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Protein Interactions and Nanomaterials: A Key Role of the Protein Corona in Nanobiocompatibility

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http://dx.doi.org/10.5772/intechopen.75501

Abstract

The protein corona is still somewhat of a mysterious consequence of the nanoparticles' application in theranostics. In this review, several critical aspects related to the protein corona are described, in particular which influences more specifically its formation, how to evaluate/characterize it, and what interactions to expect when the nanoparticle and the protein corona are inside the cell. Despite these issues, which have been studied in a general way, it has been verified that there's still much information missing when it comes to specific nanoparticles. Here, a few proteins are also highlighted as examples, which have been identified as part of the protein corona; in addition, several factors related to the formation of protein corona are discussed due to their important role in the different adsorbed proteins.

Keywords: protein corona, nanoparticles, nanobiocompatibility, proteomics

1. Introduction

Nanotechnology is becoming everyday a more valuable resource in developing strategies of diagnostics and therapeutics; in fact, a new area is arising which is named nanomedicine [1]. From the use of nanoparticles [2] to nanorobots [3] or nanosensors [4], there is no shortage of ways to apply it to nanomedicine's benefit. Nanoparticles are particularly useful as theranostic agents, as a multifunctional platform which combine both therapeutic and diagnostic applications simultaneously [5]. However, nanoparticles must gather a number
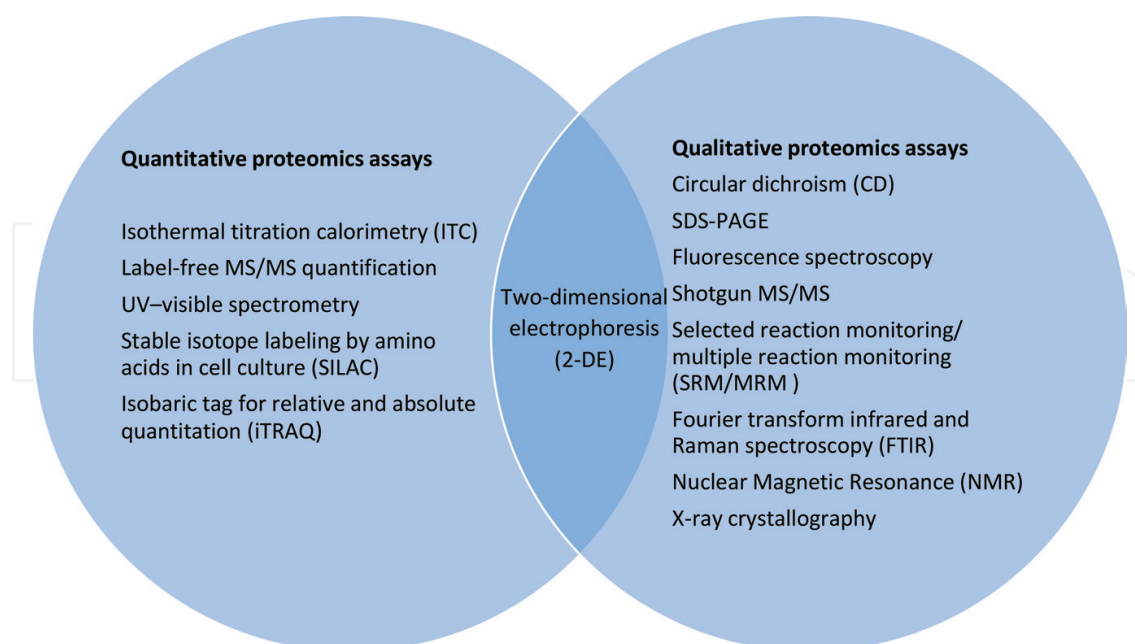
of characteristics in order to be considered as good theranostic agents, such as suitable size [6] and shape [7] for cell penetration, biocompatibility, surface charge, efficient targeting [8], and fluorescence, among others [9]. Despite of these advantages and promising applications, there are still many problems associated to the entrance of the nanoparticle in a physiological environment, which may be justified with different intrinsic characteristics of the nanoparticles [10]. In general, there are two different nanoparticle identities, such as "synthetic identity," which refers to their intentional physicochemical properties [11], and a "biological identity," which is related to the physicochemical properties shown by the nanoparticle after its application in a physiological environment and interaction with the presented biomolecules [10]. This "biological identity" is profoundly related to the formation of the protein corona, as it significantly alters the size, shape, and surface charge of the nanoparticle [12, 13]. The protein corona is formed after the entrance of the nanoparticle in a physiological environment, such as the bloodstream and/or peripheral blood, where the presence of thousands of proteins [14] (among other biomolecules) causes their adsorption onto the nanoparticle surface [15], in a corona shape [16]. The formation of this corona is energetically favorable, with a decrease of enthalpy and increase of entropy [15]. It can be divided into two categories: a "hard" corona, and a "soft" corona. The "soft" corona is based on abundant proteins that firstly bind to the nanoparticle through low-affinity bonds, and the "hard" corona is more dense [17], based on sparse proteins that replace the "soft" proteins over time, due to their higher affinity bonds [11], which is known as the Vroman effect [18]. The composition of the corona is directly dependent on the biomolecular composition of the physiological environment that surrounds it [19, 20], the time of exposure [11, 17, 21], and also incubation conditions (such as temperature or mild stirring), among others. Moreover, it is clear that the protein corona is not static and varies in the course of time; in other words, it's dynamic [17, 21]. Eventually, it will reach a state of equilibrium, steady stochastic state, where the association and dissociation rates for each protein occur equally [21], unless it is further incubated in a different biological media or proximal biological fluid, with a formation of a new corona [22]. The great majority of the proteins that form the corona are independent of the size and surface charge of the nanoparticle but are very influenced by the chemical properties of the material that constitutes the nanoparticle [23]. However, there are still sensitive proteins to size and surface charge, whose nature can change and consequently alter the interactions between nanoparticles and cells with the consequent alteration of the biological outcome and biological impacts [23]. For instance, if opsonins bind to the nanoparticle in the protein corona, they will be recognized as a "threat," and consequently are phagocytosed by macrophages [24]. It is crucial to prevent opsonization, "camouflage" the nanoparticle to avoid the phagocytosis, and keep the nanoparticles in circulation, which can be achieved by the application of a polymer coating, such as poly(ethylene glycol) (PEG) [25]. This polymer coating also prevents the formation of the protein corona, which can later compromise the nanoparticle internalization by the cells [25]. It is thus important to study the influence of the protein corona in the internalization of the nanoparticle, as the interactions of the nanoparticles with cells in in vivo studies are much different from the in vitro ones [26–28], which can prove to be an obstacle in the generalized application of this theranostic approach based on nanomaterials.

2. Characterization of protein corona by proteomic strategies

Since the formation of the protein corona has a great impact on the nanoparticle's performance when applied to a biological system, it is important to assess its structure and composition, in order to minimize the adverse effects it may have on the nanoparticle's use. Any alteration in shape, size, electron transfer, or others may come from the binding of the protein corona to the nanoparticle and may be used as parameters of comparison to be tested between nanoparticles, before and after administration to a biological fluid [29]. However, it is necessary to separate the nanoparticle-protein complex from the excess of plasma proteins [30] before assessing the composition of the protein corona. This is frequently made by centrifugation [30, 31], but it can have many adverse effects in the corona, due to the alterations caused by washing steps as well as gradient and volume variations [30, 32, 33]. In order to avoid loss of proteins from the corona, or even tainting the protein corona sample with the proteins in excess from the plasma, centrifugation can be accompanied by other procedures, such as size exclusion chromatography [32] or microfiltration [33, 34]. In the case of magnetic nanoparticles, a one-step centrifugation does not work, as it agglomerates the nanoparticles, making it necessary to perform a magnetic separation [35, 36]. As said by Megido et al. [33], the main methods of evaluation can be held as qualitative or quantitative, being summarized in **Figure 1**.

2.1. Quantitative proteomics

Quantitative proteomics is the collection of techniques that allow the determination of the number of proteins in a sample, which may be its absolute amount or just the relative change

Quantitative proteomics assays

Isothermal titration calorimetry (ITC)
Label-free MS/MS quantification
UV–visible spectrometry
Stable isotope labeling by amino acids in cell culture (SILAC)
Isobaric tag for relative and absolute quantitation (iTRAQ)

Two-dimensional electrophoresis (2-DE)

Qualitative proteomics assays
Circular dichroism (CD)
SDS-PAGE
Fluorescence spectroscopy
Shotgun MS/MS
Selected reaction monitoring/ multiple reaction monitoring (SRM/MRM)
Fourier transform infrared and Raman spectroscopy (FTIR)
Nuclear Magnetic Resonance (NMR)
X-ray crystallography

Figure 1. Summary of methodological approaches useful for the characterization of the protein corona (figure based on Megido et al. [33]).

in amounts between two states [37]. There are many problems associated to the methods used for these assays, such as difficulties in reproducing the results and lack of precision in the measurements [38, 39], but recent technologies have allowed to minimize such issues [40], increasing the depth and coverage [38], which can also be done by using several techniques simultaneously and by defining standards for reproducibility [39]. The most commonly used assays are isothermal titration calorimetry (ITC), UV-visible spectrometry, stable isotope labeling by amino acids in cell culture (SILAC), isobaric tag for relative and absolute quantitation (iTRAQ), and label-free MS/MS quantification. There are also quantitative approaches that make use of two-dimensional electrophoresis (2-DE) [41], but it's mostly used for qualitative proteomics [39], due to the current limitations in performing quantitative assays .

2.1.1. Isothermal titration calorimetry (ITC)

Isothermal titration calorimetry is a method that allows the determination of thermodynamic parameters in a solution (binding affinity, binding stoichiometry, and binding enthalpy change [33]), in particular the ones coming from interactions between biological macromolecules [42]. This process is based on the changes in heat caused by the protein adsorption to the nanoparticle [43]. The main advantage of this method relies on the fact that it allows the characterization while still in the incubation medium [43], which consequently allows a greater optimization of the nanoparticle.

2.1.2. UV-visible spectrometry

UV-visible spectrometry is a process based on the ratio between the passed light measured and the incident light in the UV-visible wavelength [33]. The presence of the protein corona induces changes on the absorption spectrum [17], which makes it an easy, fast, and applicable approach, as it requires no other chemicals or resources other than the protein corona itself [33]. However, it is an unreliable method, as the radiation energy reaching the sample is low [44]; it is very influenced by parameters such as size, temperature, pH [33], and equipment errors, which have a much bigger impact, as there is no other chemical or technique applied to lower the risk of incorrect results [44].

2.1.3. Stable isotope labeling by amino acids in cell culture (SILAC)

SILAC, an acronym to stable isotope labeling by amino acids in cell culture, is a procedure where an essential a.a. has been replaced by its stable isotope counterpart in the cells' growth medium, making this "heavy" amino acid incorporated into all expressed proteins [45]. This causes the growth of two populations of cells: the ones growing in "light" medium containing the natural isotope in the amino acids and the ones growing in "heavy" medium containing stable isotope-labeled amino acids [33, 46]. After complete labeling, equal amounts of labeled and unlabeled cells or protein extracts are mixed in the cell population. The samples are then digested into peptides and then analyzed with mass spectrometry. The quantification of SILAC is thus based on the ratio of introduced isotope-labeled peptides to unlabeled peptides [46]. The many advantages of SILAC are its easy implementation, reasonable quantitative accuracy, and high reproducibility [46].

2.1.4. Isobaric tag for relative and absolute quantitation (iTRAQ)

Isobaric tag for relative and absolute quantitation, also known as iTRAQ, is a widely used method in proteomics for quantification. It is based on mass spectrometry (MS) [47] and is useful in situations where the proteins come from different sources in the same sample [33]. This technique makes use of amine-reactive reagents with different isotopic masses between them [47], labeling the peptides differently and allowing for a clear distinction when analyzing MS scans, as various peptides appear each in a single peak [33]. However, this method has a great disadvantage concerning its cost [48], which makes it impracticable when compared to cheaper alternatives.

2.1.5. Label-free MS/MS quantification

Label-free quantification methods make no use of labeling on the proteins, relying only on the measurement of ion intensity changes in chromatography or on spectrum counting of fragments of peptides in a given protein [46]. This procedure is especially suited for biomarker discovery in large sample sets, as it is not needed labeling in any protein [49]. Labeling also limits the dynamic range, resulting in loss of signal and possible omission of proteins [50]. Therefore, using a label-free quantification approach allows the gathering of reliable information, with great reproducibility [49].

2.2. Qualitative proteomics

Qualitative proteomics refers to the assays designed for identification of proteins in a sample and are often performed not only for identification but also for quantitative purposes, such as the abovementioned 2-DE electrophoresis [39]. Other assessments that allow identification of proteins are circular dichroism (CD), SDS-PAGE, fluorescence spectroscopy, shotgun MS/MS, selected reaction monitoring (SRM)/multiple reaction monitoring (MRM), Fourier transform infrared and Raman spectroscopy, nuclear magnetic resonance (NMR), and X-ray [33].

2.2.1. Circular dichroism

Circular dichroism is an assay based on the determination of the secondary structure, folding and binding properties of proteins [51], using the difference between the absorption of left and right circularly polarized light [52]. This method is based on the optic properties shown by the conformation of the protein, which can be altered when interacting with a nanoparticle. The nanoparticle itself shows no influence in the light, as it is not a chiral compound [33], and it can also be used with small amounts of proteins (20 μg) in a short amount of time [51], making it a viable way of assessment. However, it has some limitations, such as unfeasibility with complex mixtures of proteins [33] and impossibility in obtaining residue-specific information [51].

2.2.2. SDS-PAGE

One of the most used methods in proteomics, electrophoresis, is a procedure that separates proteins in a sample according to their charge. Using a gel of polyacrylamide, a protein solution is applied, and in relation to their charge, proteins will migrate across the gel [53]. The proteins

get sorted by molecular weight [53], and staining is needed with an appropriate pigment, for instance, Coomassie blue [54]. The molecular weight is then compared with the one shown by the markers, and a densitometry analysis is performed [33]. This procedure is suitable to characterize the proteins that form the corona, comparing the proteins obtained from the plasma with the ones that are found in the corona. It is thus possible to verify exactly which ones get adsorbed to its surface and, therefore, the ones that have greater affinity to the nanoparticle [43].

2.2.3. Fluorescence spectroscopy

Fluorescence spectroscopy is a method that allows the measurement of the fluorescence of a compound, when excited at a given wavelength [33]. The fluorescence may come from the protein (intrinsic probes), the nanoparticle, or even a fluorophore added to the complex (extrinsic probes) [33, 55], which will be picked up by the amino groups and then detected in the fluorescence spectrometer [56].

2.2.4. Shotgun MS/MS

Shotgun proteomics is a widely used technique in proteomics for identifying proteins [57], with great sensitivity, making it a great influence in the discovery of clinically actionable biomarkers [58]. To perform it, a complex mixture of proteins is separated by sequence-specific proteolysis, forming peptides that will then be separated in smaller fragments to be later analyzed by mass spectrometry [57]. Each peptide will have a mass associated to it, but since peptides may have the same a.a., but in a different arrangement, it is important to assess the sequence, which can be done by the ratios of its mass spectrum, that is, by MS/MS [57].

This method is often disregarded by researchers, due to its early problems in reproducibility and fallible nature [58, 59]. However, the development of bioinformatic tools has allowed a decrease in these problems, making it a viable option for proteomic research [57–59].

2.2.5. Selected reaction monitoring (SRM)/multiple reaction monitoring (MRM)

One of the ways frequently used in replacement of shotgun MS/MS is selected reaction monitoring (SRM) [60], also known as multiple reaction monitoring (MRM). This assay makes use of a triple-quadrupole mass spectrometer [60], where peptides from a previously digested protein go through. In the first analyzer, molecular ions with similar mass to the peptide are selected, followed by fragmentation of the peptide bonds in the second analyzer. Lastly, in the third and final analyzer, the fragmented ions from the peptide pieces are measured, originating a transition signal [61]. This method does not record any full-mass spectrum, increasing its sensitivity and allowing the detection of scarce proteins in complex mixtures [60].

2.2.6. Fourier transform infrared and Raman spectroscopy

FTIR is a procedure that gives information about the surface properties of the nanoparticle and the protein corona, as it allows the detection of its attachment [33]. Standard Raman

spectroscopy is not normally used independently, and it's usually meant to complement other methods, such as FTIR [62]. Together, they provide information about protein's secondary structure [63], plus vibrational and rotational parameters.

2.2.7. Nuclear magnetic resonance (NMR)

NMR, which stands for nuclear magnetic resonance, is a form of evaluation of proteins, widely used to describe dendrimers, polymers, and fullerenes derivatives, characterizing structure, purity, and functionality [64] and their possible effects in membrane disruption [65]. Usually, it is used to analyze lipids, as they show high affinity for the nanoparticles [66], after a size exclusion chromatography [33]. It still has disadvantages; for instance, it cannot distinguish the distribution of targeting agent density on a population of nanoparticles [64].

2.2.8. X-ray crystallography

Considered one of the primary sources of structural information about the protein-ligand complex [67], and it is based on the positions and intensities of the reflections as measured in the diffraction pattern of the crystal [67]. This method has big challenges associated to it, as the quantity of radiation needed may be excessive and cause damages to the proteins before a signal is obtained [68], and there are many uncertainties when applying it, such as identity or location of the proteins to be evaluated [67].

3. Nanoparticle's intracellular trafficking

After the nanoparticle's entry in the biological fluid, it is important to ensure its internalization into the cells and intracellular transport, as the formation of the protein corona influences directly the cellular uptake and may also have a significant role on the success of the nanoparticle or lack thereof [69]. Most of mammalian cells internalize the nanoparticles through pinocytosis, although big, specialized cells (such as macrophages) are able to do it by phagocytosis [70, 71], which is the uptake of large particles [70]. Some nanoparticles can also do it by passive penetration of the cellular membrane; however, if the nanoparticle is not small enough, it may deform the membrane [72] by forming holes or thinning it [73], increasing the cytotoxicity [71, 74]. Still, this mechanism is useful in drug delivery, as the nanoparticle travels directly to the cytosol, without making use of endocytic vesicles [74], promoting the reach of the intracellular targets [75]. Hence, it is necessary to take that into account, when designing the nanoparticle, as it may be possible to optimally design the surface of the selected nanoparticle for drug delivery and avoid the membrane's deformation [74]. As for the pinocytosis internalization, which is the cellular uptake of small particles (fluids and solutes), it has four different types of mechanisms [70] (**Figure 2**).

The physical properties of the nanoparticle such as size, net surface charge, and chemical composition determine which endocytosis process is chosen for the internalization [76],
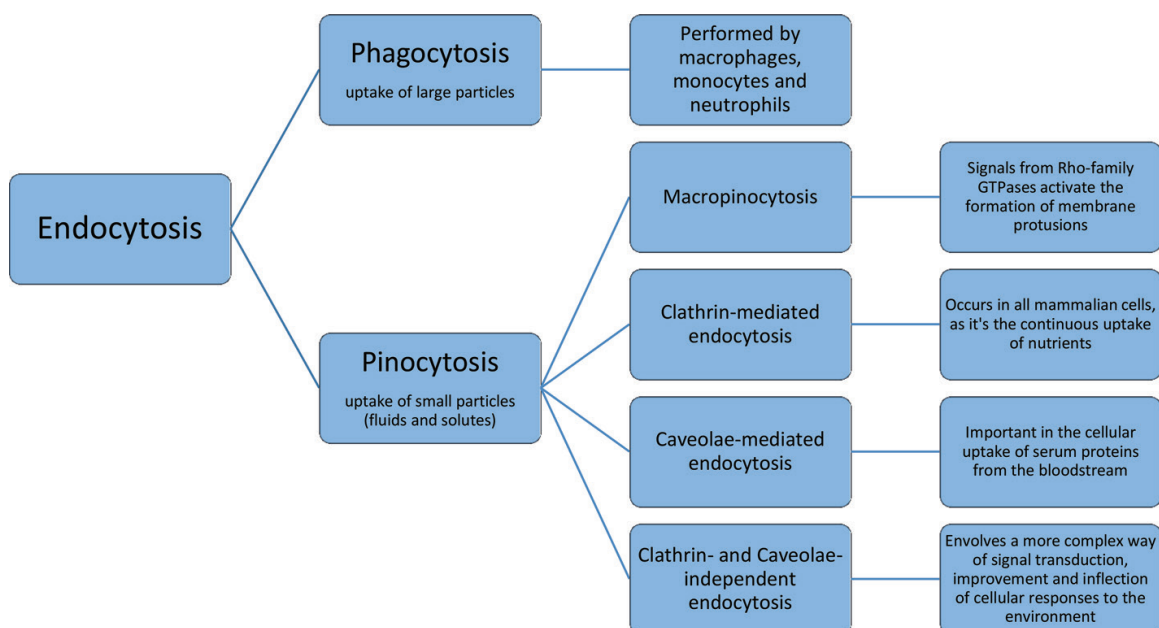
Figure 2. Summary of the different kinds of endocytosis (information based on Conner and Schmid [71]).

although more than one mechanism can be used for the same nanoparticle [77, 78], and the formation of the protein corona may have a great influence in this choice [71]. The proteins adsorbed on the nanoparticle give it its biological identity, and they may present a sequence that is not recognized by the cell as relevant or needed, preventing its endocytosis [79]. It was verified that nanoparticles without a protein corona have higher rates of cellular uptake but can also cause more damage to the cell and alter the cellular metabolism and cell cycle [79, 80]. Nevertheless, if the sequence of proteins in the protein corona is identified as relevant, the endocytosis mechanisms are activated, and the nanoparticle is internalized [79]. When binding to the cellular membrane, the protein corona does not separate itself from the nanoparticle [81], nor does it detach when inside the cell, being internalized as a single complex [82]. After internalization, the nanoparticle's course must be followed by capturing its fluorescence, which can come from the nanoparticle itself or from a fluorescent dye added posteriorly. According to Guarnieri et al. [83], polystyrene nanoparticles follow a fairly diffuse pattern once inside the cell, which suggested no interaction between the nanoparticle and the cytosolic structures, in both situations with and without protein corona. This diffuse pattern can be explained by the nanoparticles being transported within the endocytic vesicles, whose movements are associated to the molecular motors, such as kinesin, myosin, and dynein [83, 84]. Therefore, Guarnieri et al. [83] report that, although the protein corona has some influence in the mechanisms of cellular uptake, it does not show an impact on the intracellular pathways taken by nanoparticles internalized by endocytosis. While leaving the cell, exocytosis mechanisms are activated, and they are dependent on proteins in the medium, because the proteins forming the corona interacted with biological systems inside the cells [76]. The exocytosis is also size, surface coating, and shape dependent, as smaller nanoparticles showed faster exocytosis rates and rod-shaped

nanoparticles showed more efficiency when compared to spherical nanoparticles [76, 85]. After performing its function within the cell, the nanoparticle is eventually cleared by the liver and spleen, where they can be kept for a long time, increasing the expected cytotoxicity of the nanoparticle [76].

4. Interaction of nanoparticles with cell interfaces

After internalization, it is important not only to guarantee the achievement of the nanoparticle's function but also to evaluate its effects on cellular organelles [86], as the toxicity cannot be too high, or it will ultimately exclude its use in nanomedicine. The understanding of the nanoparticles' interaction with each cellular organelle is still fairly underdeveloped, as researchers tend to overlook the possible connections between the nanoparticle's composition and the cellular response, focusing considerably more on its uptake [86]. Nonetheless, some studies have already been made to counteract this tendency, in order to give more information and also a better understanding of the nanoparticles' real impact in the cell. According to the experiment performed by Bertoli et al. [78], it is possible to separate the organelles retaining the nanoparticle through magnetism, if the particle is designed to have magnetic properties. Their experiment [78] was based on separating the nanoparticle from the cell after internalization, in order to identify the proteins adsorbed to it, and determining their origin, according to the characteristic proteins from each cellular organelle. The nanoparticles were verified to have the majority of proteins (over 44%) coming from the endocytic pathway, while fewer than 5% came from each of the different organelles studied, such as nucleus, mitochondria, or peroxisomes. However, some proteins can overlap by belonging to more than one organelle [87], acting like a contamination, as they can induce errors in the examination results. Nevertheless, it can be inferred that the majority
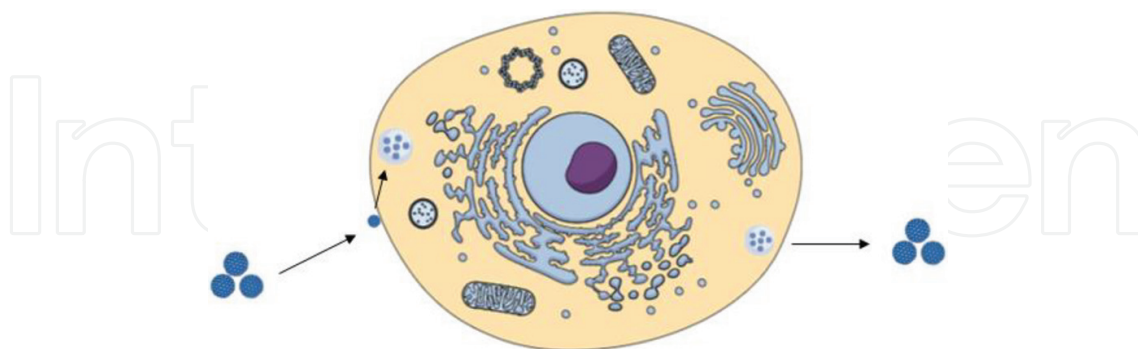
Figure 3. Summarization of the nanoparticle's cellular internalization. The nanoparticles interact with the cell, which can lead to cellular uptake or accumulation of the nanoparticle in the cell membrane. If uptake occurs, the cell engulfs the nanoparticle within endocytic vesicles, which transport the nanoparticle while inside the cell. According to the experiments performed by Bertoli et al. [79], the majority of nanoparticles does not leave the endocytic vesicles to interact directly with other organelles. The nanoparticles are exocytosed afterwards, through lysosomes, to be cleared by the liver or spleen.

of nanoparticles does not leave the endocytic vehicles to associate itself with the different organelles that are present on the cell, which can become a problem, if there is a specific intracellular target for the nanoparticle.

In order to find the internalization's time distribution, Bertoli et al. [78] also performed a time-dependent experiment, evaluating the locations of the nanoparticles after submitting the cells to a short exposure. It was verified that, after only a few minutes, the presence of the nanoparticles was greater in early endocytosis' organelles, with the total absence of lysosomal markers. Still, a more prolonged exposure (24 h) revealed a stronger nanoparticle existence in lysosomes, without any proteins present in early endocytic organelles, indicating their concluding exocytosis from the cells. A similar experiment was previously performed by Shapero et al. [88], where by electron and fluorescence microscopy, the internalization pathway of SiO_2 fluorescent-labeled nanoparticles was characterized. The results obtained also showed a greater number of nanoparticles in the early endocytic organelles after a short exposure, lessening those numbers as time passed and as the nanoparticle's location progressed to lysosomal structures, suggesting its clearance mechanism from the cell. Shapero et al. [88] also verified a nonexistent association between the nanoparticles and the cellular organelles, consolidating the theory that the great majority of nanoparticles does not leave or circulates outside the endocytic vehicles to interact with other organelles (**Figure 3**).

5. A selection of relevant proteins identified in the protein corona

As mentioned before, the constitution of the protein corona is mainly dependent on the composition of the biological medium where it's inserted [19, 20], as different physiological environments have different proteins that compose them. The protein corona is also dependent on the time of exposure [11, 17, 20], chemical properties of the nanoparticle applied [23], and, in some cases, size and surface of the nanoparticle [23]. It is impossible to have a standard protein corona for a given nanoparticle, as each one will have a different composition [15]. The best approach possible then is to characterize the most occurring proteins, in order to predict the behavior of the nanoparticle when inside the physiological system. This issue of research is still emergent, with promising outcomes to better adjust the nanoparticle to its function and environment. An example of protein corona evaluation is the work presented by Mirshafiee et al. [89], who assessed the differences between nanoparticles with different coatings without a protein corona and the same nanoparticles with it adsorbed. Three different types of nanoparticles were used: the bare nanoparticle (with no coating), a nanoparticle with human serum albumin coating (HSA), and a nanoparticle with gamma-globulin coating (GG). The results indicated that there was a different protein corona associated to each of the various coatings, identifying the proteins by LC-MS/MS. For instance, a greater number of lipoproteins and a low quantity of complement factors and immunoglobulins were found in HSA-coated nanoparticles, while the opposite

occurred in GG-coated nanoparticles, with higher levels of complement factors and opsonins, especially immunoglobulins, and low levels of lipoproteins. The presence of opsonins made the uptake more difficult, and when comparing with nanoparticles without protein corona, the differences in uptake were very significant, as nanoparticles without it entered the cells more easily.

Another work that explored the composition of the protein corona was provided by Mahmoudi et al. [90], who verified the alteration of the proteins that formed the corona after submitting it to plasmonic heat induction. In this experiment, gold nanorods were used and immersed in fetal bovine serum (FBS) at different concentrations – 10% to mimic in vitro milieu and 100% that mimic in vivo milieu. The protein corona was then evaluated before and after exposure to plasmonic heat induction in both concentrations, and by LC-MS/MS, it was found that, at room temperature, at 10% FBS the most abundant proteins were apolipoprotein A-I precursor and the hemoglobin fetal subunit beta, while the least abundant was the apolipoprotein C-III precursor. At room temperature at 100% FBS, the protein corona was rich in the apolipoprotein A-II precursor and also in hemoglobin fetal subunit beta, while the most scarce was the apolipoprotein C-III precursor as well. After exposure to plasmonic heat induction, at 10% FBS the most abundant proteins became the α-2-HS-glycoprotein precursor and the apolipoprotein A-II precursor, while hemoglobin fetal subunit beta maintaining its numerousness and hemoglobin became the least frequent protein. At 100% FBS after plasmonic heat induction, α-2-HS-glycoprotein precursor also became the most frequent one, followed by hemoglobin fetal subunit beta as well, having a very significant decrease in the quantity of apolipoprotein A-II precursor but still having the apolipoprotein C-III precursor as the least prevalent of all the proteins evaluated. Accordingly with these results [90], it is then possible to conclude that the protein corona's composition is dependent on the medium where the nanoparticle is inserted, which, in this case, also translates to a difference between in vitro and in vivo applications of the nanoparticle, being demonstrated as well its dependency on a physical factor (temperature), which must be taken into account when dealing with hyperthermic nanoparticles as a therapeutic method against tumors.

6. Conclusions

The study of the protein corona is still in a very embryonic stage, with many problems and questions yet to be answered, such as the composition when formed in most nanoparticles, exact description of the uptake and clearance mechanisms, and extensive reports on the consequences of its formation. Some steps have been taken with the purpose of answering these questions, especially in resorting to bioinformatic approaches, allowing an easier and more efficient analysis and sharing of the data obtained. Nevertheless, these are without a doubt interesting research topics, leading the way to improve what is already a very auspicious field in nanomedicine.

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