cell but they interact with proteins that are absorbed at the cell. Nanoparticles sizes

greater than 6 nm cannot be excreted by the kindness and accumulate in specific organs [55]. For example, cadmium selenide quantum dots contact stays in the tissue causing hepatotoxicity [56]

Sonavane et al. carried out studies on the bio-distribution and bioaccumulation in the blood of gold nanoparticles of different sizes. They observed that smaller one was stayed longer in the bloodstream and accumulated to a greater extent in all organs [57].

3.2.2. Shape

Shape is a physicochemical property that influences the toxicity of materials [58]. Nanoparticles have different shapes and structures such has tubes, fibers, spheres, planes, etc. so, it may also influence their endocytosis process, internalization, biodistribution and elimination. For example, spherical nanoparticles of similar size have been found to be easier and faster internalized by endocytosis than rod-shaped nanoparticles, which is explained by greater membrane wrapping time required for the elongated particles. In addition, the spherical ones are relatively less toxic [59].

3.2.3. Surface modification

The nanoparticle and cells interactions and solubility depend on the nature of the nanoparticle surface [60]. Nanoparticles surface coating alteration can modify their magnetic, electrical, chemical and optical properties, affecting their cytotoxic properties by influencing pharmacokinetics, distribution, accumulation and toxicity [61].

Surface charges determine the response of the organism to changes in nanoparticles shape and size in the form of cellular accumulation, called colloidal behaviour [62]. The effect of surface chemistry on nanoparticles affects absorption [63], colloidal behaviour, plasma protein binding [64] and crossing the blood-brain barrier [65]. The cytotoxicity of nanoparticles increased with an increase in surface charge [66]. This suggests that higher positive charges get greater electrostatic interactions with the cell and consequently greater endocytic uptake. However, the uptake of positively charged nanoparticles may increase toxicity than negatively charged [67]. Nanoparticles with a positively charged surface tended to accumulate more in tumours than negatively charged ones that most probably because positively charged density can be more easily separated in the interstitial space and therefore internalized by tumour cells [68].

Surface chemical modification is an important effect utilized in biomedical applications to decreases toxicity, increase stability and to control and modulate cellular internalization [69]. Surface functionalization is predominantly comprising of PEG, the negative carboxyl group, neutral groups like hydroxyl group and amine groups [66]. For example, surface nanoparticle can be functionalized by proper polymers such as polyethylene glycol (PEG) to reduce nonspecific binding and to get specifically bindings to receptors at cell [70]

Hydrophobicity is a key factor that also affects the pharmacokinetics and biodistribution [70]. Nanoparticles with a more hydrophobic surface tend to absorb plasma proteins, reducing the time spend in the bloodstream [71]. A computer molecular simulation studio has revealed that the surface membrane uptake of hydrophobic C60 agglomerates is thermodynamically favoured than semi-hydrophilic because of the hydrophobicity of the interior membrane space in cells [72].

3.2.4. Composition and crystalline structure

Nanomaterial shape, size and surface contribute significantly to their interaction with cells, however, the nanoparticle chemistry is another fundamental factor. Regarding particle chemistry, Griffitt et al. [73] observed different toxicity in zebrafish, daphnids and algae species for silver and copper nanoparticles with respect to titanium oxide, which resulted in no toxicity problems.

In addition to these characteristic properties of nanoparticles, their state of aggregation must be also taken into account. Aggregation depends on surface load, material type and size, among other factors. It has been shown that higher concentrations of nanoparticles result in high aggregation and consequently lower toxicity [74]. According, macrophages remove large particles more efficiently and easily than small ones, which evade this defence mechanism more easily [75].

3.2.5. Protein corona

Since nanoparticles are injected into bloodstream they are exposed to a large amount of biomolecules that form a corona around them [76]. Protein corona is mainly composed by proteins with different affinity interactions: immunoglobulin G, serum albumin, fibrinogen, clusterin and apolipoproteins are generally present after exposure to plasma [77]. So, nanoparticles experiment changes in their physicochemical properties and their biological identity. Therefore, in order to know the possible adverse effects of the physicochemical, kinetic, dynamic and thermodynamic interactions of nanoparticles, the characterization of nanoparticle-protein interactions has become one of the main challenges of nanomedicine.

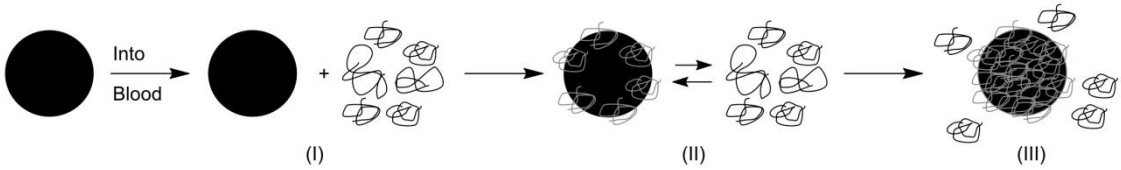

Figure 5. Schematic protein corona formation. First, takes place the introduction of a nanoparticle to fluid/medium enriched in protein content (I). Then, the nanoparticle is coated with proteins which are abundant and highly mobile (II). Finally, the protein species are exchanged over time resulting in hard corona of strongly bound proteins (III).

When nanoparticles are introduced into a biological medium a competitive dynamic process take place to form the protein corona (Figure 5). This process is based on the affinity adsorption of proteins on nanoparticles surface and on protein-protein interactions. According to the Vroman effect [78], first, proteins with high concentration and low affinity are bound to nanoparticle surface and then they are gradually replaced by higher affinity proteins present in low concentrations. The protein corona is classified into hard and soft depending on the duration of protein exchanges. Hard corona is a bound layer of proteins with high affinity and which has a long exchange time. Proteins of the hard corona form the closest layer to the nanoparticle surface, so they are susceptible to thermodynamically favourable conformational changes (irreversible) depending on the chemistry functionalization, the hydrophobicity or hydrophily, the nature of proximal biological fluid and the temperature [79]. Soft corona is a low affinity layer of proteins which has a fast exchange over time. A recently model [80] suggests that hard corona is bound in a hard way to the nanoparticle surface and the soft corona is not directly bound to the nanoparticle but with low degree of biomolecule interactions. As a result, the protein concentration, particle size, type of nanomaterial and the surface properties are factors that impact on the several layers of biomolecules and the density of the protein corona [81]. The biological environment is another key factor that plays a determinant role in the protein corona formation: the media components, temperature, pH and the physiological state of the medium. Depending on the type of administrative routes nanoparticles are subjected to interactions with different kind of biomolecules [82].

The "*in vivo*" protein corona formation of biomedical liposomes seems to be more complex than "*in vitro*" [83]. In consequence, the "in vivo" protein corona characterization is fundamental for biomedical applications.

Different methods and techniques are needed to determine proteins interactions in different biological media because of the large number of proteins in different concentrations that compete to functionalize at nanoparticle surface [84]. Techniques usually described for protein corona evaluation are based on proteomic analysis [79]: centrifugation, isothermal calorimetry titration, UV-Visible spectrometry, MS/MS quantification, SDS-PAGE electrophoresis [85].

For all that, it is essential to understand the relationship between the different properties of nanomaterials and a concrete biological environment in order to understand their behaviour and the results obtained in the different areas of research.

4. Applications of nanoparticles

In this section, it is briefly described some of the nanoparticles applications in order to understand their broad potentials. Although we have focused on nanomedical applications, we should not forget all those other important non-biological applications that have improved the quality of human life [86].

Nanoparticles have attracted a great interest to nanobiotechnology applications (Table 1). The design of nanostructures by controlling their surface properties is a strategy to achieve improved responses aimed at a medical application. Nanobiotechnology plays a central role in nanomedicine and other areas which aspire to develop highly functional biosensors, molecular switches and tissue analogs for organs of the body among others. The nanobiotechnological applications to disease treatment, diagnosis, monitoring by bioimaging, biosensing and drug delivery have been referred as nanomedicine. Nanomedicine holds significant potential to improve the efficacy of cancer immunotherapy.

Table: Main applications of nanoparticles in nanomedicine

4.1. Nanomedical applications: immunotherapy

Immunotherapy has become one of the effective treatment modalities for cancer: cytokine therapy, checkpoint-blockade therapy, adoptive T-cell transfer and chimeric antigen receptor T(CAR-T) cell therapy [121]. Immunotherapy not only treats primary tumors, but also prevents metastasis and recurrence. Other opportunity for combinatorial immunotherapy is based on nanoparticle platforms because of their improved methods for tumor-cell detection, tumor imaging and their ability to efficiently deliver drugs to target sites and protect drugs from endogenous enzymes [122]. Nanoparticles can release agents in response to biochemical changes in the target microenvironment (pH, redox potential and enzymes) or to external stimuli (light, electrical and magnetic fields) [123]. Owing to that, targeted delivery of nanoparticles and controlled drug release can allow the activation of immunotherapies in the action sites [124]. The use of nanoparticles for delivery antigens, adjuvants and other therapeutic agents resulted in more specific targeting and better outcome in contrast to conventional immunotherapy. Advanced biomaterials and drug delivery systems, such as nanoparticles and the use of T cells have been designed to improve immunotherapy [125]. Moreover, nanoparticles can deliver cytotoxic agents to tumour cells killing most or all the target cells with low concentrations of immune-stimulating drugs thanks to their potential to amplify T cell responses [121].

Nanoparticle physicochemical properties can be tuned to stimulate the innate immune cells and to promote nanoparticle-immune cell interactions thus being a good therapeutic option [126]. For example, nano-sized nanoparticle has the advantage of accumulating within the tumor microenvironment with specific targeting minimizing thus off-target toxicity. The most common nanocarriers allowing specificity are liposomes, micelles, dendrimers, gold nanoparticles, iron oxide nanoparticles, carbon nanoparticles and quantum dots (nanoparticles for tumor immunotherapy). According, the most studied functionalization for cancer immunotherapy is PLGA nanoparticles because of their acceptance and biodegradability. Rosalia et al. [127] studied PLGA nanoparticles functionalized with a αCD40-monoclonal antibody (mAb) agonistic vaccine targeting dendritic cells (DCs). Two different adjuvants targeting the toll like receptor (TLR) were encapsulated into PLGA NPs to induce potent CD8+ T cell responses. *In vivo* experiments in murine melanoma-OVA mouse model indicated that active targeting of DCs and vaccine delivery resulted in efficient priming of CD8+ T cells, tumor control, and prolonged survival of the tumor-bearing mice.

Several programs work in integrated and interconnected research focused on therapeutically modifying the tumour micro-environment and (re)activation of anti-cancer immunity and corresponding drug delivery system (DDS) [128]. Initially, they aim to develop new tumour-targeted drugs to selectively block key innate and adaptive immune checkpoints, such as PD-1, TIM-3 and

CD47, in the tumour micro-environment. Further, they aim to develop new tumour-targeted drugs to selectively activate key co-stimulatory receptors of the TNFRSF in the tumour micro-environment.

On the other hand, suitable DDS systems could be developed using modern drug formulations based on nanotechnology and surface chemistry to achieve tumor-localized release and optimal localized co-stimulation of anti-cancer immunity. These developments will be attended by label free detection of protein interactions by means of advanced bioanalysis methods [129]. That could ensure induction and execution of anticancer immune responses in the absence of systemic immune-related side-effects.

Finally, the knowledge and manipulation of immune system by immunotherapies and nanotechnology may be the cause of engineering remarkable mechanisms to produce an effective and long-lasting immune response against cancer.

5. Conclusions and perspectives

Nanotechnology has significantly impacted medicine. In the past decade, studies about biological response to nanoparticles are greatly investigated in parallel with nano-bio interactions which have influenced nanoparticles design. Both were in concordance with the evolution of nanoparticles for biomedical applications. Many studies have investigated and demonstrated that nanoparticles enter into the human system. So, the effect of nanoparticles on biological systems, such as their physicochemical properties (size, shape, surface, coating and morphology, surface charge, hydrophobicity, chemical composition, crystalline structure and the state of agglomeration), the types of biomolecules presents, and the bio-identity of nanoparticle (protein corona) after enter into contact with a biological environment, will be important to characterize in order to know how they finally interact with cells, organisms, biological medium, biomolecules, and other biological systems or even with other nanomaterials. These studies helped to determine their possible biocompatibility and toxicity in biological microenvironments and to engineer nontoxic nanomaterials which may be used in biomedical applications.

With the potentially wide application of nanoparticles in the future, nanoparticles may be extensively used in various fields, especially in immunotherapy for clinical diagnosis and therapy based on their size, biocompatibility, surface chemistry and adjustable toxicity. Immunotherapy combined with nanomedicines may be used for the treatment of different types of cancer due to their excellent efficacy in penetration, specific retention and killing of tumor cells.

The human proteome study [130] can be an arduous and discouraging task due to the high number of proteins, encoded by around 25,000 different genes, from which multiple protein variants are generated by post-transdutional modification. The concept of proteomics involves a comprehensive study on the structures, localizations, post-translational modifications, functions, and interactions of all proteins expressed by an organism at a certain time and under certain conditions. Nanotechnology field has been expanded by providing innovative methods capable of responding to proteomics demands. In this sense, nanotechnologies applications to proteomics has established a novel technical platform termed “nanoproteomics”. For example, currently, detection methodologies have become a great interest in order to overcome the limitations associated with the common used of labels. Detection techniques without labels are useful in the study of protein interaction kinetics, thanks to avoid steric impediments caused by the presence of labels. The design and development of new multifunctional platforms based on nanomedicine could be of great interest in the unlabeled detection of protein-protein interactions given the possibility of synthesizing de novo proteins in vitro in the presence of these nanosystems.

In conclusion, the progress in nano-bio studies can potentially improve nanomedical applications and ensure a sustainable future.

Author Contributions: The manuscript has been approved by all the co-authors.

Funding: We gratefully acknowledge financial support from the Spanish Health Institute Carlos III (ISCIII) for the grants: FIS PI17/01930 and CB16/12/00400. Fundación Solórzano FS/38-2017. The Proteomics Unit belongs to

ProteoRed, PRB3-ISCIII, supported by grant PT17/0019/0023, of the PE I + D + I 2017-2020, funded by ISCIII and FEDER.

Acknowledgments: We gratefully acknowledge financial support from the Spanish Health Institute Carlos III (ISCIII) for the grants: FIS PI17/01930 and CB16/12/00400. Fundación Solórzano FS/38-2017. The Proteomics Unit belongs to ProteoRed, PRB3-ISCIII, supported by grant PT17/0019/0023, of the PE I + D + I 2017-2020, funded by ISCIII and FEDER. A. Landeira-Viñuela is supported by VIII Centenario USAL-BancoSantander PhD Grant.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Figure 1. Schematic representation of method to couple nanoparticles and biomolecules or other nanoparticles.

Figure 2. The small size of the nanoparticles allows them to enter the human body by inhalation, ingestion or through the skin. Once in the extracellular fluid they are conjugated with the present biomolecules, which allows them to internalize in the cells through different mechanisms (phagocytosis, micropinocytosis, clathrin-dependent endocytosis, caveolae-dependent endocytosis or by direct penetration).

Figure 3. Schematic illustration of a multifunctional nanoparticle in biology applications.

Figure 4. Schematic illustration of the main physicochemical properties of nanoparticles governing interaction mechanisms in biological systems.

Figure 5. Schematic protein corona formation. First, takes place the introduction of a nanoparticle to protein rich conditions (I). Then, the nanoparticle is cored with proteins which are abundant and highly mobile (II). Finally, the protein species are exchanged over time resulting in hard corona of strongly bound proteins (III).

References

1. Dean, S.; Mansoori, G.; Fauzi Soelaiman, T. Nanotechnology — An Introduction for the Standards Community. *J. ASTM Int.* **2005**, *2* (6), 13110.
2. Schmidt, K. F. Nanofrontiers; Visions for the Future of Nanotechnology. *Woodrow Wilson Int. Cent. Sch. Pen 6* **2007**, No. March.
3. National Nanotechnology Initiative. Available online: http://www.nano.gov (accessed on 14 Juin 2019)
4. Davies, J. C. Nanotechnology Oversight. *Washington, DC Proj. Emerg. Nanotechnologies* **2008**, No. July
5. Bera, A.; Belhaj, H. Application of Nanotechnology by Means of Nanoparticles and Nanodispersions in Oil Recovery - A Comprehensive Review. *Journal of Natural Gas Science and Engineering* **2016**, *34*, 1284–1309.
6. Manuscript, A. Nanomedicine – Challenge and Perspectives Kristina. **2014**, *48* (5), 872–897.
7. Sanchez, F.; Sobolev, K. Nanotechnology in Concrete - A Review. *Constr. Build. Mater.* **2010**, *24* (11), 2060–2071.
8. Ingle, A. P.; Duran, N.; Rai, M. Bioactivity, Mechanism of Action, and Cytotoxicity of Copper-Based Nanoparticles: A Review. *Appl. Microbiol. Biotechnol.* **2014**, *98* (3), 1001–1009.
9. Verma, A.; Stellacci, F. Effect of Surface Properties on Nanoparticle-Cell Interactions. *Small* **2010**, *6* (1), 12–21.
10. Singh, T.; Jyoti, K.; Patnaik, A.; Singh, A.; Chauhan, R.; Chandel, S. S. Biosynthesis, Characterization and Antibacterial Activity of Silver Nanoparticles Using an Endophytic Fungal Supernatant of Raphanus Sativus. *J. Genet. Eng. Biotechnol.* **2017**, *15* (1), 31–39.

11. Phogat, N.; Kohl, M.; Uddin, I. *Interaction of Nanoparticles With Biomolecules, Protein, Enzymes, and Its Applications*; Elsevier Inc., 2018.
12. Dutta, P. P.; Bordoloi, M.; Gogoi, K.; Roy, S.; Narzary, B.; Bhattacharyya, D. R.; Mohapatra, P. K.; Mazumder, B. Antimalarial Silver and Gold Nanoparticles: Green Synthesis, Characterization and in Vitro Study. *Biomed. Pharmacother.* **2017**, *91*, 567–580.
13. Vigneshwaran, N.; Jain, P. Metal Nanoparticles in Microbiology. **2011**, 135–150.
14. Yameen, B.; Choi, W. I.; Vilos, C.; Swami, A.; Shi, J.; Farokhzad, O. C. Insight into Nanoparticle Cellular Uptake and Intracellular Targeting. *Journal of Controlled Release* 2014, *190*, 485–499.
15. Hu, G.; Jiao, B.; Shi, X.; Valle, R. P.; Fan, Q.; Zuo, Y. Y. Physicochemical Properties of Nanoparticles Regulate Translocation across Pulmonary Surfactant Monolayer and Formation of Lipoprotein Corona. *ACS Nano* **2013**, *7* (12), 10525–10533.
16. Shang, L.; Nienhaus, K.; Nienhaus, G. Engineered Nanoparticles Interacting with Cells: Size Matters. *J Nanobiotechnol* **2014**, *12* (1), 5.
17. Zhao, J.; Stenzel, M. H. Entry of Nanoparticles into Cells: The Importance of Nanoparticle Properties. *Polym. Chem.* **2018**, *9* (3), 259–272.
18. Wilhelm, C.; Billotey, C.; Roger, J.; Pons, J. N.; Bacri, J.; Gazeau, F. Intracellular Uptake of Anionic Superparamagnetic Nanoparticles as a Function of Their Surface Coating. **2003**, *24*, 1001–1011.
19. Champion, J. A.; Mitragotri, S. Role of Target Geometry in Phagocytosis. *Proc. Natl. Acad. Sci.* **2006**, *103* (13), 4930–4934
20. Maeda, H. Toward a Full Understanding of the EPR Effect in Primary and Metastatic Tumors as Well as Issues Related to Its Heterogeneity. *Adv. Drug Deliv. Rev.* **2015**, 1–4.
21. Buzea, C.; Pacheco, I. I.; Robbie, K. Nanomaterials and Nanoparticles: Sources and Toxicity. *Biointerphases* **2007**, *2* (4), MR17–MR71.
22. Pirollo, K. F.; Chang, E. H. Does a Targeting Ligand Influence Nanoparticle Tumor Localization or Uptake? *Trends in Biotechnology* **2008**, *26* (10), 552–558.
23. Maeda, H.; Wu, J.; Sawa, T.; Matsumura, Y.; Hori, K. Tumor Vascular Permeability and the EPR Effect in Macromolecular Therapeutics: A Review. **2000**, *65*, 271–284
24. Manuscript, A. NIH Public Access. **2014**, *172* (3), 782–794.
25. Otsuka, H.; Nagasaki, Y.; Kataoka, K. P EGylated Nanoparticles for Biological and Pharmaceutical Applications. **2003**, *55*, 403–419.
26. Boczkowski, J.; Lanone, S. Respiratory Toxicities of Nanomaterials — A Focus on Carbon Nanotubes. *Advanced Drug Delivery Reviews* **2012**, *64* (15), 1694–1699.
27. Mu, Q.; Jiang, G.; Chen, L.; Zhou, H.; Fourches, D.; Tropsha, A.; Yan, B. Chemical Basis of Interactions Between Engineered Nanoparticles and Biological Systems. *Chem. Rev.* **2014**, *114* (15), 7740–7781.
28. Lau, C. P.; Abdul-Wahab, M. F.; Jaafar, J.; Chan, G. F.; Rashid, N. A. A. Effect of PH and Biological Media on Polyvinylpyrrolidone-Capped Silver Nanoparticles. *AIP Conf. Proc.* **2016**, *1756*, 1–8.
29. Carrillo-Carrión, C.; Nazarenus, M.; Paradinas, S. S.; Carregal-Romero, S.; Almendral, M. J.; Fuentes, M.; Pelaz, B.; del Pino, P.; Hussain, I.; Clift, M. J.; et al. Metal Ions in the Context of Nanoparticles toward Biological Applications. *Current Opinion in Chemical Engineering* **2014**, *4*, 88–96.
30. Sharma, V. K.; Sayes, C. M.; Guo, B.; Pillai, S.; Parsons, J. G.; Wang, C.; Yan, B.; Ma, X. Interactions between Silver Nanoparticles and Other Metal Nanoparticles under Environmentally Relevant Conditions: A Review. *Science of The Total Environment* **2019**, *653*, 1042–1051.
31. Peer, D. Immunotoxicity Derived from Manipulating Leukocytes with Lipid-Based Nanoparticles. *Advanced Drug Delivery Reviews* **2012**, *64* (15), 1738–1748.

32. Albanese†, A.; Chang, WC. Effect of gold nanoparticle aggregation on cell uptake and toxicity. *ACS nano* **2011**, *57*, 5478-89.
33. Nichols, G.; Byard, S.; Bloxham, M. J.; Botterill, J.; Dawson, N. J.; Dennis, A.; Diart, V.; North, N. C.; Sherwood, J. D. A Review of the Terms Agglomerate and Aggregate with a Recommendation for Nomenclature Used in Powder and Particle Characterization. *Journal of Pharmaceutical Sciences* **2002**, *91* (10), 2103–2109.
34. Pellegrino, F.; Pellutiè, L.; Sordello, F.; Minero, C.; Ortel, E.; Hodoroaba, V. D.; Maurino, V. Influence of Agglomeration and Aggregation on the Photocatalytic Activity of TiO 2 Nanoparticles. *Appl. Catal. B Environ.* **2017**, *216*, 80–87.
35. Zook, J. M.; MacCuspie, R. I.; Locascio, L. E.; Halter, M. D.; Elliott, J. T. Stable Nanoparticle Aggregates/Agglomerates of Different Sizes and the Effect of Their Size on Hemolytic Cytotoxicity. *Nanotoxicology* **2011**, *5* (4), 517–530.
36. Yan, C.; Wang, T. A New View for Nanoparticle Assemblies: From Crystalline to Binary Cooperative Complementarity. *Chem. Soc. Rev.* **2017**, *46* (5), 1483–1509.
37. Singamaneni, S.; Bliznyuk, V. N.; Binek, C.; Tsymbal, E. Y. Magnetic Nanoparticles: Recent Advances in Synthesis, Self-Assembly and Applications. *J. Mater. Chem.* **2011**, *21* (42), 16819.
38. Mørup, S.; Hansen, M. F.; Frandsen, C. Magnetic Interactions between Nanoparticles. *Beilstein J. Nanotechnol.* **2010**, *1*, 182–190.
39. Dyawanapelly, S.; Mehrotra, P.; Ghosh, G.; Jagtap, D. D.; Dandekar, P.; Jain, R. How the Surface Functionalized Nanoparticles Affect Conformation and Activity of Proteins: Exploring through Protein-Nanoparticle Interactions. *Bioorganic Chemistry* **2019**, *82*, 17–25.
40. Gehr, P. Interaction of Nanoparticles with Biological Systems. *Colloids and Surfaces B: Biointerfaces* **2018**, *172*, 395–399.
41. Firkowska-Boden, I.; Zhang, X.; Jandt, K. D. Controlling Protein Adsorption through Nanostructured Polymeric Surfaces. *Advanced Healthcare Materials* **2018**, *7* (1), 1700995.
42. Hemmelmann, P. Nanoparticles, Proteins, and Nucleic Acids: Biotechnology Meets Materials Science. *Angew. Chem. Int. Ed.* **2001**, 31.
43. Arsalan, A.; Younus, H. Enzymes and Nanoparticles: Modulation of Enzymatic Activity via Nanoparticles. *International Journal of Biological Macromolecules* **2018**, *118*, 1833–1847.
44. Saallah, S.; Lenggoro, I. W. Nanoparticles Carrying Biological Molecules: Recent Advances and Applications. *KONA Powder Part. J.* **2018**, *2018* (35), 89–111.
45. Singh, B.; Mitragotri, S. Harnessing Cells to Deliver Nanoparticle Drugs to Treat Cancer. *Biotechnology Advances* **2019**.
46. Zhao, Z.; Ukidve, A.; Krishnan, V.; Mitragotri, S. Effect of Physicochemical and Surface Properties on in Vivo Fate of Drug Nanocarriers. *Advanced Drug Delivery Reviews* **2019**.
47. Centi, S.; Tatini, F.; Ratto, F.; Gnerucci, A.; Mercatelli, R.; Romano, G.; Landini, I.; Nobili, S.; Ravalli, A.; Marrazza, G.; et al. In Vitro Assessment of Antibody-Conjugated Gold Nanorods for Systemic Injections. **2014**, 1–10.
48. Tatini, F.; National, I.; Massai, L. Size Dependent Biological Profiles of PEGylated Gold Nanorods. **2014**, No. October 2015.
49. Pérez-Juste, J.; Pastoriza-Santos, I.; Liz-Marzán, L. M.; Mulvaney, P. Gold Nanorods: Synthesis, Characterization and Applications. *Coord. Chem. Rev.* **2005**, *249* (17-18 SPEC. ISS.), 1870–1901.
50. Khan, I.; Saeed, K.; Khan, I. Nanoparticles: Properties, Applications and Toxicities. *Arabian Journal of Chemistry* **2017**.

51. Aillon, K. L.; Xie, Y.; El-Gendy, N.; Berkland, C. J.; Forrest, M. L. Effects of Nanomaterial Physicochemical Properties on in Vivo Toxicity. *Adv. Drug Deliv. Rev.* **2009**, *61* (6), 457–466.
52. Huang, Y. The Toxicity of Nanoparticles Depends on Multiple Molecular and Physicochemical Mechanisms. **2019**.
53. Id, M. A.; Moosavi, M. A.; Rahmati, M.; Falahati, M. Health Concerns of Various Nanoparticles: A Review of Their in Vitro and in Vivo Toxicity. **2018**, No. Figure 1, 1–28.
54. Hoshyar, N.; Gray, S.; Han, H.; Bao, G. The Effect of Nanoparticle Size on in Vivo Pharmacokinetics and Cellular Interaction. *Nanomedicine* **2016**, *11* (6), 673–692.
55. Albanese, A.; Albanese, A.; Tang, P. S.; Chan, W. C. W. The Effect of Nanoparticle Size, Shape, and Surface Chemistry on Biological Systems. **2014**, No. April 2012.
56. Ballou, B.; Lagerholm, B. C.; Ernst, L. A.; Bruchez, M. P.; Waggoner, A. S. Noninvasive Imaging of Quantum Dots in Mice. **2004**, 79–86.
57. Sonavane, G.; Tomoda, K.; Makino, K. Biodistribution of Colloidal Gold Nanoparticles after Intravenous Administration: Effect of Particle Size. *Colloids Surfaces B Biointerfaces* **2008**, *66* (2), 274–280.
58. Lee, Y. J.; Ahn, E.; Park, Y. Shape-Dependent Cytotoxicity and Cellular Uptake of Gold Nanoparticles Synthesized Using Green Tea Extract. **2019**, 1–14.
59. Champion, J. A.; Mitragotri, S. Role of Target Geometry in Phagocytosis. *Proc. Natl. Acad. Sci.* **2006**, *103* (13), 4930–4934.
60. Risom, L.; Møller, P.; Loft, S. Oxidative Stress-Induced DNA Damage by Particulate Air Pollution. *Mutat. Res. - Fundam. Mol. Mech. Mutagen.* **2005**, *592* (1–2), 119–137.
61. Ospina, S. P.; Favi, P. M.; Gao, M.; Johana, L.; Morales, M.; Pavon, J. J.; Webster, T. J. Shape and Surface Effects on the Cytotoxicity of Nanoparticles : Gold Nanospheres versus Gold Nanostars. **2015**, *0811170*, 1–14.
62. Curtis, A. C.; Toghani, D.; Wong, B. SC. *Colloids Surfaces B Biointerfaces* **2018**.
63. Hoshino, A.; Fujioka, K.; Oku, T.; Suga, M.; Sasaki, Y. F.; Ohta, T.; Yasuhara, M.; Suzuki, K.; Yamamoto, K. Physicochemical Properties and Cellular Toxicity of Nanocrystal Quantum Dots Depend on Their Surface Modification. *Nano Lett.* **2004**, *4* (11), 2163–2169.
64. Pietroiusti, A.; Massimiani, M.; Fenoglio, I.; Colonna, M.; Valentini, F.; Palleschi, G.; Camaioni, A.; Magrini, A.; Siracusa, G.; Bergamaschi, A.; et al. Low Doses of Pristine and Oxidized Single-Wall Carbon Nanotubes Affect Mammalian Embryonic Development. *ACS Nano* **2011**, *5* (6), 4624–4633.
65. Georgieva, J. V; Kalicharan, D.; Couraud, P.; Romero, I. A.; Weksler, B.; Hoekstra, D.; Zuhorn, I. S. Surface Characteristics of Nanoparticles Determine Their Intracellular Fate in and Processing by Human Blood – Brain Barrier Endothelial Cells In Vitro. *Mol. Ther.* **2009**, *19* (2), 318–325.
66. Foroozandeh, P.; Aziz, A. A. Insight into Cellular Uptake and Intracellular Trafficking of Nanoparticles. **2018**, *6*.
67. Hu, D.; Kantner, X. K.; Geidel, X. C.; Brandholt, S.; Cock, I. De; Soenen, S. J. H.; Gil, P. R.; Montenegro, J.; Braeckmans, K.; Nienhaus, G. U.; et al. Polymer-Coated Nanoparticles Interacting with Proteins and Cells: Focusing on the Sign of the Net Charge. **2013**, No.
68. Hoshyar, N.; Gray, S.; Han, H.; Bao, G. The Effect of Nanoparticle Size on in Vivo Pharmacokinetics and Cellular Interaction. *Nanomedicine* **2016**, *11* (6), 673–692.
69. Macarthy, D. J. NIH Public Access. **2011**, *6* (20), 2246–2249.
70. Mahmoudi, M.; Lynch, I.; Ejtehadi, M. R.; Monopoli, M. P.; Bombelli, F. B.; Laurent, S. Protein À Nanoparticle Interactions: Opportunities and Challenges. **2011**, 5610–5637.

71. Duan, X.; Li, Y. Physicochemical Characteristics of Nanoparticles Affect Circulation, Biodistribution, Cellular Internalization, and Trafficking. *Small* **2013**, *9* (9–10), 1521–1532.
72. Li, Y.; Chen, X.; Gu, N. Computational Investigation of Interaction between Nanoparticles and Membranes: Hydrophobic / Hydrophilic Effect. **2008**, 16647–16653.
73. Griffitt, R. J.; Luo, J.; Gao, J.; Bonzongo, J. C.; Barber, D. S. Effects of Particle Composition and Species on Toxicity of Metallic Nanomaterials in Aquatic Organisms. *Environ. Toxicol. Chem.* **2008**, *27* (9), 1972–1978.
74. Gatoo, M. A.; Naseem, S.; Arfat, M. Y.; Mahmood Dar, A.; Qasim, K.; Zubair, S. Physicochemical Properties of Nanomaterials: Implication in Associated Toxic Manifestations. *Biomed Res. Int.* **2014**, *2014*.
75. Van Der Zande, M.; Vandebriel, R. J.; Van Doren, E.; Kramer, E.; Herrera Rivera, Z.; Serrano-Rojero, C. S.; Gremmer, E. R.; Mast, J.; Peters, R. J. B.; Hollman, P. C. H.; et al. Distribution, Elimination, and Toxicity of Silver Nanoparticles and Silver Ions in Rats after 28-Day Oral Exposure. *ACS Nano* **2012**, *6* (8), 7427–7442.
76. Peng, Q.; Zhang, S.; Yang, Q.; Zhang, T.; Wei, X. Q.; Jiang, L.; Zhang, C. L.; Chen, Q. M.; Zhang, Z. R.; Lin, Y. F. Preformed Albumin Corona, a Protective Coating for Nanoparticles Based Drug Delivery System. *Biomaterials* **2013**, *34* (33), 8521–8530.
77. Choi, E.; Webster, T. J.; Kim, S. Effect of the Protein Corona on Nanoparticles for Modulating Cytotoxicity and Immunotoxicity. **2015**, 97–113.
78. Hirsh, S. L.; Mckenzie, D. R.; Nosworthy, N. J.; Denman, J. A.; Sezerman, O. U.; Bilek, M. M. M. Colloids and Surfaces B: Biointerfaces The Vroman Effect : Competitive Protein Exchange with Dynamic Multilayer Protein Aggregates. *Colloids Surfaces B Biointerfaces* **2013**, *103*, 395–404.
79. Megido, L.; Díez, P.; Fuentes, M. Nanoproteomics approaches in biomarcker Discovery: the critical role of protein corona on nanoparticles as drug carriers. In *Nanotechnologies in preventive and regenerative medicine*, Uskokovic, V., Uskokovic, P. D., Elsevier; Matthew Deans: Cambridge, United States, 2018, pp.225-239.
80. Liu, W.; Rose, J.; Plantevin, S.; Auffan, M.; Bottero, J. Y.; Vidaud, C. Protein Corona Formation for Nanomaterials and Proteins of a Similar Size: Hard or Soft Corona? *Nanoscale* **2013**, *5* (4), 1658–1665
81. Xiao, W.; Gao, H. The Impact of Protein Corona on the Behavior and Targeting Capability of Nanoparticle-Based Delivery System. *Int. J. Pharm.* **2018**, *552* (1–2), 328–339.
82. Konduru, N. V.; Molina, R. M.; Swami, A.; Damiani, F.; Pyrgiotakis, G.; Lin, P.; Andreozzi, P.; Donaghey, T. C.; Demokritou, P.; Krol, S.; et al. Protein Corona: Implications for Nanoparticle Interactions with Pulmonary Cells. *Part. Fibre Toxicol.* **2017**, *14* (1), 1–12.
83. Hadjidemetriou, M.; Mcadam, S.; Garner, G.; Thackeray, C.; Knight, D.; Smith, D.; Al-ahmady, Z.; Mazza, M.; Rogan, J. The Human In Vivo Biomolecule Corona onto PEGylated Liposomes: A Proof-of-Concept Clinical Study. **2018**, *1803335*, 1–9.
84. Pino, P. Del; Pelaz, B.; Zhang, Q.; Maffre, P.; Nienhaus, G. U.; Parak, W. J. Protein Corona Formation around Nanoparticles - From the Past to the Future. *Mater. Horizons* **2014**, *1* (3), 301–313
85. Strojan, K.; Leonardi, A.; Bregar, V. B.; Kriz, I. Dispersion of Nanoparticles in Different Media Importantly Determines the Composition of Their Protein Corona. **2017**, 1–21.
86. Soc, C. Chem Soc Rev Industrial Applications of Nanoparticles. **2015**, 5793–5805.
87. Hasan, A.; Morshed, M.; Memic, A.; Hassan, S.; Webster, T. J.; Marei, H. E. S. Nanoparticles in Tissue Engineering: Applications, Challenges and Prospects. *Int. J. Nanomedicine* **2018**, *13*, 5637–5655
88. Danie Kingsley, J.; Ranjan, S.; Dasgupta, N.; Saha, P. Nanotechnology for Tissue Engineering: Need, Techniques and Applications. *J. Pharm. Res.* **2013**, *7* (2), 200–204
89. Ellis-Behnke, RG.; Liang, YX.; You, SW.; Tay, DK.; Zhang, S.; So, KF.; Schneider, GE. Nano neuro knitting: peptide nanofiber scaffold for brain repair and axon regeneration with functional return of vision. *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103*, 5054.

90. DE KWAADSTENIET, M.; BOTES, M.; CLOETE, T. E. Application of Nanotechnology in Antimicrobial Coatings in the Water Industry. *Nano* **2011**, *06* (05), 395–407.
91. Li, Y.; Leung, P.; Yao, L.; Song, Q. W.; Newton, E. Antimicrobial Effect of Surgical Masks Coated with Nanoparticles. *J. Hosp. Infect.* **2006**, *62* (1), 58–63.
92. Theivasanthi, T.; Alagar, M. Anti-Bacterial Studies of Silver Nanoparticles. **2011**, No. 1.
93. Kargozar, S.; Mozafari, M. Nanotechnology and Nanomedicine: Start Small, Think Big. *Mater. Today Proc.* **2018**, *5* (7), 15492–15500.
94. Carey, J. D. Engineering the next Generation of Large-Area Displays: Prospects and Pitfalls. *Philos. Trans. R. Soc. A Math. Phys. Eng. Sci.* **2003**, *361* (1813), 2891–2907.
95. Roy, I.; Stachowiak, M. K.; Bergey, E. J. Nonviral Gene Transfection Nanoparticles: Function and Applications in the Brain. **2008**, *4*, 89–97.
96. Fatima, H.; Kim, K. S. Magnetic Nanoparticles for Bioseparation. *Korean J. Chem. Eng*. **2017**, *34* (3), 589–599
97. Adeniyi, OM.; Azimov, U.; Burluka, A. Algae biofuel: Current status and future applications. *Renewable Sustainable Energy Rev* **2018**, *90*, 316–35
98. Sekoai, P. T.; Ouma, C. N. M.; du Preez, S. P.; Modisha, P.; Engelbrecht, N.; Bessarabov, D. G.; Ghimire, A. Application of Nanoparticles in Biofuels: An Overview. *Fuel* **2019**, *237*.
99. Yang, G.; Wang, J. Improving Mechanisms of Biohydrogen Production from Grass Using Zero-Valent Iron Nanoparticles. *Bioresour. Technol.* **2018**, *266* (May), 413–420.
100. Timothy, C. J.; Kogularamanan, S.; Sthepen, L. The Next Generation of Platinum Drugs: Targeted Pt (II) Agents, Nanoparticle Delivery, and Pt(IV) Prodrugs. *Chem Rev.* **2016**, *116*, 343-348643.
101. Díez, P.; González-Muñoz, M.; González-González, M.; Dégano, R. M.; Jara-Acevedo, R.; Sánchez-Paradinas, S.; Piñol, R.; Murillo, J. L.; Lou, G.; Palacio, F.; et al. Functional Insights into the Cellular Response Triggered by a Bile-Acid Platinum Compound Conjugated to Biocompatible Ferric Nanoparticles Using Quantitative Proteomic Approaches. *Nanoscale* **2017**, *9* (28), 9960–9972.
102. Delivery, L. D. HHS Public Access. **2017**, *44* (6), 2049–2061.
103. Dizaj, S. M.; Barzegar-jalali, M.; Zarrintan, M. H.; Adibkia, K.; Lotfipour, F. Calcium Carbonate Nanoparticles as Cancer Drug Delivery System. **2015**, 1–12.
104. Choi, D. G.; Venkatesan, J.; Shim, M. S. Selective Anticancer Therapy Using Pro-Oxidant Drug-Loaded Chitosan – Fucoidan Nanoparticles. **2019**.
105. Centi, S.; Tatini, F.; Ratto, F.; Gnerucci, A.; Mercatelli, R.; Romano, G.; Landini, I.; Nobili, S.; Ravalli, A.; Marrazza, G.; et al. In Vitro Assessment of Antibody-Conjugated Gold Nanorods for Systemic Injections. **2014**, 1–10.
106. Tatini, F.; Landini, I.; Scaletti, F.; Massai, L.; Centi, S.; Ratto, F.; Nobili, S.; Romano, G.; Fusi, F.; Messori, L.; et al. Size Dependent Biological Profiles of PEGylated Gold Nanorods. *J. Mater. Chem. B* **2014**, *2* (36), 6072–6080.
107. De Clercq, E. Highlights in Antiviral Drug Research: Antivirals at the Horizon. *Med. Res. Rev.* **2013**, *33* (6), 1215–1248.
108. Guidi, F.; Landini, I.; Puglia, M.; Magherini, F.; Gabbiani, C.; Cinellu, M. A.; Nobili, S.; Fiaschi, T.; Bini, L.; Mini, E.; et al. Proteomic Analysis of Ovarian Cancer Cell Responses to Cytotoxic Gold Compounds. *Metallomics* **2012**, *4* (3), 307–314.
109. Magherini, F.; Fiaschi, T.; Valocchia, E.; Becatti, M.; Pratesi, A.; Marzo, T.; Massai, L.; Gabbiani, C.; Landini, I.; Nobili, S.; et al. Antiproliferative Effects of Two Gold(I)-N-Heterocyclic Carbene Complexes in A2780 Human Ovarian Cancer Cells: A Comparative Proteomic Study. *Oncotarget* **2018**, *9* (46), 28042–28068.

110. Mügge, C.; Rothenburger, C.; Beyer, A.; Görls, H.; Gabbiani, C.; Casini, A.; Michelucci, E.; Landini, I.; Nobili, S.; Mini, E.; et al. Structure, Solution Chemistry, Antiproliferative Actions and Protein Binding Properties of Non-Conventional Platinum(ii) Compounds with Sulfur and Phosphorus Donors. *Dalt. Trans.* **2011**, *40* (9), 2006–2016.
111. Marzo, T.; Massai, L.; Pratesi, A.; Stefanini, M.; Cirri, D.; Magherini, F.; Becatti, M.; Landini, I.; Nobili, S.; Mini, E.; et al. Replacement of the Thiosugar of Auranofin with Iodide Enhances the Anticancer Potency in a Mouse Model of Ovarian Cancer. *ACS Med. Chem. Lett.* **2019**, *10* (4), 656–660.
112. Maiore, L.; Cinellu, M. A.; Nobili, S.; Landini, I.; Mini, E.; Gabbiani, C.; Messori, L. Gold(III) Complexes with 2-Substituted Pyridines as Experimental Anticancer Agents: Solution Behavior, Reactions with Model Proteins, Antiproliferative Properties. *J. Inorg. Biochem.* **2012**, *108*, 123–127.
113. Maiore, L.; Cinellu, M. A.; Michelucci, E.; Moneti, G.; Nobili, S.; Landini, I.; Mini, E.; Guerri, A.; Gabbiani, C.; Messori, L. Structural and Solution Chemistry, Protein Binding and Antiproliferative Profiles of Gold(I)/(III) Complexes Bearing the Saccharinato Ligand. *J. Inorg. Biochem.* **2011**, *105* (3), 348–355.
114. Landini, I.; Lapucci, A.; Pratesi, A.; Massai, L.; Napoli, C.; Perrone, G.; Pinzani, P.; Messori, L.; Mini, E. Selection and Characterization of a Human Ovarian Cancer Cell Line Resistant to Auranofin. **2017**, *8* (56), 96062–96078.
115. Guidi, F.; Modesti, A.; Landini, I.; Nobili, S.; Mini, E.; Bini, L.; Puglia, M.; Casini, A.; Dyson, P. J.; Gabbiani, C.; et al. The Molecular Mechanisms of Antimetastatic Ruthenium Compounds Explored through DIGE Proteomics. *J. Inorg. Biochem.* **2013**, *118*, 94–99.
116. Gamberi, T.; Massai, L.; Magherini, F.; Landini, I.; Fiaschi, T.; Scaletti, F.; Gabbiani, C.; Bianchi, L.; Bini, L.; Nobili, S.; et al. ScienceDirect Proteomic Analysis of A2780 / S Ovarian Cancer Cell Response to the Cytotoxic Organogold (III) Compound Aubipy C. *J. Proteomics* **2014**, *103*, 103–120.
117. Sharifi, M.; Avadi, MR.; Attar, F.; Dashtestani, F.; Ghorchian, H.; Rezayat, SM.; Saboury, AA.; Falahati, M. Cancer diagnosis using nanomaterials based electrochemical nanobiosensors. *Biosensors and Bioelectronics* **2019**, *126*, 773-784
118. Ambrosi, A.; Airò, F.; Merkoçi, A. Enhanced Gold Nanoparticle Based ELISA for a Breast Cancer Biomarker. *Anal. Chem*. **2010**, *82* (3), 1151-1156
119. Ramos, M.; Castillo, C. Aplicaciones biomédicas de las nanopartículas magnéticas. *Ide@s CONCYTEG* **2011**, *6* (72), 629-646
120. Haun, JB.; Yoon, TJ.; Lee, H.; Weissleder, R. Magnetic nanoparticle biosensors. *WIREs NanomedNanobiotechnol* **2010**, *2*, 291-304
121. Riley, R. S.; June, C. H. Delivery Technologies for Cancer Immunotherapy. *Nat. Rev. Drug Discov.* **2013**.
122. Park, W.; Heo, Y.; Han, D. K. New Opportunities for Nanoparticles in Cancer Immunotherapy. **2018**, 1–10.
123. Moon, J. J. Cancer Nanomedicine for Combination. *Nat. Rev. Mater.* **2019**, *4* (June).
124. Wang, Z.; Liu, W.; Shi, J.; Chen, N.; Fan, C. Nanoscale Delivery Systems for Cancer Immunotherapy. *Mater. Horiz.* **2018**, *5* (3), 344–362.
125. Mi, Y.; Smith, C. C.; Yang, F.; Qi, Y.; Roche, K. C.; Serody, J. S.; Vincent, B. G.; Wang, A. Z. A Dual Immunotherapy Nanoparticle Improves T-Cell Activation and Cancer Immunotherapy. **2018**, *1706098*, 1–9.
126. Surendran, S. P.; Moon, M. J.; Park, R.; Jeong, Y. Y. Bioactive Nanoparticles for Cancer Immunotherapy Bioactive Nanoparticles for Cancer Immunotherapy. **2019**, No. December 2018.

127. Rosalia, R. A.; Cruz, L. J.; Duikeren, S. Van; Tromp, A. T.; Oostendorp, J.; Silva, A. L.; Jiskoot, W.; Gruijl, T. De; Clemens, L.; Burg, S. H. Van Der; et al. Biomaterials CD40-Targeted Dendritic Cell Delivery of PLGA-Nanoparticle Vaccines Induce Potent Anti-Tumor Responses. **2014**.

128. Nakamura, T.; Harashima, H. Integration of Nano Drug-Delivery System with Cancer Immunotherapy. **2017**, *8*, 987–1000.

129. Latterich, M.; Corbeil, J. A Nano-Porous Silicon-Based Detection Method. **2008**, *11*, 1–11.

130. Jia, L.; Lu, Y.; Shao, J.; Liang, X.; Xu, Y. Nanoproteomics: A New Sprout from Emerging Links between Nanotechnology and Proteomics. **2013**, *31* (2), 99–107.