Advances in Nanomedicine and Nanotechnology Research
ANNR, 2(1): 93-105
www.scitcentral.com
ISSN: 2688-5476
Original Research Article: Open Access

The Protein Corona: A Tangled Puzzle

Pederzoli F, Ruozi B*, Tosi G and Duskey J

*Department of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy.

Received October 09, 2019; Accepted October 10, 2019; Published June 27, 2020

ABSTRACT

Nanoparticles (NPs) are one of the most important tools in the emerging area of nanomedicine. The behavior of NPs in relevant biological environments, as preclinical setting, may be quite complex due to both their interactions with biological fluids and the formation of a protein layer, called protein corona (PC). PC remarkably affects the physicochemical properties of NPs (size, shape, surface chemistry, aggregation state, etc.) and consequently their biological fate including their pharmacokinetics, biodistribution, toxicity and therapeutic efficacy. Strong efforts were applied to correlate PC composition and observed effect after *in vitro*/*in vivo* experiments, but unfortunately poor reproducibility of data is often assessed. Biological, chemical and physical properties of NPs along with proteins composition of PC and features of pathological environments hardly complicate the study of interactions between PC-NP complexes and their interaction with target cells. In this contest, in order to quantitatively and qualitatively evaluate PC adsorbed onto NPs, the optimization of purification/separation procedures and rigorous and standardized analytical methods become a priority request to further design tailored nanomaterial able to interact with proteins and cells in a tunable manner.

Keywords: Nanocarriers, Molecular size, Nanoparticles, Pharmacokinetics, NP complexes

INTRODUCTION

Nanomedicine is one of the most active research areas of nanotechnology involving the application of nanocarriers for the medical prevention, diagnosis, and treatment of diseases [1]. Polymeric or inorganic nanoparticles (NPs) have the capacity to incorporate active substances of various characteristics and protect them from the inhospitable biological environment [2]. Due to their nanometrical size, NPs show ability to cross tissue barriers and the cell membrane, thus allowing interaction with the smaller components such as cellular proteins and other macromolecules [3]. The production of PEG-decorated NPs could reduce the reticuloendothelial system (RES) uptake, accumulation in liver, spleen or bone marrow, increasing circulation time and limit non-specific target uptake ultimately leading to a decrease in toxicity [4]. Further functionalization with targeting ligands, which possess the inherent ability to facilitate selective binding to cell types, can confer "smart" properties to NPs [5].

Generally, to rationally design an efficient nanoparticle-based therapeutic tool able to selectively transfer drugs to the target site, thus minimizing side effects and increasing therapeutic compliance, the combination of the nanocarriers formulative aspects along with a fundamental understanding of the molecular mechanism involved in regulating nanocarrier-biological interactions is highly required. In fact, immediately after NPs come into contact with protein-containing media (such as biological fluids), a layer of proteins, called the protein corona (PC), is formed on the particle surface. This PC could remarkably alter the original Np molecular identity affecting their clearance by RES, cellular uptake, biodistribution and also toxicity. Furthermore, if the NPs are surface-functionalized with selective ligands, the absorption of proteins could mask the targeting ability, inhibiting their biological effects. Thus, it is more than evident that the PC plays a key role in the interaction of particles with cells after systemic administration [6].

The PC has recently been the subject of extensive studies aiming to investigate it as complex and multiple layer entity characterized by proteins that exchange dynamically between the surface and the surrounding environment (soft corona-SC) and proteins more firmly adsorbed on the surface of the NPs (hard corona-HC) [7,8]. An unsolved

Corresponding author: Barbara Ruozi, University of Modena and Reggio Emilia, E-mail: barbara.ruozi@unimore.it

Citation: Pederzoli F, Ruozi B, Tosi G & Duskey J. (2020) The Protein Corona: A Tangled Puzzle. Adv Nanomed Nanotechnol Res, 2(1): 93-105.

issue remains in identifying both the stability and the role of these protein layers in biodistribution and the efficacy effects in transferring drugs to target tissues and cells. The highlights summary listed in **Table 1** on the evidence of biological effects in function of the PC's on the NPs (also including liposomes and nanotubes) strongly confirms this difficulty. These data obtained generally by *in vitro* experiments, simulating *in vivo* biological conditions, are affected by both the nanocarrier features (material, size, surface, charge, shape) and environment (composition, exposure time, pH, temperature, shear stress). Unfortunately, considering all these variables, the data obtained by different research groups seem to be poorly reproducible, frequently not useful or premonitory for completely clarifying the role of the PC and to better tailor design and produce really efficacious drug targeting carriers.

As evidence, conflicting reports on cytotoxicity and biological fates were reported even when similar NPs were tested [9-12]. For example, Ogawara et al. [13] reported that the PC adsorbed onto polystyrene NPs prolongs their circulation time, while Nagayama et al. [14] showed the PC on similar polystyrene NPs is responsible of increased clearance due to rapid recognition by the scavenger receptors and internalization by Kupffer cells. Examples of conflicting results can be found for other biological effects as well (e.g. cytotoxicity, targeting activity, etc.) [15-18].

Table 1. PC on carriers' surface vs. biological effects.

Biological effects	Type of NPs	Experiment	PC composition	Results	References
Interaction with cells of the immune system	Nonporous polymer (methacrylic acid) NPs	*In vitro* assay of cellular uptake on human monocytic leukemia cell line THP-1	Mainly BSA	PC on NP surface induced a reduction of cellular uptake by monocytic cells and macrophages.	[19]
	C-PS particles	Flow cytometry experiments by means of fluorescent labeled particles (cellular uptake on DCs)	Depending on the composition of the incubation medium: HSA and/or α2-GP and/or IgG	A PC of α2-GP induces an increased uptake by DCs if compared with the PC composed by only IgG. HSA (alone or with the other proteins) induces a significant inhibition of uptake by the DCs.	[20]
	TiO_2-NPs	ILs identification after human macrophages incubation of NPs (ELISA)	Proteins with many N-/O-glycosylations and phosphorylations.	PC on NPs induced the release of different ILs, particularly IL-6	[21]
	PS-NPs modified with PEG or PEEP	*In vitro* uptake of NPs in murine macrophage cell line (RAW264.7) and human cervix carcinoma cells (HeLa) (flow	Predominantly cluster in protein	Reduced protein absorption by presence of PEG and PEEP on the NP surface. Cluster in protein prevents the non-specific cellular	[22]

		cytometry experiments)		uptake by macrophages.	
Biodistribution	HMS-NPs	*In vivo* biodistribution study (mice) (magnetic resonance measurements)	Elevated amounts of apolipoprotein (ApoA1 and ApoA2)	HMSNPs covered by ApoA1 and ApoA2 preferentially accumulated in liver.	[23]
	PS-NPs with and without HSA coating	*In vivo* biodistribution study (male Wistar rats) (fluorescent spectrometry)	Not reported	Injected HSA-coated NPs showed prolonged circulation time.	[13]
	PS-NPs		Fetuin	Recognition and internalization of NPs by Kupffer cells via scavenger receptors.	[14]
Hemo-compatibility	Carboxylated multi-walled CNT	*In vitro* incubation of CNT with human blood platelets (aggregation assays)	Mono-protein PC (HSA/IgG/FBG/H1) depending on the adopted incubation medium.	HSA as well as FBG corona attenuates the platelets aggregation activity of CNT. IgG corona causes platelets fragmentation inhibiting their aggregation activity. H1 corona induces a strong platelets aggregation.	[24]
	Silica NPs	*In vitro* hemolytic assay on human red blood cells	Several plasma protein types	PC protective effect towards silica NPs-induced hemolysis.	[25]
Targeting efficiency	Tfr-functionalized Silica NPs	*In vitro* cellular interaction study on adenocarcinomic human alveolar basal epithelial cells (A549 cells) (fluorescence imaging)	Different plasma protein types	PC reduces the targeting capability of NPs	[26]
	NPs functionalized with cyclic RGD peptides and covered by PEG chains	*In vitro* competitive-cellular uptake-assay on A549 cells	Different plasma protein types	Targeting efficiency of RGD-NPs reduced by shielding effect of	[15]

				the absorbed proteins. NPs functionalized with short PEG molecules, and moderate cyclic RGD density performed better in targeting efficiency assays.	
	Poly(methacrylic acid) microcapsules functionalized with humanized A33 monoclonal antibody	Flow cytometry on the LIM2405 cell line (A33-antigen positive and negative cell mixture)	Not reported	PC of NPs does not influence their targeting ability toward human colon target cells.	[16]
	Core-shell chitosan NPs covered by SN-38 and functionalized with MUC1 aptamer	*In vitro* apoptosis evaluation (flow cytometry test with Annexin V-FITC/PI) on HT29 cells	Not reported	PC on NPs surface hampered the cytotoxicity of NPs in human colon cancer cells, HT29 (PC shields MUC1 that can't interact with mucin receptor).	[27]
Cellular up-take	Liposome (HSPC/CHOL/PEG, DSPE) loaded with DOX	*In vitro* uptake study (FACS) after liposome incubation with MCF7 and MDA-MB-435S cell lines	Mainly composed by Apo	Reduction of cellular internalization of liposomes	[28]
	Silica-NPs labeled with fluorescent molecules (IRIS)	*In vitro* uptake experiments on human mesenchymal stem cells (hMSCs) (CLSM, TEM and flow cytometry)	Not reported	Cellular uptake of the Silica-NPs by hMSCs appears not to be influenced by PC	[29]
	SiO_2-NPs	Nanoparticle uptake experiments (flow cytometry) on A540 cell line	Several protein types	The PC induces a lower cell adhesion/uptake of NPs in different cell types (lung epithelial cells, cervix	[30]

				epithelium HeLa cells, glial astrocytoma cells, and brain capillary endothelial cells).	
	liposome composed by cationic/neutral/zwitter ionic/ anionic lipids	*In vitro* uptake study (FACS) after liposome incubation with Human pancreatic carcinoma cell line (PANC-1)	Each liposome exhibits a specific protein pattern dictated by its specific lipid composition.	The PC of liposome (50:50 DOPE: DOTAB) contains Vitronectin, ApoA1, A2, B, C2-, Vitamin K-dependent protein and Integrin β_3, correlated to the promotion of cellular uptake.	[31]
Cytotoxicity	Single-wall CNTs	Cellular viability (CCK-8 assay kits) on two different cell lines: two cell lines, THP-1 and HUVECs	Mono- protein PC (FBG, BSA, Ig, Tfr, ferritin) depending on the adopted incubation medium	FBG-, BSA-, Tf- and Ig-PCs cause less cytotoxicity than uncoated CNTs in human acute monocytic leukemia cell line and human umbilical vein endothelial cells. PC with only FBG results in no toxicity.	[32]
	SiO_2-NPs (ultrafine particles)	Cellular viability (CCK-8 assay kits) on human corneal epithelial cells (hCECs)	Not reported	The PC around SiO_2-NPs significantly reduces the toxicity in human cornea cells.	[17]
	PAA-NPs	Cytokines determination (cytometric bead array kit) after in vitro incubation of PAA-NPs with different cell types (human leukemia cells, human embryonic kidney	Different proteins including FBG that undergoes to an unfolding conformation	The unfolded conformation of the FBG in PC triggers the release of inflammatory cytokines through its interaction with MAC-1 receptor that induces the NF-kB signaling pathway in	[18]

		cells, human monocytic cells)		different cell types (human leukemia cells, human embryonic kidney cells, human monocytic cells).	
Drug release profile	Abraxane (NPs loaded with Paclitaxel-Albumin)	Drug release studies using different incubation media HP 100%, FBS 100%, HP 10% (90% PBS+10% HP) or FBS 10% (90% PBS+10% FBS)	Not reported	Significantly reduction of the burst effect of Abraxane.	[33]
	Doxoves (PEGylated-liposome composed by HSPC/CHOL/mPEG2000-DSPE and loaded with DOX)	Drug release evaluation (FLIM) after liposome immobilization in agarose gel matrix	Alb, coagulation factors, Ig, acute phase proteins, complement	PC interferes with the integrity of Doxoves, leading to a fraction of DOX leakage.	[28]

BSA: Bovin Serum Albumin; C-PS: Carboxylated Polystyrene; DCs: Dendritic Cells; HAS: Human Serum Albumin; α2-GP: α2-Glycol-Protein; Ig: Immunoglobulin g; TiO_2: Titanium Dioxide; ILs: Interleukins; ELISA: Enzyme-Linked Immunosorbent Assay; PS: Polystyrene; PEG: Poly-Ethylene Glycol; PEEP: Poly-(Ethyl-Ethylene-Phosphate); HMS: Hollow Mesoporous Silica; CNT: Carbon Nanotube; FBG: Fibrinogen; H1: Histone H1; Tfr: Transferrin; RGD: Arginyl-Glycyl-Aspartic Acid; CPT: Camptothecin; SN-38: Active Metabolite of CPT Conjugated with HA; HA: Hyaluronic Acid; FITC/PI: Fluorescein Isothiocyanate/Propidium Iodide; HSPC: Hydrogenated Soy Phosphatidylcholine; CHOL: Cholesterol; DSPE: 1,2-Distearoyl-sn-Glycero-3-Phosphorylethanolamine; DOX: Doxorubicin; FACS: Fluorescence-Activated Cell Sorting; DOPE: Dioleoylphosphatidylethanolamine; DOTAB: Dodecyltrimethylammonium Bromide; CLSM: Confocal Laser Scanning Microscopy; TEM: Transmission Electron Microscopy; CCK-8: Cell Counting Kit 8; SiO_2: Silicon Dioxide; PAA: Poly-(Acrylic Acid); HP : Human Plasma; FBS: Fetal Bovine Serum; FLIM: Fluorescence Life Time Imaging Microscopy; Alb: Albumin; MAC-1: Macrophage-1 Antigen; NF-kB: Nuclear Factor KB

Purification and analyses of PC-NP complexes

To ameliorate the difficulty interpreting the biological response observed by using NPs, researchers focalized the attention optimizing the procedure to characterize the PC around NPs. In particular, evaluating the overall quantity, density, thickness composition, relative abundance of each protein, protein binding affinity, and protein conformation. Firstly, PC-NP complexes were separated from the protein solution. This operation is not simple as the protein-NPs interaction is regulated by dynamic exchanges and equilibrium that are particularly sensitive to purification processes, altering the real contribution of the PC on the NP surface.

Commonly, sequential cycles of centrifugation/washing represent the most used method, because it is simple, suitable and gives reliable results [34,35]. However, multiple purification steps can alter the equilibrium of complexes and may lead to modification in corona compositions as previously explain [36]. In particular, purification techniques adopted to isolate and to study the SC, which is governed by more dynamic exchanges, must be accurately selected and optimized in terms of operative conditions (time, temperature, stress, etc.). Moreover, due to high variability, in order to validate the data, several replicates must be collected and the results must be statistically relevant.

For this reason, the development of methodologies that minimize the number of purification steps of the PC-NP complexes in order to lower the possible impact of the process on their properties is broadly considered as an urgent issue [37]. Beyond centrifugation, other techniques, summarized in **Table 2**, have also been applied to separate and study PC-NP complexes.

Table 2. Purification process of PC-NP complexes.

Isolation techniques	Advantages	Limits	References
Centrifugation	Simple and quick	Loss of the weakly binding protein and multiple purification steps can alter the equilibrium of the system	[38-41]
Ultra-centrifugation in sucrose gradient	Possibility to isolate and recover different PC-NP complexes with high size resolution from different biological media and lower impact on protein structure	Preparation of the sucrose gradient	[37]
Size exclusion chromatography (SEC)	Weakly bound proteins may still be retrieved after the separation	Longer than centrifugation in time and recovery of PC-NP complex diluted in the mobile phase	[42,43]
Magnetic separation/**magnetic flow** field fractionation (MgFFF)	Allows the screening of proteins with distinct exchange kinetics in the corona around NPs.	Applicable only to paramagnetic NPs	[44,45]
Sodium Dodecyl Sulphate – Poly Acrylamide Gel Electrophoresis (SDS-PAGE)	Allows to isolate and fractionate the proteins to submit to mass spectrometry analysis	Proteins must be previously denatured and negatively charged by an anionic detergent	[45,46]

These techniques were successfully applied to isolate PC-NPs complexes in ex-vivo experiments, namely by incubating NPs in biological medium simulating relevant biological conditions. In total, only a few experiments reported reproducible results in terms of identification of PC isolated from plasma after *in vivo* administration. Actually, the idea is to exploit NP features and to adapt or combine different methodologies in function of the type of NPs. As an example, Sakulkhu et al. [45] demonstrated that the *in vitro* PC profile of polyvinyl alcohol-coated SPIONs differs from *in vivo* ones, after *in vivo* administration, by separating the NPs using a strong external magnetic field and therefore exploiting the unique magnetic properties of the particles. As another example, a combined approach was proposed by Hadjidemetriou et al. [47] to recover lipid-based NPs from the blood circulation of rodents after intravenous administration to investigate the *in vivo* PC formation as well as its evolution. In this case size exclusion chromatography followed by membrane ultrafiltration allowed the isolation of the PC-NP complex and to recover reproducible samples to identify the PC components.

After purification, several different approaches were proposed to characterize PC-NP complex in terms size, thickness, quantity, density, thickness composition, relative abundance of each protein, protein binding affinity and protein conformation **(Table 3)**.

Table 3. Instrumental analyses of PC-NP complexes.

Techniques	Analysed Parameter	Advantages	Limits	References
Dynamic Light Scattering (DLS)	Hydrodynamic diameter of NP with/without PC and NP diffusion coefficient	Possibility to perform *in situ* analysis	Dis-homogeneous shape and/or poly-disperse population of PC/NPs complexes could affect the measurement; influenced by NP agglomeration	[38,41,48,49]
Differential Centrifugal Sedimentation (DCS)	Size and density of NPs with/without PC	Possibility to perform *in situ* analysis	Risks of contaminations; poor recovery of sample	[41]
Transmission Electron Microscopy (TEM)	Thickness of the PC	High-resolution, two-dimensional images of PC-NPs complexes	Artifacts may still occur in the sample during preparation	[41,50]
BCA assay	Determination of the total amount of adsorbed proteins	Highly sensitive and compatible with several reagents	Longer time of reaction with respect to other colorimetric assays	[51-53]
Bradford assay	Determination of the total amount of adsorbed proteins	Possibility to test several samples in a short amount of time	Un-compatible with the use of some buffers/detergents	[54,55]
Thermogravimetric analysis (TGA)	Mass weight of the proteins adsorbed onto inorganic NP	Sensitive and quick	Applicable only for the inorganic NPs	[56]
Fluorescence correlation spectroscopy (FCS)	Protein binding affinity	Provides important information on both kinetic and thermodynamic properties of a specific protein of the PC	Requires the use of fluorescent proteins in solution	[57]
Isothermal Titration Calorimetry (ITC)	Stoichiometry, affinity and enthalpy of NP-protein interaction	Capability to determinate the number of bound protein molecules per particle	Necessity to perform previous analysis (i.e., determination of NP and protein concentration) and applicable only for single protein solution	[58,59]
Surface Plasmon Resonance (SPR)	Association and dissociation constants	Fine characterization of the binding stoichiometry	Necessity to immobilize NPs onto a gold surface on a sensor chip	[38,58]
Quartz crystal microbalance (QCM)	Association and dissociation constants	Real-time and quantitative NP-protein binding profiles are obtained	Necessity to immobilize NPs onto a gold surface on a quartz crystal	[60]
Zeta- potential (Z-pot)	Zeta potentials and the isoelectric points of the NPs with/without PC	Possibility to perform *in situ* analysis	Poor reproducibility and strongly dependence on environment/buffers	[48,61,62]
Computer simulation	Orientation and	Possibility to quickly	*In silico* simulation that requires	[63]

	Conformation of the adsorbed proteins	predict different experimental conditions as function of NP surface ligand structure, surface curvature	experimental confirmation	
Circular Dichroism (CD)	Determination of changes in secondary structure of the adsorbed protein	Possibility to perform *in situ* analysis	cannot be used for a mixture of proteins due to its spectral complexity	[61,64]
Raman spectroscopy (RS) and Fourier transform infrared (FTIR) spectroscopy	Determination of changes in secondary structure of the adsorbed protein	Detection of instable formulations already at a very early stage as well as is able to highlight conformational changes during the ongoing aggregation process	Not applicable to complex protein mixtures	[64]
Nuclear magnetic resonance (NMR)	Protein structure and residue-specific structural information	Possibility to obtain localized conformational information regarding some adsorbed proteins	Analysis of only a few selected proteins	[65]
Differential Scanning Calorimetry (DSC)	Thermal denaturation/stability of the adsorbed proteins	Possibility to perform *in situ* analysis	Information obtained by micro-DSC is on a macroscopical level	[66]
Sodium Dodecyl Sulphate - PolyAcrylamide Gel Electrophoresis (SDS-PAGE and 2D-PAGE)	Identification and quantification of the proteins composing the PC	Densitometry analysis allows to quantify protein abundance and, in case of simple incubation media, also allows to rapidly identify the adsorbed proteins	Proteins must be previously denatured and negatively charged by an anionic detergent	[51,64,67,68]
Mass spectrometry	Qualitative and semi-quantitative analysis of proteins composing the PC	Allow the identification of the proteins composing the PC also after the incubation of the NPs in complex matrix (i.e., plasma and serum)	Proteins need to be first digested into smaller peptides with a proteolytic enzyme and the recognition of protein identity needs the use of specific protein-sequence database and software	[34,69-72]

The interaction between proteins and the NPs is another relevant issue that could be addressed by different procedures. As an example, it is possible to consider physical changes in morphology, size and zeta potential of the complexes with respect to free NPs as proof of a PC-NP complex presence. Microscopical analyses using transmission electron microscopy (TEM) and in some cases, atomic force microscopy (AFM), could allow the visualization of differences in shape, density, surface and also dimensions of NPs before and after incubation while dynamic light scattering (DLS) rapidly identifies changes in dimensional distribution and poly-dispersity.

The interaction in terms of affinity, association and dissociation constant, and the stability of protein adsorbed were analyzed by using a plenty of techniques including isothermal titration calorimetry (ITC), circular dichroism, surface plasmon resonance, quartz crystal microbalance frequently combined together. Gel filtration has been proposed not only to separate the protein-NP complexes in incubation media as previously reported, but also to isolate proteins from the NP surfaces as well as to provide useful information on kinetic exchange rates for adsorbed proteins [58].

Besides proof of the PC-NPs complex presence, defining the PC represents a major topic: several researchers reported the use of electrophoresis as the preferred technology, while protein *quantity determination* was proposed to be characterized by mass spectrometry, in which protein samples were digested into small peptides and simply injected into the analysis instrument [73].

In summary, as evident, the identification and characterization of the PC cannot be obtained by a single analytical protocol, but different and complementary technologies are generally combined. In order to reach the highest level of quality and standardization in PC identification and characterization, the key point relies on the choice of the techniques in function of both the type of nanocarriers and on the analytical parameter to investigate. Using multiple characterization techniques is therefore crucial to analyze different aspects of the PC (i.e., presence of complex, composition of PC, reliability in *in vivo* conditions, etc.) and to get a better understanding of this biological entity. Remarkably, some techniques allow to detect the protein corona *in situ* (ITC), while other procedures require the detachment of bound proteins from the nanocarriers before measurements, thus still representing a controversial issue on PC analysis, since the adoption of purification methods may change equilibrium properties of the PC.

CONCLUSION

In recent years, the advance of nanomedicine as applied science in disease treatments highlighted a deeper need in understanding the interactions between nanocarriers and the biological environment aiming to improve their effectiveness and safety profiles. In this context, the study of the PC connected to any kind of NP drug carrier and its impact on both biodistribution and interaction with the target site is of extreme importance. Relevant information of the PC composition could also be exploited in sample screening at early research stages. This interest generated a wide number of attempted experiments, but at a deeper analysis of the results, even if remarkable, the lack of reproducibility and defined protocols in PC analysis still remain an urgent issue. In particular, as pointed out in this brief review, those data often obtained *in vitro* by simulating biological environments are affected by a high number of variables and seem not to be reliable and predictive for *in vivo* readouts and therefore their translatability. As also pointed out, numerous purification and investigation techniques were applied to evaluate and characterize the PC demonstrating that some technologies could really be useful in analysing some aspects of PC. Thus, in order to concretely exploit PC-NPs complexes data, the scientific path in this field will surely pass through an optimization of the protocols with a rational combination of the different techniques to finalize a systematic and more reproducible PC study approach.

ACKNOWLEDGEMENT

Supported by MAECI grant (PI Tosi, Nanomedicine for BBB-crossing in CNS oncologic pathologies) and Emilia Romagna Region grant (Step-by-step screening: RCUP E56C18002000002).

REFERENCES

1. Bamrungsap S, Zhao Z, Chen T, Wang L, Li C, et al. (2012) Nanotechnology in therapeutics: A focus on nanoparticles as a drug delivery system. Nanomedicine 7: 1253-1271.
2. Fornaguera C, Garcia-Celma MJ (2017) Personalized nanomedicine: A revolution at the nanoscale. J Pers Med 7.
3. Wilczewska AZ, Niemirowicz K, Markiewicz KH, Car H (2012) Nanoparticles as drug delivery systems. Pharmacol Rep 64: 1020-1037.
4. Knop K, Hoogenboom R, Fischer D, Schubert US (2010) Poly(ethylene glycol) in drug delivery: Pros and cons as well as potential alternatives. Angewandte Chemie Int 49: 6288-6308.
5. Friedman AD, Claypool SE, Liu R (2013) The smart targeting of nanoparticles. Curr Pharm Design 19: 6315-6329.
6. Pederzoli F, Tosi G, Vandelli MA, Belletti D, Forni F, et al. (2017) Protein corona and nanoparticles: How can we investigate on? Wiley Interdiscip Rev Nanomed Nanobiotechnol 9: e1467.
7. Milani S, Bombelli FB, Pitek AS, Dawson KA, RäDler J (2012) Reversible versus irreversible binding of transferrin to polystyrene nanoparticles: Soft and hard corona. ACS Nano 6: 2532-2541.
8. Monopoli MP, Aberg C, Salvati A, Dawson KA (2012) Biomolecular coronas provide the biological identity of nanosized materials. Nat Nanotechnol 7: 779-786.
9. Sharifi S, Behzadi S, Laurent S, Forrest ML, Stroeve P, et al. (2012) Toxicity of nanomaterials. Chem Soc Rev 41: 2323-2343.
10. Mahmoudi M, Lynch I, Ejtehadi MR, Monopoli MP, Bombelli FB, et al. (2011) Protein-nanoparticle

interactions: Opportunities and challenges. Chem Rev 111: 5610-5637.

11. Mao HY, Laurent S, Chen W, Akhavan O, Imani M, et al. (2013) Graphene: Promises, facts, opportunities and challenges in nanomedicine. Chem Rev 113: 3407-3424.
12. Hajipour MJ, Raheb J, Akhavan O, Arjmand S, Mashinchian O, et al. (2015) Personalized disease-specific protein corona influences the therapeutic impact of graphene oxide. Nanoscale 7: 8978-8994.
13. Ogawara K, Furumoto K, Nagayama S, Minato K, Higaki K, et al. (2004) Pre-coating with serum albumin reduces receptor-mediated hepatic disposition of polystyrene nanosphere: Implications for rational design of nanoparticles. J Control Release 100: 451-455.
14. Nagayama S, Ogawara K, Minato K, Fukuoka Y, Takakura Y, et al. (2007) Fetuin mediates hepatic uptake of negatively charged nanoparticles via scavenger receptor. Int J Pharm 329: 192-198.
15. Su G, Jiang H, Xu B, Yu Y, Chen X (2018) Effects of protein corona on active and passive targeting of cyclic RGD peptide-functionalized pegylation nanoparticles. Mol Pharm 15: 5019-5030.
16. Dai Q, Yan Y, Ang CS, Kempe K, Kamphuis MM, et al. (2015) Monoclonal antibody-functionalized multilayered particles: Targeting cancer cells in the presence of protein coronas. ACS Nano 9: 2876-2885.
17. Sun D, Gong L, Xie J, Gu X, Li Y, et al. (2018) Toxicity of silicon dioxide nanoparticles with varying sizes on the cornea and protein corona as a strategy for therapy. Sci Bull 63: 907-916.
18. Deng ZJ, Liang M, Monteiro M, Toth I, Minchin RF (2011) Nanoparticle-induced unfolding of fibrinogen promotes Mac-1 receptor activation and inflammation. Nat Nanotechnol 6: 39-44.
19. Yan Y, Gause KT, Kamphuis MMJ, Ang CS, O'brien-Simpson NM, et al. (2013) Differential roles of the protein corona in the cellular uptake of nanoporous polymer particles by monocyte and macrophage cell lines. ACS Nano 7: 10960-10970.
20. Thiele L, Diederichs JE, Reszka R, Merkle HP, Walter E (2003) Competitive adsorption of serum proteins at microparticles affects phagocytosis by dendritic cells. Biomaterials 24: 1409-1418.
21. Borgognoni CF, Mormann M, Qu Y, Schafer M, Langer K, et al. (2015) Reaction of human macrophages on protein corona covered TiO(2) nanoparticles. Nanomedicine 11: 275-282.
22. Schöttler S, Becker G, Winzen S, Steinbach T, Mohr K, et al. (2016) Protein adsorption is required for stealth effect of poly(ethylene glycol)-and poly(phosphoester)-coated nanocarriers. Nat Nanotechnol 11: 372-377.
23. Pochert A, Vernikouskaya I, Pascher F, Rasche V, Linden M (2017) Cargo-influences on the biodistribution of hollow mesoporous silica nanoparticles as studied by quantitative (19)F-magnetic resonance imaging. J Colloid Interface Sci 488: 1-9.
24. De Paoli SH, Diduch LL, Tegegn TZ, Orecna M, Strader MB, et al. (2014) The effect of protein corona composition on the interaction of carbon nanotubes with human blood platelets. Biomaterials 35: 6182-6194.
25. Shi J, Hedberg Y, Lundin M, Odnevall Wallinder I, Karlsson H, et al. (2012) Hemolytic properties of synthetic nano and porous silica particles: The effect of surface properties and the protection by the plasma corona. Acta Biomater 8: 3478-3490.
26. Salvati E, Re F, Sesana S, Cambianica I, Sancini G, et al. (2013) Liposomes functionalized to overcome the blood-brain barrier and to target amyloid-beta peptide: The chemical design affects the permeability across an in vitro model. Int J Nanomed 8: 1749-1758.
27. Varnamkhasti BS, Hosseinzadeh H, Azhdarzadeh M, Vafaei SY, Esfandyari-Manesh M, et al. (2015) Protein corona hampers targeting potential of MUC1 aptamer functionalized SN-38 core-shell nanoparticles. Int J Pharm 494: 430-444.
28. Caracciolo G, Palchetti S, Digiacomo L, Chiozzi RZZ, Capriotti AL, et al. (2018) Human biomolecular corona of liposomal doxorubicin: The overlooked factor in anticancer drug delivery. ACS Appl Mater Interfaces 10: 22951-22962.
29. Catalano F, Accomasso L, Alberto G, Gallina C, Raimondo S, et al. (2015) Factors ruling the uptake of silica nanoparticles by mesenchymal stem cells: Agglomeration versus dispersions, absence versus presence of serum proteins. Small 11: 2919-2928.
30. Lesniak A, Fenaroli F, Monopoli MP, Aberg C, Dawson KA, et al. (2012) Effects of the presence or absence of a protein corona on silica nanoparticle uptake and impact on cells. ACS Nano 6: 5845-5857.
31. Palchetti S, Caputo D, Digiacomo L, Capriotti AL, Coppola R, et al. (2019) Protein corona fingerprints of liposomes: New opportunities for targeted drug delivery and early detection in pancreatic cancer. Pharmaceutics 11.
32. Ge C, Du J, Zhao L, Wang L, Liu Y, et al. (2011) Binding of blood proteins to carbon nanotubes reduces cytotoxicity. Proc Natl Acad Sci U S A 108: 16968-16973.

33. Behzadi S, Serpooshan V, Sakhtianchi R, Muller B, Landfester K, et al. (2014) Protein corona change the drug release profile of nanocarriers: The "overlooked" factor at the nanobiointerface. Colloids Surf B Biointerfaces 123:143-149.

34. Sempf K, Arrey T, Gelperina S, Schorge T, Meyer B, et al. (2013) Adsorption of plasma proteins on uncoated PLGA nanoparticles. Eur J Pharm Biopharm 85: 53-60.

35. Walkey CD, Chan WC (2012) Understanding and controlling the interaction of nanomaterials with proteins in a physiological environment. Chem Soc Rev 41: 2780-2799.

36. Carrillo-Carrion C, Carril M, Parak WJ (2017) Techniques for the experimental investigation of the protein corona. Curr Opin Biotechnol 46: 106-113.

37. Di Silvio D, Rigby N, Bajka B, Mayes A, Mackie A, et al. (2015) Technical tip: High-resolution isolation of nanoparticle-protein corona complexes from physiological fluids. Nanoscale 7: 11980-11990.

38. Casals E, Pfaller T, Duschl A, Oostingh GJ, Puntes V (2010) Time evolution of the nanoparticle protein corona. ACS Nano 4: 3623-3632.

39. Lundqvist M, Stigler J, Cedervall T, Berggard T, Flanagan MB, et al. (2011) The evolution of the protein corona around nanoparticles: A test study. ACS Nano 5: 7503-7509.

40. Monopoli MP, Pitek AS, Lynch I, Dawson KA (2013) Formation and characterization of the nanoparticle-protein corona. Methods Mol Biol 1025: 137-55.

41. Walczyk D, Bombelli FB, Monopoli MP, Lynch I, Dawson KA (2010) What the cell "sees" in bionanoscience? J Am Chem Soc 132: 5761-5768.

42. Lynch I, Cedervall T, Lundqvist M, Cabaleiro-Lago C, Linse S, et al. (2007) The nanoparticle-protein complex as a biological entity; a complex fluids and surface science challenge for the 21st century. Adv Colloid Interface Sci 31: 167-174.

43. Cedervall T, Lynch I, Foy M, Berggard T, Donnelly SC, et al. (2007) Detailed identification of plasma proteins adsorbed on copolymer nanoparticles. Angew Chem Int Ed Engl 46: 5754-5756.

44. Ashby J, Pan S, Zhong W (2014) Size and surface functionalization of iron oxide nanoparticles influence the composition and dynamic nature of their protein corona. ACS Appl Mater Interfaces 6: 15412-15419.

45. Sakulkhu U, Mahmoudi M, Maurizi L, Salaklang J, Hofmann H (2014) Protein corona composition of superparamagnetic iron oxide nanoparticles with various physico-chemical properties and coatings. Sci Rep 4: 5020.

46. Lundqvist M, Augustsson C, Lilja M, Lundkvist K, Dahlback B, et al. (2017) The nanoparticle protein corona formed in human blood or human blood fractions. PLoS One 12.

47. Hadjidemetriou M, Al-Ahmady Z, Kostarelos K (2016) Time-evolution of *in vivo* protein corona onto blood-circulating PEGylated liposomal doxorubicin (DOXIL) nanoparticles. Nanoscale 8: 6948-6957.

48. Monopoli MP, Walczyk D, Campbell A, Elia G, Lynch I, et al. (2011) Physical-chemical aspects of protein corona: Relevance to *in vitro* and *in vivo* biological impacts of nanoparticles. J Am Chem Soc 133: 2525-2534.

49. Maiorano G, Sabella S, Sorce B, Brunetti V, Malvindi MA, et al. (2010) Effects of cell culture media on the dynamic formation of protein-nanoparticle complexes and influence on the cellular response. ACS Nano 4: 7481-7491.

50. Mahmoudi M, Abdelmonem AM, Behzadi S, Clement JH, Dutz S, et al. (2013) Temperature: The "ignored" factor at the nanobio interface. ACS Nano 7: 6555-6562.

51. Gessner A, Lieske A, Paulke BR, Müller RH (2003) Functional groups on polystyrene model nanoparticles: Influence on protein adsorption. J Biomed Mater Res Part A 65: 319-326.

52. Goppert TM, Muller RH (2005) Protein adsorption patterns on poloxamer and poloxamine-stabilized solid lipid nanoparticles (SLN). Eur J Pharm Biopharm 60: 361-72.

53. Sohaebuddin SK, Thevenot PT, Baker D, Eaton JW, Tang L (2010) Nanomaterial cytotoxicity is composition, size and cell type dependent. Particle Fibre Toxicol 7: 1-22.

54. Arvizo RR, Giri K, Moyano D, Miranda OR, Madden B, et al. (2012) Identifying new therapeutic targets via modulation of protein corona formation by engineered nanoparticles. PLoS One 7.

55. Mbeh DA, Javanbakht T, Tabet L, Merhi Y, Maghni K, et al. (2015) Protein corona formation on magnetite nanoparticles: Effects of culture medium composition and its consequences on super paramagnetic nanoparticle cytotoxicity. J Biomed Nanotechnol 11: 828-840.

56. Clemments AM, Botella P, Landry CC (2015) Protein adsorption from biofluids on silica nanoparticles: Corona analysis as a function of particle diameter and porosity. ACS Appl Mater Interfaces 7: 21682-21689.

57. Nienhaus GU, Maffre P, Nienhaus K (2013) Studying the protein corona on nanoparticles by FCS. Methods Enzymol 519: 115-137.

58. Cedervall T, Lynch I, Lindman S, Berggard T, Thulin E, et al. (2007) Understanding the nanoparticle-protein corona using methods to quantify exchange rates and affinities of proteins for nanoparticles. Proc Natl Acad Sci U S A 104: 2050-2055.

59. Winzen S, Schoettler S, Baier G, Rosenauer C, Mailaender V, et al. (2015) Complementary analysis of the hard and soft protein corona: Sample preparation critically effects corona composition. Nanoscale 7: 2992-3001.

60. Kaufman ED, Belyea J, Johnson MC, Nicholson ZM, Ricks JL, et al. (2007) Probing protein adsorption onto mercaptoundecanoic acid stabilized gold nanoparticles and surfaces by quartz crystal microbalance and ζ-potential measurements. Langmuir 23: 6053-6062.

61. Fleischer CC, Payne CK (2014) Nanoparticle-cell interactions: Molecular structure of the protein corona and cellular outcomes. Acc Chem Res 47: 2651-2659.

62. Yallapu MM, Chauhan N, Othman SF, Khalilzad-Sharghi V, Ebeling MC, et al. (2015) Implications of protein corona on physico-chemical and biological properties of magnetic nanoparticles. Biomaterials 46: 1-12.

63. Ding HM, Ma YQ (2014) Computer simulation of the role of protein corona in cellular delivery of nanoparticles. Biomaterials 35: 8703-8710.

64. Mahmoudi M, Shokrgozar MA, Sardari S, Moghadam MK, Vali H, et al. (2011) Irreversible changes in protein conformation due to interaction with superparamagnetic iron oxide nanoparticles. Nanoscale 3: 1127-1138.

65. Lundqvist M, Sethson I, Jonsson BH (2004) Protein adsorption onto silica nanoparticles: Conformational changes depend on the particles' curvature and the protein stability. Langmuir 20: 10639-10647.

66. Brandes N, Welzel PB, Werner C, Kroh LW (2006) Adsorption-induced conformational changes of proteins onto ceramic particles: Differential scanning calorimetry and FTIR analysis. J Colloid Interface Sci 299: 56-69.

67. Gessner A, Lieske A, Paulke BR, Müller R (2002) Influence of surface charge density on protein adsorption on polymeric nanoparticles: Analysis by two-dimensional electrophoresis. Eur J Pharm Biopharm 54: 165-170.

68. Göppert TM, Müller RH (2005) Polysorbate-stabilized solid lipid nanoparticles as colloidal carriers for intravenous targeting of drugs to the brain: Comparison of plasma protein adsorption patterns. J Drug Target 13: 179-187.

69. Pozzi D, Caracciolo G, Capriotti AL, Cavaliere C, La Barbera G, et al. (2015) Surface chemistry and serum type both determine the nanoparticle-protein corona. J Proteomics 119: 209-217.

70. Tenzer S, Docter D, Rosfa S, Wlodarski A, Kuharev JR, et al. (2011) Nanoparticle size is a critical physicochemical determinant of the human blood plasma corona: A comprehensive quantitative proteomic analysis. ACS Nano 5: 7155-7167.

71. Docter D, Distler U, Storck W, Kuharev J, Wunsch D, et al. (2014) Quantitative profiling of the protein coronas that form around nanoparticles. Nat Protoc 9: 2030-2044.

72. Pederzoli F, Tosi G, Genovese F, Belletti D, Vandelli Ma, et al. (2018) Qualitative and semi quantitative analysis of the protein coronas associated to different functionalized nanoparticles. Nanomedicine (Lond) 13: 407-422.

73. Nguyen VH, Lee BJ (2017) Protein corona: A new approach for nanomedicine design. Int J Nanomed 12: 3137-3151.