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Formation of a protein corona influences the biological identity of nanomaterials☆

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ABSTRACT

The development and testing of nanomaterials is an area of interest due to promising diagnostic and therapeutic applications in the treatment of diseases like cancer or cardiovascular disease. While extensive studies of the physicochemical properties of nanoparticles (NPs) are available, the investigation of the protein corona (PC) that is formed on NPs in biofluids is a relatively new area of research. The fact that few NPs are in clinical use indicates that the biological identity of NPs, which is in large part due to the PC formed in blood or other bodily fluids, may be altered in ways yet to be fully understood. Herein, we review the recent advances in PC research with the intent to highlight the current state of the field. We discuss the dynamic processes that control the formation of the PC on NPs, which involve the transient soft corona and more stable hard corona. Critical factors, like the environment and disease-state that affect the composition and stability of the PC are presented, with the intent of showcasing promising applications for utilizing the PC for disease diagnosis and the identification of disease-related biomarkers. This review summarizes the unique challenges presented by the nanoparticle corona and indicates future directions for investigation.

1. Factors that control the formation of the protein corona on nanoparticles

1.1. *Hard and soft coronas*

When nanoparticles (NPs) circulate through the body, these are exposed to a complex fluid environment, such as blood or lymph, and interact with resident biomolecules. For example, in the blood, NPs encounter abundant proteins like serum albumin or apolipoproteins. These interactions over time result in the resident biomolecule(s) binding to and coating the NPs, forming a protein corona (PC). Protein "coronas", originally coined by Cedervall et al.,[1] are complex structures, sometimes 20–30 nm thick, that consist of soft and hard layers, termed the soft and hard corona. The soft corona (SC) is composed of proteins involved in transient low-affinity interactions, while the hard corona (HC) depends on more

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permanent high-affinity interactions. Generally, the higher affinity proteins forming the HC initially interact with NPs. SC proteins may secondarily interact with NPs as a consequence of the presence of the HC proteins, rather than the core NP surface.[2] An alternative concept is that SC and HC proteins may interact with NPs at different binding affinities. Cedervall and colleagues reported that the SC components bind to NPs for only a few minutes, while HC components bind to NPs for several hours, with proteins having the highest affinity being the smallest in molecular weight.[1] Because of a longer interaction time with NPs, Walkey et al. proposed that the HC proteins may be more important than the SC proteins in defining the biological response of the nanomaterials.[3] Hence, the SC–HC interface is likely critical to establishing the biological identity of NPs. Most research relates to the HC, due to challenges in isolating the more transient SC. One study utilized iron oxide NPs incubated in fetal bovine serum (FBS) to study the SC and found that it was composed of mostly complement proteins, antithrombin, and alpha-antiproteinase.[4] In another SC study, iron oxide NPs were incubated in human blood or lymph serum.[5] In human blood, SC-specific molecules identified were angiotensinogen, annexins, cathepsins, and collagen-based. Interestingly, complement proteins were mainly found in the HC, rather than the SC[5]; as in the previous study.[4] A possible explanation is that the NPs' surface chemistry differed; consisting of polyvinyl alcohol[4] or no modifications.[5] Components of the HC, on the other hand, are well established. Apolipoproteins, serum albumin, fibrinogen, and immunoglobulins are generally the most common, even among different nanoparticle classifications, such as metalloids,[6] liposomes,[7] and polymers.[8]

1.2. *Physicochemical and dynamic properties that influence corona formation*

Physicochemical attributes of NPs, such as shape, size, and charge, can affect formation of the PC on NPs. An interesting study that examined the effect of nanoparticle shape on the PC is the *in vivo* study by García-Álvarez et al. Gold nanoparticles in nanorod and nanostar configurations were incubated in mouse blood, and proteins specific to each nanoparticle were analyzed.[9] PC proteins unique to nanorods were beta-globulin and plasminogen, while PC proteins unique to nanostars were murinoglobulin-2, serine protease inhibitor A3N and apolipoprotein A-I. While, the majority of the PC constituents were shared between both shaped particles, differences in the abundance of several proteins demonstrated that the shape of the NPs was a critical factor in the composition of the PC. For example, nanostar coronas had at least twice the amount of serum albumin, alpha-2-macroglobulin, and serine protease inhibitor A3K, than nanorod coronas.[9] To explore the effect of nanoparticle size on the PC, iron oxide particles of 30, 200, and 400 nm were incubated in human plasma.[10] Only 20% of corona proteins were shared among the three sizes, indicating that the size of NPs was important in forming the PC. The 30 nm particles uniquely associated with cell cycle proteins, whereas the 200 nm particles bound to proteins with reproduction, localization, and homeostatic process-related functions.[10] The 400 nm NPs had no exclusive functional associations.[10] Degrees of protein abundance in the PC were also shown to differ among the three constructs. To illustrate, the extent of 30 nm construct binding to platelet factor 4 was twice that of the larger particles, while the binding of apolipoprotein A-I and serum albumin to 200 nm particles was at least twice as abundant as 30 and 400 nm constructs.[10] For investigating nanoparticle charge, Lundqvist et al. used positive-(amine-conjugated) and negatively-charged (unmodified or carboxylated) polystyrene NPs incubated in human plasma and found significant differences.[11] PC proteins specific to positive NPs were apolipoprotein F, complement C1r, and mannose-binding protein, while the PC proteins specific to negative NPs were the majority of complement, Ig gamma, and Ig kappa.[11] Despite such differences, the PC tends to give NPs a zeta potential in the range of −10 mV to −20 mV that seems independent of the NPs' physicochemistry. For example, in the study by Alkilany et al., anionic and cationic polyelectrolyte-coated gold nanorods, incubated in biological media with bovine serum albumin (BSA), had the same zeta potential (−20 mV).[12] Hence, the formation of the PC depends on multiple factors in addition to the physicochemical properties of the NPs.

Biological dynamics can impact PC formation on NPs. One dynamic aspect is termed the "Vroman effect", originally studied by Leo Vroman.[13] The Vroman effect describes the phenomenon that certain proteins that initially associate with the nanoparticle PC, over time, within minutes or hours, are exchanged with a new set of proteins that possess higher affinities for the nanoparticle's surface or the corona.[14] This trafficking of proteins may occur within the SC in seconds to minutes due to the SC proteins' low affinity for each other and for the HC proteins. In contrast, components of the HC are strongly bound to NPs and may take hours to exchange with higher affinity proteins, if at all.[15] This was studied by Tenzer et al., employing silica-based particles in human plasma. The degree of adsorption onto NPs of members of the same protein class was dependent on incubation time as well as nanoparticle charge.[16] For instance, the level of coagulation protein adsorption of prothrombin was stable over two hours for positively-charged NPs but showed a 6-fold increase with negatively-charged NPs, while kininogen-1 decreased overtime on negative particles and was stable with positive particles.[16] In an investigation by Palchetti et al., liposomes were incubated in FBS for 5 and 90 min.[17] Under static conditions, the extent of FBS protein binding onto NPs decreased by at least a factor of two, while under dynamic flow conditions, in a peristaltic pump, the degree of binding dramatically increased by at least three times.[17] Further, an *in vivo* study by Chen et al. showed the dynamics of complement C3 binding using iron oxide nanoworms.[18] When particles were pre-incubated in human plasma with C3, injected into a C3-deficient mouse, and then recovered after 5 min, the C3 was completely absent from the particles. Only native murine C3 was retained post-injection on non-precoated nanoworms, showing the complex dynamics of complement protein adsorption and de-adsorption on NPs.[18]

"Fingerprinting" is a dynamic concept that describes the unique coronal pattern formed on NPs in response to the environment in which particles were previously or currently exposed to. An early example of this was reported by Lundqvist et al. Silica NPs were treated with human plasma, cytosolic fluid, or both and their coronal differences examined.[19] The main coronal components of the NPs exposed to human plasma and then cytosolic fluid were more representative of the proteins from plasma. Using two different nanoparticle types, silica, and polystyrene, the presence of apolipoprotein A-I on the PC was reduced during transitioning from plasma to cytosolic fluid.[19] In another study, iron oxide NPs were incubated in human blood, lymph, or both, and the transition between fluids resulted in a 35–45% change in the NPs' PC.[20] The authors concluded that in most cases, the PC, after exposure to two different mediums, was mostly composed of proteins from the initial medium. For example, apolipoprotein B-100 and complement C3, which were highly adsorbed to NPs in blood, remained highly absorbed after a transition to lymph, with the C3 presence only slightly reduced.[20]

Coronal dynamics can also be reflective of coronal layers (a.k.a. thickness). The SC is so dynamic that a new term "protein cloud"[21] signifies the idea that the SC is weak, fragile, and easily changed due to the slightest interaction. This SC cloud could consist of layers of protein stacks, which is supported by the fact that plasma proteins generally have diameters of 3–15 nm,[22] while coronal layers can be over 30 nm thick.[23] Moreover, the protein cloud's thickness can be much larger than the HC, due to reports of higher protein concentrations occupying the SC as opposed to the HC. This was shown by Alex et al., using gold nanorods incubated with specific concentrations of HSA, immunoglobulin G (IgG), or transferrin to analyze protein adsorption.[24] The SC consistently exhibited higher protein concentrations than the HC. For example, HSA had a 5-times greater concentration in the SC vs. the HC, in the case of positively-charged nanorods, while transferrin had a 5-times greater concentration in the SC *vs.* the HC, in neutral nanorods.[24]

Another parameter of biological dynamics concerns whether the most abundant proteins occupying the PC directly correlate with the most abundant proteins in the biological fluid to which the NPs are exposed - the current consensus being that the two do not always correlate. To demonstrate, the concentration ratios of the top ten most-abundant human serum proteins within the PC were compared to that of their native concentrations in the blood.[20] While serum albumin was consistently the highest coronal component from blood, other proteins, like alpha-1-antitrypsin, displayed a higher concentration in the PC as compared to blood. When Bonvin's group did this assessment with a focus on the biological function of the twenty most-abundant proteins, a greater disparity between coronal and blood protein ratios was seen.[20] Protein classes that increased in the PC were complement, coagulation factors, and lipoproteins. Protein classes that decreased in the corona were molecular transport-related. Such results suggest that the *vitro* to *in vivo* consistency of the biofluids surrounding NPs is a necessary consideration when evaluating the formation of the PC and its impact on the biological activity of nanomaterials.

2. Impact of the corona on the biological properties of nanoparticles

2.1. *Effect of the corona on the targeting and uptake of nanomaterials by cells*

An important consideration with nanomedicine is the optimal delivery of the drug cargo to its target. Functionalizing the nanoparticle surface with chemicals, such as polyethylene glycol (PEG) to reduce immune-mediated NP destruction[25] or folic acid to target cancer cells overexpressing folate receptors,[26–28] improves drug delivery. But an emerging question in the field is how the *in vivo* coronal components, particularly proteins present in the blood, affect nanoparticle delivery. NPs accumulate in tissues through two generalized mechanisms: passive and active targeting. Passive targeting relies mostly on blood vessel permeability. In the tumor environment, the abnormal growth of blood vessels (angiogenesis) can result in a leaky vasculature. This phenomenon, along with lymphatic changes, is known as the enhanced permeability and retention (EPR) effect.[29] Untargeted NPs can accumulate in tumor tissue *via* the EPR effect[30]; nonetheless, the EPR effect is highly variable due to a number of factors including tumor type, stage, size and systolic blood pressure, among others.[31] Alternatively, active targeting has the advantage of cellular internalization *via* receptor-mediated mechanisms. The nanoparticle surface is decorated with specific molecules (*e.g.*, folic acid, transferrin, antibodies) that bind to receptors on the target cell membrane. Tuning the optimal conditions of the ligands on the nanoparticle surface is essential for effective targeting. Factors such as density, orientation, affinity, and accessibility of the ligand on the nanoparticle surface are critical for target recognition. Obstruction of the targeting ligand by other ligands or molecules absorbed onto the NPs presents a challenge that may affect the ability of the ligand to bind to its receptor. While surface functional groups (*i.e.* coating, targeting moieties) are intended to dictate the physiological response of the nanocarrier, the bio-corona or PC will impact the targeting abilities and ultimately the biodistribution of the NPs. For instance, in active targeting, the smaller the ligand is, the greater the chance that it will be impeded by the PC. Salvati and collaborators revealed that the targeting capabilities of transferrin (Tf)-functionalized silica NPs disappeared in biological fluids, likely as a consequence of the PC formation.[32] Moreover, Dai et al. showed that silica-poly(methacrylic acid)-PEG-Anti-HER2 NPs lose their targeting ability when a PC formed after incubation with human serum.[33] Interestingly, when the same NPs were incubated with HSA, only the HSA-PC enhanced the targeting capabilities of the NPs,[33] suggesting that the PC can have positive or negative influences on the accessibility of the targeting ligands.

To prevent aggregation and uptake by phagocytes and to enhance systemic circulation time, antifouling polymers like PEG are commonly used to coat NPs. As a result, PEGylation has become the gold standard in the field. Multiple reports demonstrate that attaching PEG to NPs reduces the nonspecific biding of serum proteins, improving passive targeting to tumor tissue *via* the EPR effect.[34,35] A critical factor is to

select the most appropriate PEG length and surface density for the nanoparticle delivery system. As demonstrated by Pozzi et al., different PEG-liposome formulations produced distinct coronas on NPs and in some instances inhibited cellular uptake.[7] Increasing the PEG length reduced protein adsorption by the liposomes, as well as the affinity for apolipoproteins and likewise the total amount of opsonins absorbed.[7] In contrast, Papi and his team found that PEGylation of the liposomal FDA approved drug, Onivide, was less important for stealth effect, than the particle-surface chemistry.[36] While increased circulation time is critical for achieving active targeting, effective internalization of nanoparticle cargo by the target cell is essential for therapeutic efficacy. The formation of the PC on NPs may fundamentally change the cellular uptake and intracellular dynamics of the NPs. For example, Digiacomo and colleagues reported that multi-component lipid NPs in human plasma developed a PC that changed the cellular uptake mechanism from macropinocytosis to clathrin-dependent endocytosis.[37] Likewise, Cracciolo et al. demonstrated that the PC formed after incubation in FBS controlled the cell internalization mechanism of modified lipoplexes. The PC induced the formation of large aggregates resulting in a switch of the uptake pathway from clathrin-dependent to caveolae-mediated.[38] It is therefore important to consider multiple factors, such NP stability as well as internalization, when applying surface modifications like PEGylation to offset some of the negative effects of the PC that forms on NPs.

2.2. *Toxic effects of nanomaterials due to corona formation*

The biological identity of NPs due to the formation of the PC can result in deleterious effects such as immunotoxicity. To this end, Borgognoni et al. investigated the effect of the PC of titanium dioxide NPs formed in response to BSA on human macrophages. An increase in the secretion of inflammatory cytokines, such as IL-1β and IL-6, from human macrophages was noted upon stimulation with PC-containing titanium dioxide NPs in a concentration dependent manner.[39] Secondary modified proteins found in the PC likely interacted with the surface receptors on macrophages that activated signaling proteins inducing cytokine production.[39] This suggested that the formation of the PC could alter the recognition of NPs by phagocytic cells like macrophages, promoting inflammation. Similarly, Yan et al. described how BSA, during the formation of the PC on disulfide-stabilized poly(methacrylic) acid nanoporous polymer NPs in 10% FBS-containing media, underwent a conformational change (denaturation) that decreased the internalization efficiency of NPs by human monocytes (THP-1). In contrast, this unfolded BSA activated class A scavenger receptor (SR-A)-mediated phagocytosis in differentiated THP-1 cells (dTHP-1), which did not affect internalization of NPs.[40] The resulting secretion of inflammatory cytokines, such as IL-1β and tumor necrosis factor α (TNFα), by dTHP-1 cells was evidence of active phagocytosis that was relevant to *in vivo* biological interactions of the PC on NPs.[40] Complement proteins may also bind to the PC and activate the complement cascade that leads to inflammation. In this regard, Kumar and colleagues found that, after being inhaled through the lungs, the PC the formed on poly(vinyl) acetate NPs contained innate immune proteins such as complement (C1q and C3).[41] When activated, these complement components can act as opsonins that promote the clearance of NPs from the lungs through phagocytosis and but can also trigger damaging inflammatory pathways. A further concern with NP toxicity is the incorporation of bacterial endotoxin (lipopolysaccharide, LPS) in the PC. LPS is found in the outer membrane of gram negative bacteria like *Escherichia coli* and is thus a common environmental contaminant. Toll-like receptor 4 (TLR4) is a well-characterized transmembrane receptor for LPS and is found on many cells, such as macrophages, dendritic cells and B cells. Stimulation of TLR4 by LPS activates a signaling cascade that triggers the release of cytokines and inflammatory mediators.[42] Hence the presence of LPS in the PC of NPs could be detrimental. Li and collaborators reported the importance of using nanomaterials that are free of bacterial endotoxin, by showing that LPS can be absorbed onto the nanoparticle surface and impact PC development. LPS incorporated by gold NPs reduced the formation of the PC in human plasma and induced an inflammatory response *in vitro*.[43] Bianchi et al. also demonstrated that LPS on titanium oxide NPs significantly heighten the pro-inflammatory effects of the NPs on murine macrophages. The formation of an LPS-containing PC enhanced the release of inflammatory cytokines through the activation of the transcription factors, nuclear factor-κB (NF-κB) and interferon regulatory factor 3 (IRF3).[44] Such results indicate the importance of characterizing the PC formed on NPs in different biofluids to prevent toxicity due to unintended biological behaviors.

3. Influence of the disease state on corona formation and function of nanoparticles

3.1. *Biofluids modulate the composition of the corona of nanomaterials*

NPs face a complex environment when introduced into biofluids like blood. Plasma, the acellular component of blood that lacks clotting factors, contains thousands of different proteins[45] as well as lipids and nucleic acids.[46] A few hundred plasma-derived biomolecules can coat NPs, forming a complex layer on the PC that has both stable and dynamic components as was previously described. While the physicochemical properties of NPs can affect the formation and stability of the PC, the biological environment surrounding the NPs may be the most important factor driving the composition of the PC. The adsorption of biomolecules from fluids can alter the biodistribution and ability of NPs to be taken up by target cells. Using sulfonated polystyrene and silica NPs, an early study of the HC formed on NPs in plasma showed that protein adsorption evolves based on the concentration of protein in the surrounding media. Since the concentrations of proteins in fluids vary significantly in *in vitro* compared to *in vivo* conditions, this study was one of the first to imply that the same NPs may function differently due to alterations in the PC that are influenced by the environment.[23] Walkey et al. further suggested that the concentration of proteins in fluids could affect the aggregation of NPs due the speed

at which the PC forms: faster in fluids with higher protein concentrations (less nanoparticle aggregation) and slowly in fluids with lower protein concentrations (more nanoparticle aggregation).[3] Other studies revealed that subtle differences, such as how blood is collected, whole (untreated) or with EDTA to prevent clotting, or the use of different media (*e.g.* RPMI, PBS) could also influence the PC formed on NPs.[47,48] Interspecies differences are another factor that confounds the evaluation of the PC. Functionalized silica NPs revealed that a human PC formed containing immunoglobulins, complement and apolipoproteins among others, while the same NPs in mouse sera absorbed different proteins such as fibrinogen.[49] It follows from these results that alterations in the biological environment, such as occurs in disease states, can affect the identity and function of NPs, which has significant implications in the development of nanomaterials as therapeutic entities.

3.2. *The corona of nanomaterials is altered by the disease state*

Disease state or lifestyle of an individual can affect their plasma proteome and alter the PC formation. For example, in the diabetic patient, glycation of proteins increases the catabolism of low-density lipoproteins (LDLs), causing a reduction of soluble albumin.[50] Liver disorders can also change the amount of albumin detected in blood. Hence many plasma proteins, like albumin, serve as clinical biomarkers for diagnosis of pathological conditions.[51] Smoking leads to changes in the nitrotyrosine modifications of plasma proteins, which can reduce fibrinogen and surfactant protein (SP)-A levels.[52] The cancer "secretome" refers to the many proteins secreted by cancer cells and tissues into bodily fluids and encompasses both soluble proteins and exosomes or vesicles containing proteins.[53] The identification of hundreds of tumor-derived proteins, such as autoantibodies,[54] supports that the plasma proteome from disease states like cancer can lead to the formation of coronas on NPs that vary from those formed in the plasma of healthy individuals.

The impact of disease on PC formation was studied by evaluating the HC formed on polystyrene and silica NPs incubated in plasma from patients with different pathologies such as rheumatism, hypercholesterolemia, pregnancy or diabetes.[55] Findings were that the type of disease affected the composition of the nanoparticle PC formed in plasma. For example, the size and zeta potential of the NPs with HC varied when incubated in the plasma of patients with different diseases.[55] As example, the DLS (dynamic light scattering) of bare polystyrene NPs was ~100 nm, which then increased from ~18 to 44 nm depending on the diseased plasma used for incubation.[55] Hence the formation of the PC on NPs can be a multifactorial process that is influenced by the plasma protein profile and may in part be independent of the Vroman effect. The influence of the PC on the biological activity of NPs was shown using graphene oxide (GO) sheets incubated with human plasma from different disease conditions as above.[56] Two breast cancer cell lines, MCF-7 and MDA-MB-231, were used to assess cytotoxicity, generation of reactive oxygen species (ROS), and production of nitric oxide (NO). GO sheets with coronas formed in plasma from cancer patients displayed increased cytotoxicity and ROS production compared to plasmas from healthy or diabetic patients, while GO sheets incubated in plasma from individuals with thalassemia major or hypofibrinogenemia produced the highest NO levels.[56] While more data is needed to draw conclusions about the impact of specific diseases on the function of NPs, such results are supportive of the idea that PC formation on nanomaterials can be unique or "personalized" to each individual's physiology.

The therapeutic use of NPs in cancer therapy is of interest due to the high mortality of this disease and potential to generate targeted anti-cancer nanoagents with reduced off-site toxicity. Examining the plasma from patients with pancreatic cancer, Caracciolo et al. observed that the protein concentration was reduced as compared to healthy individuals, which in part could be attributed to a reduction in serum albumin and alpha and gamma globulins.[57] Positively and negatively charged lipid nanoparticles (plain or PEGylated) were incubated with plasma from healthy or pancreatic cancer patients and their physiochemical properties characterized. Using the plasma from pancreatic and healthy individuals to incubate NPs, a difference in the zeta potential was only observed with the plain, positively charged NPs, suggesting that the NPs of different charges may recruit diverse proteins from plasma.[57] Another study of plasmas from pancreatic, as well as gastric and breast cancers, used the clinically approved AmBisome-like liposomes to examine the composition of the PC formed on these nanomaterials.[58] Findings were that the HC of the NPs that were incubated in the plasma from pancreatic patients was less negatively charged and more enriched in proteins, such as immunoglobulins, compared to other cancer types.[58,59] An explanation proposed was that the increased binding of immunoglobulins to the PC of NPs could be due to the production of autoantibodies in pancreatic cancer, indicating that, by forming a PC unique to the plasma source, NPs act like a "nano-concentrator" and isolate rare proteins from plasma that have clinical relevance.[60] This concept underlies the development of diagnostic blood tests for cancer or the identification of new therapeutic targets due to the presence of cancer-relevant markers that are enriched in the PCs of nanomaterials. Examples are: carcinoembryonic antigen (CEA) for pancreatic disease[59] or hepatoma-derived growth factor (HDGF) for ovarian cancer.[61]

In addition to proteins, lipids are found in blood, usually associated with lipoproteins such as high-density lipoproteins (HDL) or LDL and can be important constituents of the PC of nanomaterials. Using polymeric NPs, Muller et al. examined how lipids become integrated into the PC, finding that lipoproteins disintegrate upon contact with NPs and lipids may then be absorbed into the PC.[62] Possible outcomes of coating NPs with lipoproteins is decreased uptake by target cells, suggesting the cholesterol level (which correlates to lipoprotein concentration) is a key factor that could affect biological responses. Metabolites like glucose or cholesterol can alter the PC by changing the binding site of fibrinogen on the surface of NPs, which impacts the immunogenicity of fibrinogen-NP complexes.[63] Underlying disease states, like cardiovascular illnesses or diabetes, can thus alter plasma components and affect the formation of the PC of NPs. In a study of the properties of carbon nanotubes (CNTs), in which ball-milling

produced structural defects, high-cholesterol mouse serum resulted in the formation of a unique PC on CNTs that was not observed with healthy mouse serum.[64] Fewer proteins, such as immunoglobulins, were found. This could be due to the increased amount of cholesterol that outcompeted other proteins from binding to NPs.[64] Hence, the contribution of lipids to the formation of the PC is relevant and should elicit further study. As an example, a report from Shannahan and colleagues revealed the potential for iron oxide NPs, treated with sera from hyperlipidemic individuals, to cause an inflammatory response in aortic endothelial cells.[65]

In contrast to blood, the lungs represent a different challenge for the therapeutic application of nanomaterials. As NPs traffic through the respiratory tract lining fluid (RTLF), these acquire a PC that reflects the contents of the RTLF, which may contain proteins involved in innate immunity, such as SP-A and complement (*e.g.* C1q and C3) proteins.[41] Interestingly, the RTLF from asthmatic patients produced a PC on NPs that was reduced in surfactant proteins or proteins involved in metal handling but had increased alpha-1-antitrypisin.[66] Hence, inhaled NPs that contact the pulmonary surfactant layer may produce a PC that is different from that formed in blood as well as different when exposed to a diseased environment. This was studied using NPs with different surface chemistries (PEG-, PLGA-, lipid-) that were incubated with porcine lung surfactant.[67] All three NPs showed minimal differences in the lipid components of the corona, however significant differences among the NPs were noted in the proteins forming the corona such as SP-A (lipid-NPs), SP-D (PEG-NPs) and apolipoprotein A1 (PLGA-NPs).[67] In a study with polystyrene NPs, which had different surface modifications, as well as titanium oxide NPs, bronchoalveolar lavage fluid (BALF) from patients with pulmonary alveolar proteinosis (PAP) was used to show that the PC formed on NPs contained "core" proteins, such SP-A, SP-B and SP-D, as well as lipids, that seemed independent of the properties of the particles.[68] As noted by others,[20] the most abundant proteins in the PC were not always the most abundant proteins in the BALF, suggesting a potential for selective enrichment of low abundance proteins on the surface of the NPs.

4. Conclusions

The PC formed on NPs is a multi-layer complex composed of proteins with different affinities that are contained within the HC and SC fractions. The physicochemical attributes of NPs play a role in the formation of the PC, affecting the concentration or density of select proteins. In this regard, the shape or curvature of the NPs may be a decisive factor. Clearly, however, the formation of the PC on NPs is a dynamic process that is far from fully understood. The characteristics of PC are representative of the environment in which particles circulate and can be either beneficial or detrimental to the function of the NPs. Positive aspects of the PC include the ability to prevent aggregation of the NPs or facilitate cellular uptake. Negative aspects of the PC involve obstructing targeting ligands on the surface of NPs and causing toxic effects, such as inflammation or complement activation. The formation of the PC on NPs is sensitive to differences in the composition of the plasma due disease-mediated changes, such as decreased albumin or increased immunoglobulins. The presence of increased cholesterol or glucose in plasma can also outcompete the binding of resident proteins to the PC and alter NP activity. Such findings necessitate improved techniques and uniformity of methods for evaluating the PC and identifying its constituents. Especially needed is the analysis of the components in the SC as well as the characterization of the interface between the SC and the HC. The non-protein elements of the PC remain poorly understood as does the proteome of other bodily fluids like those of the lungs. Such studies are fundamental next steps to advance the therapeutic development of NPs and improve their clinical relevance. The PC formed on NPs has promising diagnostic use based on its ability to bind and sequester low abundance proteins. Blood tests for cancer[59,69] and other diseases like multiple sclerosis or Alzheimer's disease,[70] based on evaluating the PC of NPs, are innovative new directions for translational research. Modifying the PC to improve the targeting of NPs, such as enriching with apolipoproteins to cross the blood-brain barrier,[71] could solve problems that currently impede the wider application of nanotherapeutics. Lastly, using libraries of NPs with different properties that form distinct coronas have powerful predictive value in the study of human cancers[72] and other diseases and could reveal new therapeutic targets and biomarkers for development.

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Conflict of interest

ARK is a shareholder in Seva Therapeutics, Inc. The other authors have no competing interests.

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