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Protein corona: Opportunities and challenges

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ABSTRACT

In contact with biological fluids diverse type of biomolecules (*e.g.*, proteins) adsorb onto nanoparticles forming protein corona. Surface properties of the coated nanoparticles, in terms of type and amount of associated proteins, dictate their interactions with biological systems and thus biological fate, therapeutic efficiency and toxicity. In this perspective, we will focus on the recent advances and pitfalls in the protein corona field.

1. Introduction

It has been known, for a decade, that the surface of nanoscale materials is masked by a layer composed of various biomolecules (*e.g.*, proteins and metabolomes) after incubation with a biological fluid. This protein-rich layer is the so-called "protein corona" (Mahmoudi et al., 2011a; Monopoli et al., 2012a). The biological fate of nanomaterials are substantially affected by the decoration of protein coronas in terms of type, amount, and conformation of associated proteins (Hamad-Schifferli, 2015). Therefore, protein coronas have recently been the subject of extensive studies (Milani et al., 2012; Monopoli et al., 2012b). In order to accurately interpret the interactions between nanoparticles and cells, it is necessary that the adsorption of protein coronas to nanoparticles be well characterized (Monopoli et al., 2013). Yet, assessment of the absolute number of bound proteins and their exchange dynamics in body fluids is an arduous task. Not to mention that there are a diverse group of biomarkers that cover nanoparticles soon after they come in contact with biological fluids (Maiolo et al., 2014). Proteins can bind to nanoparticles with either high affinity, hence known as a hard corona (HC), or low affinity, hence known as a soft corona (SC). Thus, HC-nanoparticle complexes are stable complexes with a long lifetime and SCs are more dynamic with shorter lifetimes (Maiolo et al., 2014; Hadjidemetriou et al., 2015). In the presence of the corona, cells are protected from the damaging effects that the bare nanoparticle surface can engender until the nanoparticles and protein corona are cleared through phagocytosis (Wang et al., 2013). Understanding these fundamental processes are essential as the unique combination of the nano-bio interphase directs the nature of a corona, which then determines the biological identity of the protein corona-nanoparticle complex (Saie et al., 2015; Jiang et al., 2010).

2. Challenges of the protein corona

Comprehensive understanding of the evolution of protein corona on the surface of nanoparticles should allow for the ability to control and exploit the bio-nano interface. However, a challenge that must be addressed is the potential hazards associated with use of nanoparticles. Numerous protocols and warnings of potential hazards and difficulties of using nanoparticles have been developed as a result of extensive study over the past decade (Monopoli et al., 2013). This encouraged researchers with a guideline to carry out basic experiments to investigate nanoparticle-protein corona complexes. The protein corona has been found to provide multiple protective effects to biological systems. However, interpretations of these beneficial effects can often be unclear due to the variation in reported outcomes. Conflicting reports on cytotoxicity and biological fates, even when identical nanoparticles were studied, have been reported (Sharifi et al., 2012; Mahmoudi et al., 2011b; Mao et al., 2013; Hajipour et al., 2015a). For example, Hadjidemetriou et al. (2015) demonstrated that *in vitro* plasma

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incubations could not accurately predict the molecular complexity of the *in vivo* protein corona, which was formed on clinically developed liposomes. Therefore, the structural characteristics of the corona formed *in vitro* can be significantly different from the *in vivo* corona. Investigators must ensure that proper controls and comparisons are being made. There are still many unmet challenges that are required before the design of truly efficient targeted nanoparticles is attained. Additionally, more work is needed to ensure predictable biological and *in vivo* outcomes (Azhdarzadeh et al., 2015a; Mahon et al., 2012). Should this goal be accomplished, it could potentially revolutionize multiple therapeutic areas and make significant improvements in nanomedicine (Saie et al., 2015).

2.1. Targeting

The ability to target specific cells *in vivo* using nanoparticles is required for successful therapeutic effects in the field of nanomedicine. Proteins, antibodies, and other biomolecules have been used to functionalize the surface of nanomaterials in order to target specific or overexpressed receptors in targeted cells. However, targeting therapeutic nanoparticles to specific cells *in vivo* is a major challenge for nanomedicine, because competing protein the functionality of the nanoparticles in biological environments (Maiolo et al., 2015). Salvati et al. (2013) showed that nanoparticles lose their targeting specificity in complex biological media, because the targeting molecules on the surface of nanoparticles are blocked by the protein corona and the interactions of the nanoparticles with other proteins in the medium. Some of the effects the protein corona can cause to specific targeting moieties include: loss of function due to poor orientation or displacement of the targeting moieties, structural or conformational disruption, and obscuring specific surface recognition.

In order to overcome this inherit challenge associated with protein corona, there are two proposed approaches including preparation of free corona particles and directing corona composition for targeting purposes.

2.1.1. Corona free particles

The protein corona plays an important biological role by coating the nanoparticle and masking the surface properties of the particles and can, therefore, make it challenging to distinguish the relationship between chemical functionality of nanoparticles and their biological effects (more specifically on targeting). To overcome this issue, the capability of zwitterionic nanoparticles to inhibit protein absorption to nanoparticles was proposed. The fate of corona-free nanoparticles was demonstrated by Moyano et al. (2014) presenting a series of zwitterionic nanoparticles such that only the formation of soft coronas was facilitated at physiological serum concentrations. Our increasing knowledge of the nano-bio interphase will direct the design of nanoparticles and will lead to the ability to control nanomaterials and biosystems without interference from protein coronas.

2.1.2. Using corona structure as targeting agents

One approach to overcome the masking issue associated with the protein corona to use engineered nanoparticles to direct protein corona composition for active targeting. To this end, our group assessed whether the targeting capabilities of recruited protein ligands taken directly from biological fluid during corona formation could improve the targeting capability of particles (Mirshafiee et al., 2016). To prepare such an engineered nanoparticle, the surface of the silica particles was chemically coated by gamma-globulins. This surface modification could recruit immunoglobulins and complement proteins from biological fluids due to the protein-protein interactions. The adsorption of these specific proteins should enhance the targeting with natural targeting capabilities. For example, the contribution of immunoglobulins and complement proteins should enhance macrophage targeting. Although we had a directed corona composition with an enriched amount of immunoglobulins and complement proteins, we found that the corona proteins with targeting capabilities did not substantially enhance nanoparticle uptake by macrophages (Mirshafiee et al., 2016). This is partly related to the fact that these proteins were not accessible to their partner receptors at the target cell surface. Therefore, one could expect that the functional corona with targeting capabilities should have recruited proteins and must be in a position which could enhance accessibility of their binding motifs to the receptors on the surfaces of the target cells.

In summary, when using the functional corona coated nanoparticles for targeting purposes, researchers should develop strategies that enable controlling both conformations and accessibility of the recruited proteins in the outer layer to facilitate their interactions with their binding partners on the target cells.

2.2. Protein function

Protein coronas are engaged with several biological pathways as they interact with cells and biological barriers. Therefore it is essential to fully study the functional biomolecular motifs at their interface. Kelly et al. (2015) have studied the binding sites on protein coronas and have demonstrated the feasibility of identifying the spatial location of proteins as well as their functional motifs and binding sites. Their findings can be used to describe the biological identity of various nanoparticles at the molecular level. In an *in situ* study, O'Connell et al. (2015) proposed deployment of protein arrays which are specifically designed to target particular systems and organs in order to study the biomolecular corona. They showed that the protein concentration in plasma and a small quantity of dominant protein–protein interactions are the major factors governing the nanoparticle interactome.

2.3. *Modification of* in vitro *assays*

To date, nanoparticles hold great potential in this very new field. Yet the very same qualities of nanoparticles that make them so distinguished in industry also make them toxic and harmful when considered in biological and medical contexts. The progress we have made in understanding and integrating nanotechnology comes to an abrupt stop into the nanomedicine. In order to get nanoparticles in clinical/commercial stage, there is a fundamental question/challenge in the field that needs to be addressed properly. The question is that why there is significant conflict in the biological data (e.g. nanotoxicology) even between identical nanoparticles. According to the findings, there is one main answer to this question: it is that there are several hidden factors at the nanobiointerfaces which had been ignored during the last decade. While it is know that there has been a large pool of overlooked factors, here we focus on only those factors relating to the protein corona.

2.3.1. Modifications of toxicity assays

Most of the toxicological protocols used for nanoparticles were developed decades before the first nano-based publication came out (Azhdarzadeh et al., 2015b). This means that these approaches are not specifically designed for toxicity evaluation of nanoparticles. In other words, essential modifications should be considered when applying these assays to evaluate nanoparticle toxicity. For example, in the MTT assay (a widely used viability assay in nanotoxicology) the presence of nanoparticles can change the structure of the dye into fomazan crystals which are blue in color (Berridge et al., 2005). This assay has a major shortcoming in terms of the negative impact of the protein corona in regards to useful assay results. Due to their high surface to volume ratio, nanoparticles have high