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REVIEW ARTICLE

Protein nanoparticle interaction

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HIGHLIGHTS

- Effects of protein properties such as interactions between proteins and target molecules or structures interactions, isoelectric points (pI) and pH on protein corona formation.
- Effects of nanoparticle such as size, shape, surface, charges properties on protein corona formation.
- Protein corona size, quantitative and structural analysis techniques.

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ABSTRACT

With the rapid development of nanotechnology in recent years, nanoparticles (NPs) have started to be used in the pharmaceutical industry for imaging, treatment and diagnosis. For the use of NPs in drug delivery, it is necessary to clearly determine how nanoparticle-based drugs will behave in the biological environment. After these drugs enter the biological environment, they interact rapidly with the proteins in the biological environment and forming a complex called protein corona (PC). The formation of the PC form affects many important events such as the adsorption and distribution of drugs, whether the drug reaches the target area, and the removal of the drug from the body. In order to design a more safety nanoparticle-based drug, the interaction of NPs with protein should be well understood, the physicochemical properties of the resulting PC form and its behavioral characteristics in the biological environment should be clearly clarified. In this review, the factors affecting the protein nanoparticle interaction and the analysis methods of the resulting PC form are briefly mentioned.

1. Introduction

The field of nanotechnology has advanced rapidly in the last decade. Nanoparticles (NPs) are used in various fields such as electronic components, some foods, industry, cosmetics, pharmaceuticals and medicine [1,2]. Recently, the use of NPs in the pharmaceutical field has been expanded for cellular therapy, tissue repair, drug delivery, as sensors metabolites and other biomolecules, implantable biosensors, nanosurger, tissue engineering, nanoparticle-enabled diagnostics [1,3].

The purpose of using nanoparticles in drug delivery is to improve the interaction between drug and target, that is, to create a better therapeutic possibility. Drugs with water solubility problems are either dispersed with nanospheres or enclosed in a nanocapsule by being surrounded by a single polymeric membrane. The efficacy of therapy administered in this way can be altered by varying the residence time and excretion of the drug or by changing site-specific targeting [1].

The application of NPs for diagnostic, therapeutic and imaging purposes depends on different parameters such as the physicochemical properties of these nanoparticles, drug loading efficiency, drug release, and most importantly, low or no toxicity [2].

Interaction between NPs and biomolecules causes the formation of a biological corona form. The biological corona form formed on the surface of NPs belonging to the same pile with different surface properties causes different biological results, as well as contains different proteins [4,5].

NPs with advanced bio-interface capabilities are easily taken into the cell by interacting with the cell membrane. In this way, intracellular receptors can be easily targeted. NPs can generally be taken into the cell in two ways.

First, when nanoparticles interact with the cell membrane, they can be taken up into the cell by endocytosis. By fusion of this endosome with the lysosome, degradation or denaturation of the protein cargo by acidic media or proteases can occur before it enters the cytoplasm. Second,

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when NPs come into contact with biological media, they are rapidly coated with proteins [3].

Looking at the inner and outer layers of the PC formation, the inner layer is characterized by strong bonds in slow change with the environment (hard corona) with a lifetime of several hours, while the weakly bonded proteins (soft corona) in the outer layer have a faster rate of change [4,6]. PC formation is a thermodynamic process.

In protein-nanoparticle interaction, defining the proteins adsorbed on the nanoparticle surface, the lifetimes and conformations of these formed forms, as well as preparing and stabilizing the NPs and giving the nanoparticle a feature, may result in the design of more reliable NPs.

However, in order to understand these formations, biological environments and systems are required as well as chemical approaches because they are too complex to be modeled in non-biological systems.

2. Effects of nanoparticle properties on protein corona formation

Nanoparticles (NPs) are defined as particles ranging in size from 1-100 nm. The full characterization of NPs includes various measurements such as size and size distribution, the chemistry of the material, surface area, state of distribution, surface chemistry and others.

Most importantly, the chemical composition of the material, surface functionalization, shape and curvature, charges, roughness and hydrophobicity or hydrophilicity, functional groups and targeting moieties will greatly affect the nanoparticle surface properties [1,4]. Therefore, all these features will affect the bionanointerface of NPs and the formation of PC.

The high surface/volume ratio of NPs has an active surface chemistry compared to other bulk biomaterials, and therefore they tend to reduce their surface energy by interacting with the biomolecules in the environment where they are dispersed. Because of the difference in these surface properties, NPs must form a new bio-nano interface between biomolecules and NPs so that they can interact with different proteins [4].

Nanoparticle size plays a role not only in the chemistry of the surface of the NP, but also in the formation of the PC composition and the conformation of this structure.

Also, as the size of the NPs changes, the degree of protein adsorption of the NP also changes. One study shows that the size of NPs with a diameter of 70-200 nm is greater than the size of the proteins they adsorb. Smaller sized nanoparticles (30 nm and above) can suppress the adsorption of certain proteins (possibly larger than NP) [7].

The shape of the NP affects the uptake of NP into the cell and the total amount of protein adsorbed. In one study, in vivo protein formation was followed after gold nanoparticles (nanorods, nanostras) injected into mice reached the bloodstream. As a result of the study, it was determined that the total amount of protein adsorbed on the particle surface was affected by the particle size [8].

In another study, if the shape of the prepared gold NPs is spherical, the interaction with the cell layers increases, while changing the shape to the rod geometry reduces this interaction [4,9].

Charges on the NP surface can interact with proteins electrostatically, and their presence can positively affect binding. Moreover, the adsorption of plasma proteins on the surface of NPs increases in direct proportion to the surface charge density of the NPs [10,11].

Most proteins are negatively charged (pH = 7.2) in the physiological environment, NPs with positive surface charge tend to bind proteins more strongly than negatively charged and neutral NPs due to the electrostatic force [10,12]. However, many NPs are stabilized in a physiological buffer with the help of negatively charged groups (carboxylated, sulfate, phosphate, etc.). In general, despite their negative surface charge, these NPs are immediately covered by plasma proteins when in contact with biological fluids [4]. This shows that many different factors play a role in the formation of PC.

The interaction between the NP protein depends on the hydrophilicity and hydrophobicity of the NPs surface. If the surface of the NPs is hydrophilic, it may interact with the protein via hydrogen bonding, while if it is hydrophobic, it can interact with the protein via a wan der Waals bond.

3. Effects of protein properties on PC formation

Most of the factors affecting protein adsorption are directly related to the three-dimensional conformation that protects the protein itself [10,13]. Due to differences in the structure of proteins, the binding behavior of proteins to NPs is different. Typically, specific interactions between proteins and target molecules or structures are non-covalent interactions such as hydrogen bonds, electrostatic interactions, and hydrophobic interactions. Morever, due to the charge present on the surface of proteins, it can detect electrostatic interaction with NPs depending on the isoelectric points (pI) and pH of the proteins [10,14].

4. PC analysis techniques

Along with the analysis of the modification of the physico-chemical properties of NPs, a qualitative and quantitative study of the formation of PC is required.

Because PC formation exhibits a complex and time-dependent dynamic behavior, there is no single technique that fully characterizes protein-NP interactions. The size distribution, density, composition, and molecular weight of the molecules in this structure cannot be easily and quickly analyzed with a single experimental measurement [5].

Prior to the analysis of PC formation, it also needs to be determined whether purification from unbound excess proteins that could alter the equilibrium is necessary or if in situ measurements can be made without the necessary purification. Analyzes can be divided into two direct and indirect methods, depending on whether purification is necessary or not [15].

When we look at the analysis techniques that can be used on PC formation, we can see these techniques size analysis, quantitative analysis, and structural analysis [5].

Common techniques that can be used for size analysis of NPs on PC formation include ultracentrifugation (UC), differential centrifugal sedimentation (DCS), asymmetric flow field fractionation (AF4), and light scattering-based methods. The combination of data from these techniques allows the determination of the apparent densities of the PC complex for different amounts of NP/protein. Moreover, with the use of small-angle X-ray scattering (SAXS) technique and transmission electron microscopy (TEM)

techniques, the comparison of NPs before and after corona formation can be examined, thus evaluating the thickness and size of the protein layer [5,16,17].

The combination of various techniques such as mass spectrometry (MS), liquid chromatography-mass spectrometry (LC-MS), inductively coupled plasma-mass spectrometry (ICP-MS) are effective techniques for the quantitative analysis of PC [5,15,18,19]. While the number of protein layers that make up the hard PC can be investigated using the ICP-MS technique, Protein-protein binding sites can also be identified using MS [5].

For PC, structural changes can be studied using techniques such as Fourier Transform Infrared Analysis (FTIR), X-ray crystallography (XRD), nuclear magnetic resonance (NMR), Raman spectroscopy, circular dichroism (CD), electron paramagnetic resonance (EPR), time-of-flight-secondary ion mass spectrometry (ToF-SIMS). Interactions between proteins and NPs can be characterized by isothermal microcalorimetry (ITC) and fluorescence (FL) techniques, which provide thermodynamic parameters such as binding constant (K) and Gibbs free energy (AG). UV, UV-vis spectroscopy and zeta potential analysis are other methods that can be used [5,20–23].

5. Molecular modeling for PC

Thanks to its atomic-scale resolution, molecular modeling can shed light on the formation of the PC form, highlighting key amino acids involved in protein adsorption, binding and conformational changes on the NP surface. At the same time, simulations at the molecular scale make it possible to evaluate the impact of environmental influences, NP material and surface functionalization on cellular uptake [24]. Due to the few and difficult studies to obtain this information experimentally, preliminary information for these interactions can be obtained by molecular modeling. In a study, researchers investigated protein adsorption on the NP surface and its effect on cellular transmission of NP through dissipative particle dynamics simulations [25].

6. Future perspectives for the PC research

There are many biological barriers in the human body. These barriers must be overcome in order for the nanoparticles to reach their targets. Because of their unique size and affinity for surface functionalization to combine desired properties, NPs are particularly well suited to overcome these barriers.

The key role of NPs protein interactions in the use of nanotechnology in drug discovery has become even more important with the recent investigation of NP-protein interaction.

Understanding protein-nanoparticle interactions is crucial for developing effective NP-based drugs. Considering the studies carried out, it is clear that more comprehensive studies are needed. The physicochemical properties of a nanoparticle affect the behavior of these drugs from adsorption to excretion.

Much less has been done on the complete model of the protein-NP complex, which is highly demanded for a better understanding of the binding mechanism and a more rational design of protein-NP interaction. Moreover, the formation mechanism of PC in NPs should be examined in detail, the formation process, stability, conformations and distribution of PC in the body should be investigated in detail, and more clear information about the behavior of this formed form after ingestion is needed. Most of the studies on PC formation are carried out in vitro. In the biological environment, this process is quite complex and dynamic. There are not many studies on the clinical trials of NPs. If these existing studies are taken furthermore safety-based drugs can be produced.

Conflict of Interest

No conflict of interest was declared.

References

1. Li, Y.; Lee, J.-S. Insights into Characterization Methods and Biomedical Applications of Nanoparticle–Protein Corona. *Materials (Basel).* **2020**, *13*, 3093, doi:10.3390/ma13143093.
2. Najahi-Missaoui, W.; Arnold, R.D.; Cummings, B.S. Safe Nanoparticles: Are We There Yet? *Int. J. Mol. Sci.* **2020**, *22*, 385, doi:10.3390/ijms22010385.
3. Kopp, M.; Kollenda, S.; Epple, M. Nanoparticle–Protein Interactions: Therapeutic Approaches and Supramolecular Chemistry. *Acc. Chem. Res.* **2017**, *50*, 1383–1390, doi:10.1021/acs.accounts.7b00051.
4. Mahmoudi, M.; Lynch, I.; Ejtehadi, M.R.; Monopoli, M.P.; Bombelli, F.B.; Laurent, S. Protein−Nanoparticle Interactions: Opportunities and Challenges. *Chem. Rev.* **2011**, *111*, 5610–5637, doi:10.1021/cr100440g.
5. Kopac, T. Protein corona, understanding the nanoparticle–protein interactions and future perspectives: A critical review. *Int. J. Biol. Macromol.* **2021**, *169*, 290–301, doi:10.1016/j.ijbiomac.2020.12.108.
6. Mahmoudi, M.; Simchi, A.; Imani, M.; Shokrgozar, M.A.; Milani, A.S.; Häfeli, U.O.; Stroeve, P. A new approach for the in vitro identification of the cytotoxicity of superparamagnetic iron oxide nanoparticles. *Colloids Surfaces B Biointerfaces* **2010**, *75*, 300–309, doi:10.1016/j.colsurfb.2009.08.044.
7. Klein, J. Probing the interactions of proteins and nanoparticles. *Proc. Natl. Acad. Sci.* **2007**, *104*, 2029–2030, doi:10.1073/pnas.0611610104.
8. García-Álvarez, R.; Vallet-Regí, M. Hard and Soft Protein Corona of Nanomaterials: Analysis and Relevance. *Nanomaterials* **2021**, *11*, 888, doi:10.3390/nano11040888.
9. Cox, M.C.; Barnham, K.J.; Frenkiel, T.A.; Hoeschele, J.D.; Mason, A.B.; He, Q.-Y.; Woodworth, R.C.; Sadler, P.J. Identification of platination sites on human serum transferrin using 13C and 15N NMR spectroscopy. *JBIC J. Biol. Inorg. Chem.* **1999**, *4*, 621–631, doi:10.1007/s007750050386.
10. Yu, Y.; Luan, Y.; Dai, W. Dynamic process, mechanisms, influencing factors and study methods of protein corona formation. *Int. J. Biol. Macromol.* **2022**, *205*, 731–739, doi:10.1016/j.ijbiomac.2022.03.105.
11. Gessner, A.; Lieske, A.; Paulke, B.R.; Müller, R.H. Influence of surface charge density on protein adsorption on polymeric nanoparticles: analysis by two-dimensional electrophoresis. *Eur. J. Pharm. Biopharm.* **2002**, *54*, 165–170, doi:10.1016/S0939-6411(02)00081-4.
12. Alexis, F.; Pridgen, E.; Molnar, L.K.; Farokhzad, O.C. Factors Affecting the Clearance and Biodistribution of Polymeric Nanoparticles. *Mol. Pharm.* **2008**, *5*, 505–515, doi:10.1021/mp800051m.
13. Haynes, C.A.; Norde, W. Globular proteins at solid/liquid interfaces. *Colloids Surfaces B Biointerfaces* **1994**, *2*, 517–566, doi:10.1016/0927-7765(94)80066-9.
14. Yang, S.-T.; Liu, Y.; Wang, Y.-W.; Cao, A. Biosafety and Bioapplication of Nanomaterials by Designing Protein-Nanoparticle Interactions. *Small* **2013**, *9*, 1635–1653, doi:10.1002/smll.201201492.
15. Carrillo-Carrion, C.; Carril, M.; Parak, W.J. Techniques for the experimental investigation of the protein corona. *Curr. Opin. Biotechnol.* **2017**, *46*, 106–113, doi:10.1016/j.copbio.2017.02.009.
16. Carney, R.P.; Kim, J.Y.; Qian, H.; Jin, R.; Mehenni, H.; Stellacci, F.; Bakr, O.M. Determination of nanoparticle size distribution together with density or molecular weight by 2D analytical ultracentrifugation.

Nat. Commun. **2011**, *2*, 335, doi:10.1038/ncomms1338.

17. Krpetić, Ž.; Davidson, A.M.; Volk, M.; Lévy, R.; Brust, M.; Cooper, D.L. High-Resolution Sizing of Monolayer-Protected Gold Clusters by Differential Centrifugal Sedimentation. *ACS Nano* **2013**, *7*, 8881–8890, doi:10.1021/nn403350v.
18. Tenzer, S.; Docter, D.; Rosfa, S.; Wlodarski, A.; Kuharev, J.; Rekik, A.; Knauer, S.K.; Bantz, C.; Nawroth, T.; Bier, C.; et al. Nanoparticle Size Is a Critical Physicochemical Determinant of the Human Blood Plasma Corona: A Comprehensive Quantitative Proteomic Analysis. *ACS Nano* **2011**, *5*, 7155–7167, doi:10.1021/nn201950e.
19. Docter, D.; Distler, U.; Storck, W.; Kuharev, J.; Wünsch, D.; Hahlbrock, A.; Knauer, S.K.; Tenzer, S.; Stauber, R.H. Quantitative profiling of the protein coronas that form around nanoparticles. *Nat. Protoc.* **2014**, *9*, 2030–2044, doi:10.1038/nprot.2014.139.
20. Gruian, C.; Vanea, E.; Simon, S.; Simon, V. FTIR and XPS studies of protein adsorption onto functionalized bioactive glass. *Biochim. Biophys. Acta - Proteins Proteomics* **2012**, *1824*, 873–881, doi:10.1016/j.bbapap.2012.04.008.
21. Vanea, E.; Simon, V. XPS and Raman study of zinc containing silica microparticles loaded with insulin. *Appl. Surf. Sci.* **2013**, *280*, 144–150, doi:10.1016/j.apsusc.2013.04.111.
22. Liu, H.; Shi, S.; Cao, J.; Ji, L.; He, Y.; Xi, J. Preparation and evaluation of a novel bioactive glass/lysozyme/PLGA composite microsphere. *Drug Dev. Ind. Pharm.* **2015**, *41*, 458–463, doi:10.3109/03639045.2013.877485.
23. Gruian, C.M.; Rickert, C.; Nicklisch, S.C.T.; Vanea, E.; Steinhoff, H.-J.; Simon, S. Conformational Changes and Competitive Adsorption between Serum Albumin and Hemoglobin on Bioceramic Substrates. *ChemPhysChem* **2017**, *18*, 634–642, doi:10.1002/cphc.201600886.
24. Casalini, T.; Limongelli, V.; Schmutz, M.; Som, C.; Jordan, O.; Wick, P.; Borchard, G.; Perale, G. Molecular Modeling for Nanomaterial–Biology Interactions: Opportunities, Challenges, and Perspectives. *Front. Bioeng. Biotechnol.* **2019**, *7*, doi:10.3389/fbioe.2019.00268.
25. Ding, H.; Ma, Y. Computer simulation of the role of protein corona in cellular delivery of nanoparticles. *Biomaterials* **2014**, *35*, 8703–8710, doi:10.1016/j.biomaterials.2014.06.033.