Colloids and Surfaces B: Biointerfaces 124 (2014) 17–24

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

The nano-plasma interface: Implications of the protein corona

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ARTICLE INFO

Article history:
Received 27 December 2013
Received in revised form 6 February 2014
Accepted 21 February 2014
Available online 2 March 2014

Keywords:
Nanoparticles
Protein corona
Nano-bio interface
Proteins
Plasma

ABSTRACT

The interactions between nanoparticles and macromolecules in the blood plasma dictate the biocompatibility and efficacy of nanotherapeutics. Accordingly, the properties of nanoparticles and endogenous biomolecules change at the nano-plasma interface. Here, we review the implications of such changes including toxicity, immunological recognition, molecular targeting, biodistribution, intracellular uptake, and drug release. Although this interface poses several challenges for nanomedicine, it also presents opportunities for exploiting nanoparticle–protein interactions.

1. Introduction

Nanoparticles present promising tools for diagnostic and therapeutic purposes. Accordingly, the scientific literature is replete with examples of nanosystems designed for medical applications [1–6]. Moreover, several nanotherapeutics have already received clinical approval and many are currently undergoing clinical trials [7]. These nanoparticles are intended for either local or systemic administration. The latter route provides a means for targeting tissue that is inaccessible through local infusion, making it a suitable method for treating diseases such as metastatic cancer. Upon systemic injection, nanoparticles are subjected to a variety of forces and biological reactions in the blood. These phenomena include, but are not limited to, mechanical stress due to rapid blood flow, enzymatic degradation, binding to biomolecules, and uptake by immune cells [8]. This review will focus specifically on the interactions between nanoparticles and components of blood plasma. Since the blood contains thousands of proteins [9], it is not surprising that such interactions occur. Notably, the nano-plasma interface may influence both the nanoparticles and the biomolecules that they come in contact with. In essence, the characteristics of nanoparticles as well as plasma components could markedly change at the interface. These interactions may lower or increase the toxicity of nanosystems and in turn change the biodistribution and efficacy of nanotherapeutics. Therefore, there has recently been an impetus toward understanding the impact of blood molecules on nanostructures and *vice versa.* Gaining a better understanding of the nano-plasma interface could aid in overcoming challenges in nanomedicine and provide opportunities for exploiting these kinds of interactions.

2. The protein corona

The biomolecule coating that forms around nanoparticles upon contact with biological fluids is termed a protein corona. The corona forms due to the high surface free energy of nanoparticles, resulting in adsorption of various molecules, most notably proteins. The binding forces that are responsible for such interactions include van der Waals interactions, hydrogen bonds, hydrophobic interactions, electrostatic interactions, and π–π stacking [10]. Indeed, protein shells have been reported to form around a vast array of

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http://dx.doi.org/10.1016/j.colsurfb.2014.02.035
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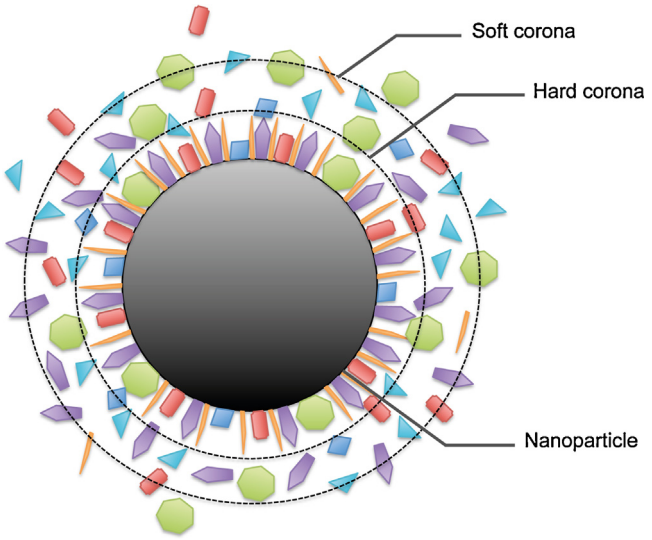

Fig. 1. Schematic representation of the current protein corona hypothesis. A hard and soft layer of proteins cover the surface of the nanoparticle. The proteins in the hard corona are more tightly associated with the particle surface, making them less dynamic than the proteins in the soft corona.

nanoparticles, including those comprised of metal [11–13], polystyrene [14–16], silica [14–17], and lipids [18]. The current hypothesis states that the corona consists of a 'hard' and 'soft' layer (Fig. 1) [19]. These components are distinguishable in how tightly the biomolecules are associated with the nanoparticles. In addition, the soft layer is thought to be more dynamic, consisting of rapidly exchanging biomolecules. There also exists controversy regarding the heterogeneity of the hard corona that forms in human blood plasma. In this regard, various sources report the presence of less than 100 different proteins [20–22], while others claim the existence of more than 100 [16,23]. Notably, this disparity may be due to the varying nature of the nanoparticles studied or differences in the methodological approaches used to identify protein profiles. In general, the most abundant proteins in the corona are albumin, apolipoprotein-1, complement proteins, and immunoglobulins [16,20,21,23].

The composition of the protein corona varies depending on the nanoparticle material, size, shape and surface charge [22,24]. For instance, hydrophobic nanoparticles, *e.g.* carbon nanotubes, usually attract proteins with several hydrophobic residues [25]. In addition, enhanced protein adsorption is observed with increased nanoparticle size [26]. This size-dependent effect can be explained by taking into account the curvature of nanoparticles. Larger nanoparticles have reduced curvature, which enables proteins to more freely interact with a larger surface area. The shape of nanoparticles also governs the type of protein corona that forms. Notable, titanium dioxide nanotubes and nanorods display different protein corona characteristics [27]. In addition, a positive surface charge is typically correlated with increased protein adsorption [28]. Moreover, while there exists a consensus concerning the dynamic nature of the protein corona, the sequential binding events at the nano-plasma interface are largely unknown. Interestingly, a recent study found that the amount of protein in the corona changes over time, while the types of bound protein remain relatively constant [16].

3. Changes to biomolecules at the nano-plasma interface

When proteins bind to nanoparticles they may undergo conformational changes. Such changes can be either reversible or irreversible. For instance, the adsorption of albumin on the surface of gold nanoparticles changes the secondary and tertiary conformation of this protein [29]. In addition, intracellular proteins, such as cytochrome c [30] and ribonuclease A [31] have also been found to undergo structural changes when exposed to nanoparticles. Notably, the extent and rate of conformational change has been linked to the overall stability of the protein [32,33]. This correlation was found by comparing protein variants with differing degrees of stability. The conformational changes were shown to occur in a step-wise manner, where the least stable variants showed the most rapid misfolding kinetics [33]. However, following prolonged incubation with nanoparticles, all protein variants eventually folded into the same state. Additionally, a correlation between nanoparticle size and protein unfolding has been observed. Larger nanoparticles with lower surface curvature cause more conformational changes in protein structure [32,34,35]. Since the structure–function relationship is strong for proteins, nanoparticle coronas may also alter the behavior of these macromolecules. As an illustration, iron oxide nanoparticles were found to change the conformation of transferrin, causing the protein to prematurely release iron [36]. Moreover, the conformational alteration is irreversible, indicating permanent damage to the function of this protein in iron transport. In addition, as proteins come in close vicinity to each other at the nanoparticle surface, they may cluster together. Indeed, one study found that the fibrillation of human β2-microglobulin increases as a result of exposure to several different nanoparticles [37].

4. Changes to nanoparticles at the nano-plasma interface

The characteristics of nanoparticles tend to change considerably upon interactions with a biological environment. In particular, properties such as the shape, size, and charge are usually affected as a consequence of the protein corona. In general, nanoparticles become larger as a result of protein interactions. This size increase is usually in the range of 20–70 nm [14,16,17,38], suggesting that the corona consist of multiple protein layers. However, the parameters that dictate the number of layers surrounding a nanoparticle remain elusive. In contrast, the size of certain lipid nanoparticles decreases in a protein-rich environment, presumably due to osmotic forces [39]. Namely, as the lipid membrane is impermeable to proteins, an osmotic pressure is created at the nano-plasma interface. Consequently, water flows out of the aqueous interior, causing nanoparticle compression. Furthermore, the protein corona may trigger nanoparticle aggregation through protein bridges, resulting in the formation of larger clusters [27,40,41]. Conversely, the presence of a protein corona may also stabilize particles and prevent aggregation [42,43]. This stabilization effect may arise due to changes in the nanoparticle surface charge or steric hindrance of inter-particle binding [43].

In addition, as most proteins are negatively charged, the formation of a protein corona will cause the nanoparticle surface to become anionic. One study showed that regardless of the zeta potential of bare nanoparticles (ranging from −28 mV to 51 mV), upon exposure to plasma proteins the zeta potential become negative (ranging from −24 mV to −6 mV) [16]. Although certain trends are evident regarding protein-induced changes in nanoparticle characteristics, it is difficult to form general rules about the behavior of particles in plasma. The vast array of nanoparticles and experimental conditions used to study the nano-plasma interface inevitably lead to variable conclusions regarding protein–particle interactions.

5. Implications of the nano-plasma interface

As discussed in the previous sections, the properties of nanoparticles and endogenous macromolecules change at the nano-plasma