Chapter 3

How do Nanoparticles (NPs) Pass Barriers?

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Abbreviations

BBBs	brain blood barriers
CME	clathrin-mediated endocytosis
CvME	caveolin-mediated endocytosis
IV	intravenous administration
NPs	nanoparticles
SWNT	single-walled carbon nanotubes
RES	reticuloendothelial system

I. Introduction

One of the essential requirements of any delivery system is its ability to move about freely in available environments and have the ability to

cross various barriers that are encountered. Nanoparticles (NPs) possess several key advantages as therapeutic materials, which allow some or all of these obstacles to be overcome. For example, the small size of some NPs allows them to pass through biological barriers and extend bioavailability in tumor tissues.[1] Another attractive characteristic of NPs is that the properties of NPs can be engineered through synthesis including size, shape, and surface characteristics. For instance, the addition of ligands or receptors onto the surface of NPs can enhance their target-specificity.[1]

The body has many natural biological barriers, which create challenges for drug administration. However, much work is underway to address this issue in an effort to bypass these barriers to better treat different disease. By deeply studying the various obstacles that NPs have to pass, it will enable engineers to design more applicable and efficient nanomaterials for drug delivery. Some of the most challenging barriers include blood vessels barriers, cell barriers, and brain blood barriers (BBBs).

Within different cell types there are specific mechanisms that select which materials can pass. One such mechanism is the endocytosis system in which only particular particle types can be transported through the cell barriers. NPs, based on their composition and cell targeting characteristics, have the advantage that they can use the endocytosis machinery to typically enter almost any cell type. However, several other physicochemical characteristics of NPs such as NP size, shape surface functional groups that give rise to charge and surface chemistry influence their cellular uptake.

Of particular importance, it should be noted that the NP properties are not necessarily applicable in biological mediums. In biological environments the surface of the NP will become coated with a layer of biomolecules especially proteins, which will form a protein corona. The composition and molecular properties of the protein corona that forms generally change the behavior of the NPs especially for the cellular uptake.

In this chapter, an understanding of how NPs pass barriers will be described; first the biological barriers and cell uptake mechanisms will

be introduced, followed by NPs properties, which affect their transport and uptake.

II. Biological Barriers

Some of the major challenges in the NP drug delivery field is to improve transport of therapeutics across biological barriers including the small intestine, nasal, skin, ocular, the mouth mucosa, BBB and cell membrane. Many natural defense mechanisms of the human body represent significant barriers for drug delivery.

Under normal conditions these barriers are designed to keep foreign material out of the body and only allow specific small molecules across. Thus, any obstacle that prevents a drug from reaching its target site of action is to be considered a barrier to drug delivery. A strong fundamental understanding of how these barriers function, will allow for the design of novel drug delivery systems based on nanotechnology.[2]

A. Blood vessel barriers

A primary barrier for systemic NP delivery are blood vessel barriers, which normally transport materials in the body. As blood moves through the vessels, they become thinner and thinner until they are finally converted to capillaries through extensive branching and narrowing. These very narrow capillaries are in close vicinity to the individual cells. Once reaching their narrowest sizes, the capillaries begin to merge, forming veins. The blood from the veins then takes the contents back to the heart where they are recirculated.[2] However, simply traveling along the vessels does not accomplish efficient drug delivery. Most importantly, the delivery system must reach the target destination. This initially requires crossing of the blood capillary wall in order to reach the extracellular fluid of the tissue and then once again crossing of other non-target cells, which can also represent a barrier until finally entering the target cell. It is clear that there are major barriers for NPs during transit, especially in going through capillaries to reach the target site.

The two routes that NPs use in order to traverse blood capillaries and other cell layers are the transcellular and paracellular routes. In passing through the transcellular route, a particulate system will enter the cell from one side and leave the cell from the opposite side in order to reach a tissue. On the other hand, in the paracellular system the NPs move between the cells using the junctions. These junctions have the advantage of not requiring a system to avoid being destroyed by the cell. Some common moieties using the paracellular pathway include ions, larger molecules, and leukocytes. This process is controlled by the association of tight and adherence junctions to the cytoskeleton, which is referred to as the apical junction complex. Tight junctions act as a barrier for regulation, while adherence junctions control the development and stabilization of tight junctions. Due to differences in structure and presence of tight junctions, epithelial and endothelial barriers have different permeability values. Thus, tight junctions determine what is transported by the paracellular pathway transport. For example, the diffusion of larger molecules will likely not be feasible, however the migration of certain cell types could be permitted. Certain regions of the body are particularly stringent, especially the epithelia and brain capillary endothelium, while the vascular endothelium in various other tissues tends to have much greater permeability. A strong fundamental understanding of this regulation mechanism is essential in order to enable the development of NPs that reach their intended target cells with minimal off target effects.

B. Blood brain barriers (BBB)

The blood-brain barrier (BBB) is a highly specialized and stringent barrier system consisting of endothelial cells that separates the blood from the body and underlying brain cells, which in turn provides protection to brain cells and overall brain homeostasis. The brain restricts the paracellular pathway as well using an endothelium containing a complex arrangement of tight junctions that restricts the passage of certain molecules. The glial processes that surround the brain serve as an additional physical barrier. The BBB is permeable to small and lipophilic molecules (up to 800 atomic mass units),

however larger molecules will not be transported across this barrier without the use of an active transport system.[3]

1. *Nanoparticles used to cross the BBB*

One important consideration for the application of nanobiotechnology in the brain is the ability to facilitate the delivery of therapeutics across the BBB. Some NPs known to pass the BBB include lipid NPs, liposome, and polymeric NPs.

A lipid NP is composed of nanometer-sized spheres that are surrounded by a lipid bilayer. The lipophilic features on the surface of these NPs facilitate traversing the BBB to enter the brain using the endocytosis pathway.[4] In order to achieve crossing the BBB with liposomes, properties such as lipid composition, size, surface charge, and the preparation method must be considered. Generally small liposomes or nanoliposomes usually measure between 25–50 nm in diameter. Additionally, liposomes can be functionalized using ApoE-derived peptides, which can facilitate cellular uptake and transport across the BBB.[5] Polymeric NPs can be engineered for targeted drug delivery across the BBB, controlled release at the target site and can be biodegradable.[2]

C. The biology of a tumor and therapeutic barriers

Delivering drugs within tumor cells can be very challenging due to the structure and environment of the tumor mass. However, there are some distinct characteristics of tumor cells such as rapid angiogenesis and local necrosis that can be used for targeted delivery. Due to this rapid angiogenesis, vessel formation is abnormal leading to vessels that are branched, short-circuited and of uneven diameter, which causes poorly constructed leaky walls. Depending on the type of tumor, the gaps between the endothelial cells are abnormally large,[2] 200 nm to 1.2 μm in comparison with the gap in healthy vessels that are ~5–10 nm.[2] Poor vascularization, as a result of abnormal vessels in these tumors leads to a lack of oxygen and nutrients driving anaerobic metabolism.[2] During anaerobic metabolism lactic acid is

produced as a result of the low pH environment, which is typical in tumors. For comparison, the pH of blood is ~7.4, while tumors can have pH values as low as 5.6.[2]

Factors such as angiogenesis, leakiness of vessels and acidic environments are properties that can be exploited for targeted delivery. However there are also a number of tumor properties that can make drug delivery extremely challenging. Tumor cells can be hard to reach, with some cells surviving even under marginal conditions and away from blood vessels. New methods in NP design in combination with an understanding of the biological systems involved will be necessary in order to design successful treatments that are effective for the eradication of the entire tumor.[2]

III. Cellular Uptake Mechanism

Prior to engineering NPs, a fundamental understanding of the mechanisms involved such as internalization mechanisms and the intracellular routing is essential. The cell membrane is an important and essential component of every cell. This essential barrier is required in order to separate the internal from the external environment of the cell.[2] Some small molecules such as ions, some small proteins, and molecules (glucose and amino acids) can be transported through specialized transporter proteins, while larger substances such as most nutrients, antigens, antibodies, signaling, growth factors, and NPs cannot diffuse through the membrane and are thus internalized through endocytosis.[2]

Endocytosis requires energy and involves folding the plasma membrane into vesicles, which are then released from the membrane, and its contents transported into the cell.[6] There are two general categories of the endocytosis mechanisms: phagocytosis and pinocytosis.

A. Phagocytosis

Phagocytosis is a common mechanism for the uptake of particles typically larger than 500 nm in diameter and is essential for the

elimination of viruses, bacteria and infected cells by the immune system using macrophages, dendritic cells, and neutrophils. Initially a particle is enclosed within the phagocytic pocket; the cell membrane is then closed forming a sealed vacuole with the particle inside, which is referred to as the phagosome.[7,8]

There are three stages of phagocytosis as shown in Figure 1) opsonization and reorganization of the particles 2) particle adhesion to the cell membrane 3) particle ingestion by the cell. Opsonins coat foreign particles, making them visible to phagocytic cells.[2] The opsonin-coated foreign particles are first recognized by receptors on the cell surface, followed by receptor binding which induces a cascade of intracellular signaling events triggering the ingestion of the

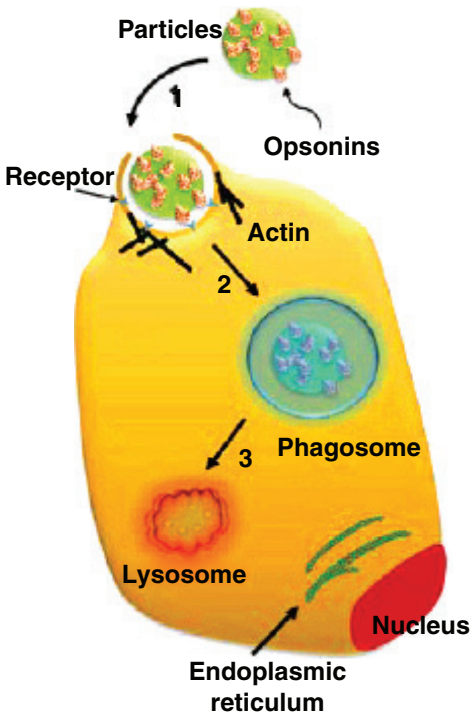

Figure 1. Simplified schematic of the phagocytosis of particles in three steps. Phagocytosis is an actin process dependent process. Taken from Ref. [23] with permission of Elsevier.

foreign particles.[8,9] The particle shape at the point of where cell attachment occurs can also trigger phagocytosis.[2]

B. Pinocytosis

Pinocytosis is defined as the uptake of fluids and molecules within small vesicles during phagocytosis, and generally engulfing particles of less than approximately 500 nm in diameter, (e.g. microorganisms and cell debris). Pinocytosis is known to occur during several different processes including macropinocytosis, clathrin-mediated endocytosis (CME), caveolin-mediated endocytosis (CvME), and clathrin- and caveolin-independent endocytosis.[8,10] The five endocytic pathways, phagocytosis included, are illustrated in Figure 2. As depicted, macropinocytosis like phagocytosis is an actin dependent process.[11,12] All the above mechanisms are described in detail as follow:

1. *Macropinocytosis*

Macropinocytosis is a non-specific mechanism where fluid contents are taken up at the same concentration as the surrounding environment. In contrast to CvME and CME, uptake by macropinocytosis is non-specific and is not receptor mediated,[2] with the vesicle size typically being 100 nm to 5 mm.

Macropinocytosis is an actin dependent process and forms protrusions at the outer cell membrane, which then can go on to

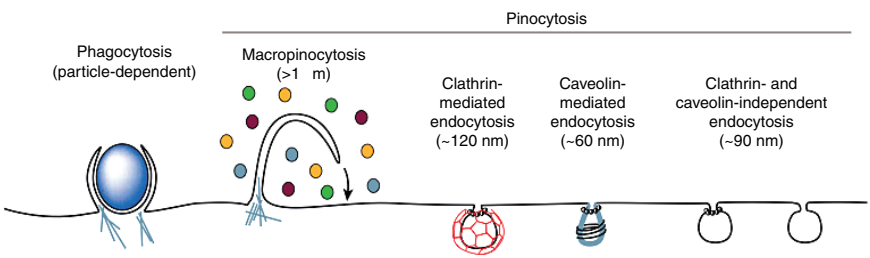

Figure 2. The different endocytic pathways in a mammal cell. The pathways differ in size and mechanism of vesicle formation. Taken from Ref. [10] with permission of Nature Publishing Group.

fuse with the cell membrane when larger fragments or debris are taken up.[2]

2. *Clathrin-mediated endocytosis (CME)*

CME is an example of receptor-mediated endocytosis having vesicle sizes of ~120 nm.[2] This type of endocytosis is well characterized and similar to other pinocytotic pathways, representing a form of receptor-mediated endocytosis. This pathway is crucial for the uptake of several molecules including low-density lipoprotein and transferrin.[2,13]

3. *Caveolin-mediated endocytosis (CvME)*

CvME is the primary mechanisms of endocytosis, which occurs after CME. This endocytosis mechanism captures small particles typically in the diameter range 60 nm.[2] Numerous cell types including the capillary endothelium, type I epithelial cells, muscle cells and fibroblasts exhibit CvME, which occurs where there are lipid rafts.[10,14]

4. *Clathrin- and caveolin-independent endocytosis*

Clathrin- and caveolin-independent endocytosis produces vesicles of ~50–100 nm and is not a selective mechanism (i.e. it is not dependent on receptors or stimuli from the internalized material).[23]

Yet, not all cell types contain the required machinery in order to perform the entire full spectrum of endocytic pathways. Therefore, these pathways are cell type specific and determine the trafficking and intracellular fate of the particles that are encountered.[2] For example, red blood cells are considered non-phagocytic cells, because they do not have any phagocytic receptors on their surface and no actin–myosin system. The non-phagocytic character of red blood cells allows them to serve as a good non-phagocytic model for how NPs penetrate through cell membrane.[2]

C. Mechanisms for the uptakes of NPs into cells

There have been numerous reported studies on NP uptake mechanisms and each study was reported to be either phagocytosis or pinocytosis driven. It is unclear whether non-endocytic uptake mechanisms, such as diffusion or active transport, can be utilized by NPs.[2] The primary factors that determine if NPs will be taken up are the size and charge of their surface. Generally, particles above 200 nm in diameter are not internalized through a single clathrin-coated vesicle and are expected to have limited internalization through classical endocytosis.[2] The uptake of larger particles occurs through phagocytosis with liquid internalization occurring by macropinocytosis.

1. *Phagocytic uptake of NPs*

The local shape and geometry of NPs determine their initial attachment to the cell membrane in phagocytosis; however binding of opsonin proteins to the NP surface is mainly what triggers phagocytic uptake of NPs. It is known that opsonins will bind to most NPs, targeting them for rapid elimination through the reticuloendothelial system (RES) when injected *in vivo*.[7,15,16] However, the most important characteristics for NP opsonization are the ionic and hydrophobic interactions that occur with the NP surface.[8,9,17] The surface hydrophobicity and negative charge are seemingly key factors for opsonization.[2] Special coatings have been specifically designed for NPs to limit phagocytic uptake by cells associated with the immune system in order to avoid removal and sequestration into the RES.[2] For example, NPs coated with PEG delays immune clearance of foreign particles.[18,19]

2. *Clathrin-mediated endocytosis (CME) pathway for uptake of NPs*

CME pathway can be utilized for NP uptake; using low density lipoprotein and transferrin receptors in combination with drug delivery devices can result in rapid and selective cellular uptake.[6,20]

Internalization via CME is dependent on NP size (50–200 nm) and cell type.[21]

3. *Caveolae-mediated endocytosis (CvME) pathway for uptake of NPs*

The second major trafficking pathway used is caveolae-mediated endocytosis (CvME), which facilitates the uptake of biomolecules and NPs.[2] There are also several classes of NPs that enter cells through caveolae.[6] Based on *in vitro* studies, the CvME pathway is postulated to have a slower uptake mechanism.[2]

IV. Macropinocytosis for Uptake of NPs

Nanoparticle uptake by endocytosis is a highly complex process involving the interplay of the energy release by the number of bound receptors, the number of vesicles that form, receptor diffusion toward the attached particle and time to complete the process. Being that for large NPs, only one particle is taken up per vesicle, attachment occurs to numerous receptors simultaneously, resulting in a substantial change in free energy. Also, larger particles require more time for membrane wrapping, with larger contact areas with the cell membrane resulting in a larger decrease in free energy.[21,22] In contrast, several smaller NPs can be taken up in one vacuole; however, only single attachment sites are used resulting in less free energy change.

A. NPs properties influencing their uptake

There are many specific physical and chemical properties that will influence cellular internalization of NPs, not to mention the surrounding environment (i.e. cell medium) (Figure 3). Some of the key properties of NPs to consider are size, shape, and surface functional groups. Therefore, great effort has been put forth towards the development of NPs with well-defined characteristics. The effect of the core composition is not discussed here, because the surface

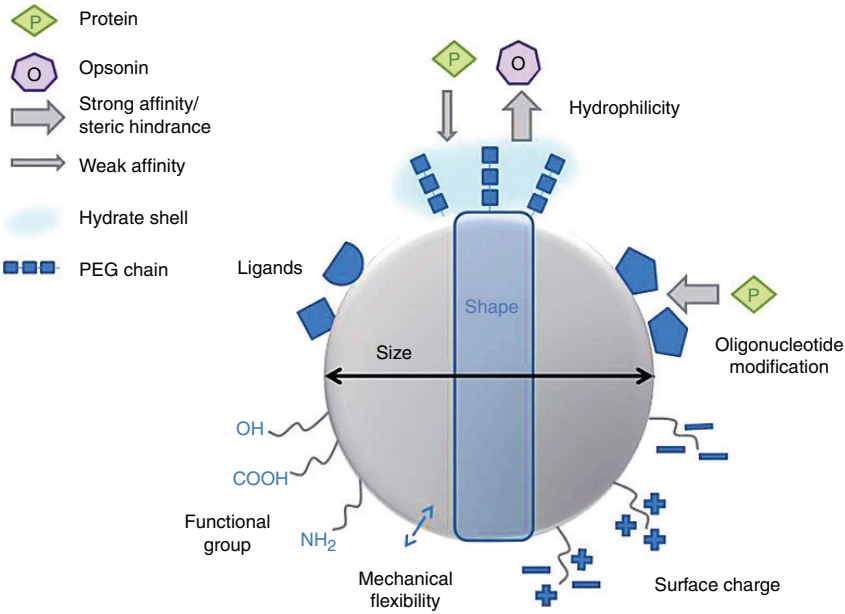

Figure 3. Determinants of nanoparticle interactions with cells as determined by experimental conditions (presence of protein and opsonins): shape (spheres, short–long rods, cubes, and triangles with different aspect ratios), cell type, size, surface chemistry, and addition of ligands. Taken from Ref. [2] with permission of Wiley & Sons.

characteristics are greatest, as the surface properties determine the protein corona and hence likely the biological impacts.[2]

B. Effect of size

Some of the most critical general issues to consider in the field of nanotechnology is the size and effect on the biodistribution, the kinetics of release and cellular uptake of NPs.[2] In particular, spherical NPs up to 500 nm in diameter have been shown to be taken up by non-phagocytic cells, whereas none of the 1000 nm NPs were taken up by these cells.[2] Small NPs have the advantage of often not being

recognized as foreign agents by macrophages and can enter macrophage cells through membrane pores; however, microparticles are easily taken up by RES.[23] Diffusion of NPs into cells can be facilitated by decreasing the size and increasing the surface area of the NPs.[2] For example, more efficient cellular and nuclear internalization was observed with 30 nm sized single-walled carbon nanotubes (SWNT) compared to 50 nm SWNTs.[2] The optimum NP size for internalization was determined to be 50 nm, and this was found to be true for a number of cell types.[2]

According to previous findings, the NP diameter was a determinant in the internalization pathway engaged. For example, clathrin coated pits control the penetration of microspheres having a diameter of less than 200 nm; however if the size is increased to 500 nm, the caveolae-mediated process is predominant for internalization.[24] Additionally, NPs with diameters of 50 nm are able to efficiently target human mesenchymal stem cells using clathrin, which is known to act independently from endocytosis.[2] Interestingly, in cancer cells, NP uptake increases as the size of the NP is decreased, which occurs regardless of surface modifications on the NPs.[25] For instance, submicron multi walls have demonstrated better cell internalization capacity than larger ones.[2]

However, the greatest efficiency for surface attachment has been demonstrated using particles with dimensions on the scale of 2–3 mm.[2] In summary, NP uptake efficiency by non-phagocytic cells illustrates a clear trend: cellular uptake initially increases as a function of NP size until reaching the optimum limit of ~50 nm and then uptake decreases when this size is exceeded. In contrast, for phagocytic cells, the uptake is not necessarily as related to just NP size.[22,24,26]

C. Effect of shape and geometry

It is well known that the shape and geometry of NPs significantly impacts their ability to pass cellular barriers and become internalized. The optimal shape can confer several advantages such as improved circulation time, biodistribution and residency time of NPs once

internalized inside cells.[27,28] Additionally, the shape of NPs can also influence cell targeting. For example, receptor-mediated endocytosis of nano-carriers is directly affected by their shape.[2] Elongated NPs have demonstrated increased efficiency for cell adherence in comparison to spherical NPs. The curve shape of spherical particles is known to limit the number of possible exposed binding sites, which are available to interact with the receptors on the target cell. In contrast, elongated NPs have more surface area available for multivalent interaction with the cell surface.[2] There, the higher aspect ratio of the elongated allow these NPs to be taken up by cells more frequently than spherical NP counterparts of the same size. For example, rod-shaped NPs which have higher aspect ratios will be taken up more efficiently by Hela and caco-2 cells, than lower aspect ratio NPs or larger 1 mm sized particles.[2]

Furthermore, rod-shaped NPs have a high affinity and internalization efficiency to endothelial cells, which allow for improved efficacy both *in vitro* and *in vivo* experiments.[2] It has also been shown, that discoid NPs are taken up better by Hela cells than rod-shaped NPs of the same volume.[2]

It should also be noted that NPs possessing sharp shapes tend to penetrate endosomal membranes and localize to the cytoplasm, leading to reduced exocytosis of these NPs compared to spherical NPs.[2] Other studies have shown differential cellular uptake of spherical NPs having different aspect ratios and resulting in a faster rate of absorption for those NPs with a high aspect ratio.[2] In addition, recent investigations have identified that ellipsoidal NPs are taken up less frequently than spherical NPs.[2]

D. Effect of surface functional group

Surface modifications can change the various physical, chemical or biological characteristics at the surface of NPs, which can be used to manipulate the charge of the NPs. Understanding the interactions between the negative membrane of cells and NPs with varying charges is essential for the design of adaptable and useful NPs.[2]

1. *Neutral modification of particle surface*

Nonionic polymers are able to increase particle stability using steric stabilization, which limits the direct interaction between cells and the phagocytic system. PEG is a commonly used neutral and hydrophilic material that forms a barrier on the surface of NPs, which can lead to the steric stabilization, low opsonization, high blood circulation time and escape from reorganization by the phagocytosis process.[2] Another use of PEG is to modify the hydrophilicity of polyester or polyalkylcyanoacrylate NPs as needed.[2] Also it is known that reducing NP aggregation has been shown to improve cellular uptake. Additionally, both PEG and modified PLGA NPs have demonstrated as much as five-fold higher cellular uptake than unmodified NPs.[2] Another advantage of PEG or folic acid NP surface modifications is that they have been shown to reduce the adsorption of proteins and thus recognition by macrophages. In terms of possible applications, these modified NPs can be easily taken up by cancer cells for efficient and selective cancer therapies.[2] As an alternative approach to the PEGylation method, polysaccharides can also be used and are known to interact with specific receptors, which allows for targeted delivery.[2]

2. *Positive modification of particles surface*

While neutral modification results in less particle aggregation, neutrally charged NPs have led to low cellular uptake.[2] An explanation for this is the low affinity of the neutral NPs toward the negatively charged cell membrane. However, positive modification of NPs causes electrostatic interactions to be formed between the surface of the NPs and the negative charge on the cell membrane.[29,30] Thus, positively charged NPs have demonstrated more efficient uptake than negatively charged NPs using the endocytosis pathways. The negatively charged proteins found on the outer surface of gastrointestinal epithelial cells allowed for increased cell permeability with the use of positively charged drug carriers.[2]

3. *Negative modification of particles surface*

The negative modification of NPs has also been shown to affect their cellular uptake. However, cellular internalization of negatively charged NPs by RME (receptor-mediated endocytosis) has yielded some opposition. Negatively charged NPs have been reported to both increase cellular uptake efficiency as a function of increased charge as well as inhibit cellular uptake as reported by He *et al.*[2] Another surface property that affects cellular uptake is hydrophobicity.

V. NPs and Biological Entities Interaction

Immediately after intravenous administration (IV), NPs come in contact with blood as part of the physiological environment. The blood plasma represents a highly complex environment containing hundreds of thousands of different proteins ranging up to 12 orders of magnitude in concentration based on the particular protein.[2] The blood plasma has a full range of proteins at different concentrations. Thus, upon IV injection of NPs entering the bloodstream, there is binding competition between various biological molecules, which determine what is adsorbed onto the NPs surface. Initially the most abundant proteins predominate adsorption on the NPs surface, however over longer periods of time these proteins will become replaced by higher affinity proteins.[2]

A. Protein corona

When NPs are introduced into biological environments, a protein-rich layer is adsorbed onto their surface, which is known as the protein corona. Understanding the process of protein corona formation is critical as subsequent downstream interactions will be mediated based on the nature of this corona. However, understanding the relationship between the original functionality of NPs and protein corona formation can be very challenging and is an area that still remains elusive.[31,32] There are several factors that contribute to how the protein corona is formed; NP surface chemistry, size[33] and charge[2]

are pivotal factors in determining the formation and nature of the resultant protein corona. The composition and molecular properties of the protein corona are known to be influential factors for the cellular uptake of NPs,[34,35] however, any direct links between structure and downstream effect still remain to be elucidated. Therefore, it is necessary to understand the adsorption of the protein corona on the NP surface to be able to reliably predict how they will then interact with cells after this has occurred.

It is important to note that there are a number of diverse biomarkers that bind and cover NPs almost immediately after they come in contact with biological fluids.[2] Protein coronas can be classified into two types: those proteins that bind to NPs with high affinity, known as a hard corona, or those with low affinity, known as a soft corona. The initial corona that is formed is considered as a "soft" protein corona, which allows for a more highly dynamic exchange of proteins, which will coat the surface of the NPs. However, over time the composition of the "soft" corona will slowly transition into a "hard", which has less dynamic exchange (Figure 4).[36,37]

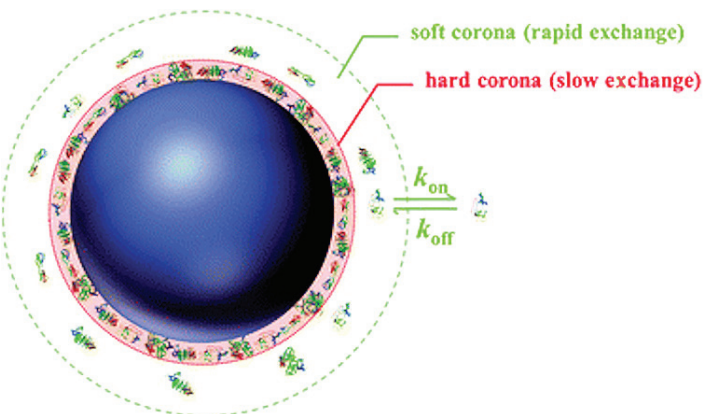

Figure 4. Illustration showing soft and hard corona on NPs in a biological environment. Taken from Ref. [45] with permission of Royal Society of Chemistry.

1. *Effect of corona on NP characteristics (size)*

While a NP may start out with at a particular size after synthesis, this size can vary after NPs are exposed to the biological environment. More specifically, the protein corona modifies several properties of the original NPs such as size and surface composition, which determine downstream events including physiological responses, kinetics, transport, accumulation, exclusion and toxicity of the NPs.

2. *Effect of corona on NP uptake*

As mentioned previously, the NP protein corona complex can influence cellular uptake. For example, changes to the adsorbed protein structure can even inhibit cellular uptake of NPs. Additionally, should the adsorbed protein corona become unfolded or undergo some other structural rearrangement, it may change accessibility of NPs to cell surface receptors.[43] However, it has been demonstrated that complete serum media and serum depletion of several abundant proteins do not influence the NP interaction with endothelial cells. There, the cellular association of NP protein complexes is a non-specific process and is mostly dependent upon the total amount of adsorbed proteins rather than the particular of type of protein coating on the NP surface.[2]

In general, even after addition of serum, the efficiency of NP uptake by phagocytes is unaffected, and contingent on the kind of protein present.[2] While many contradictory effects occur, this can be explained by a differential population of proteins in the medium, and by dynamic changes in protein corona composition over time, driven by protein abundance, affinity[38] and by opsonization. It has also been found that increasing the amount of serum present in the culture medium of non-phagocytic cells results in a reduction in cellular NP uptake. The extent of NP hydrophobicity decreases the effect of NPs while increasing hydrophobicity increases the effect. It is known that the hydrophobicity of NPs determines binding affinity to

hydrophobic pockets of bovine serum albumin. Differential NP uptake by non-phagocytic cell lines is explained by a number of properties of the cells including different binding proteins present on the cell surface, charge differences among cells and the rate of receptor turnover.[39,40]

As previously discussed, NP uptake is a multi-step process, in which NPs initially adhere to the cell membrane and then are subsequently internalized into the cell by energy dependent pathways. Typically, the protein corona strongly reduces NP adhesion to cellular membranes in contrast to bare nano-materials and thus results in a concomitant decrease in nanoparticle uptake efficiency.[2]

3. *Prevention strategies to inhibit corona formation*

The biomolecular protein corona is still largely an unpredictable complex factor, which in some cases can trigger desired outcomes, but in others can result in toxic biological responses.[44] Therefore, there is currently and has been much effort put forth in order to synthetically prevent protein adsorption. One possible solution was to functionalize NPs with polymer chains such as PEG, making the NPs' surface highly biocompatible, which limits non-specific interactions with biological components.[2] While PEGylation is able to partially mitigate protein adsorption to NPs, it has still been unable to completely prevent protein/biomolecule corona formation.[37,41] An alternative solution that could be employed is to functionalize the NP surface with zwitterions. There have been NPs designed such that hydrophobicity is tunable in which the NPs don't adsorb proteins at moderate serum levels and do not form hard coronas at physiological serum concentrations. Novel strategies such as the one just mentioned have the capacity to lead to new analytical methods to investigate the interaction of nano materials in biological environments without compounding variables such as interference from protein binding. However, even with recent gain, further development in producing "corona-free" NPs will be required and is currently underway.[42]

VI. Concluding Remarks

NPs have several unique properties such as small size and high surface area, which are distinctly advantageous in biomedicine. The small size of NPs allows them to enhance drug transport across biological barriers (leading to an increased bioavailability of the entrapped drug) or improve intracellular drug delivery which can then be applied to gene or cancer therapy. NPs can be designed to transport different substances across various barriers depending on the properties they possess. With so many possibilities it is important to understand the system one wishes to exploit and then design a nanocarrier to accomplish or exceed previous limits of already characterized systems.

In general, the following trends can be concluded from this chapter: based on the morphology of NPs, uptake by non-phagocytic cells is optimum at a NP size of ~50 nm, and for phagocytes, the results are still inconclusive. The effect of increased surface charge modification, either positive or negative, tends to favor cellular uptake in either cell type. However, when all other factors are comparable, positively charged particles are taken up more extensively.

NPs in biological medium exhibit different properties than the original synthetic NPs; in order to reduce unpredicted NP behavior in the body, surface enhancements such as PEGylation should be employed.

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