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Significance of cell "observer" and protein source in nanobiosciences

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ABSTRACT

It is well understood that when nanoparticles (NPs) enter a biological medium, their surface is coated by various proteins; thus, the interaction of the living systems with the NPs depends on the composition of the protein layer, rather than the surface characteristics of the nanoparticle itself. However, there are several neglected parameters in protein–NP interactions (e.g., the key role of the protein source) that should be addressed. The composition of the protein corona is recognized as having a crucial influence on the delivery of NPs into cells, which is important in therapeutic applications and in nanotoxicology; however, the effect of "cell observer" (cell type) is poorly understood. This study probed the effects of different protein sources (fetal bovine serum [FBS] and human plasma [HP]) on the composition and protein thickness of the hard corona formed at the surface of superparamagnetic nanoparticles (SPIONs) with various sizes and surface chemistries. The results show that the hard corona can change quite considerably as one passes from the biophysicochemical properties of nanoparticles and protein sources (e.g., FBS and HP) appropriate to *in vitro* cell/tissue studies to those appropriate for *in vivo* studies. These changes in the hard corona have deep implications for *in vitro–in vivo* extrapolations. In addition, we probed the "cell observer" effect on the uptake and toxicity of SPIONs with the same protein corona composition to highlight the effect of cell type in nanobiosciences. The particles interacted with various cell lines. We find that without consideration of the "cell observer" effect, the cellular targeting/toxicity of NPs is inherently imprecise; thus, a deep understanding of both the protein corona composition and the "cell observer" effect offer a way to predict NP dosage for therapy and for the study of nanotoxins.

1. Introduction

It is now well recognized that the surface of nanoparticles (NPs), after coming in contact with a biological fluid, interacts with the biological milieu and becomes covered with proteins. This protein coating is called the protein corona [1,2]. Depending on the physicochemical properties of the NPs, such as surface curvature, material, charges, surface modifications, and the biological fluid that they come in contact with, only a selective set of proteins with a high affinity for the NP surface will adhere and remain tightly bound for a long time [3–5]. Because it has been shown that the protein corona layer remains tightly bound to the NP surface for several hours, it is most likely that the living organism (e.g., cell) "sees" the NP protein corona rather than the bare surface of the NP [6–13].

Importantly, the corona layer is dynamic; hence, what the biological organism sees is the exact protein layer at the time it contacts the NPs. According to previous reports, the cell "sees" a nanosystem in which the core NP is covered by a "hard" corona of slowly exchanging proteins and a "soft" outer corona made up of weakly interacting and rapidly exchanging collections of proteins [14].

The membrane is the part of the cell that first makes contact with a foreign object (e.g., biomolecules, drugs, and NPs). Nevertheless, there are approximately 200 types of differentiated cells in the human body. Significant differences in cell membranes cause differences in cellular uptake and toxicity mechanisms. Thus, what the cell "sees" when it is faced with NPs is most likely dependent on the "cell observer" [15]. Nevertheless, to the best of our knowledge, there has been no comprehensive report on the differences between the membranes of various cell types. In addition, the uptake yield of biomolecules and drugs, along with their fate in the intercellular environment, are strongly related to their transport through the cellular membrane. Membrane transport depends greatly upon cellular composition.

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In this article, we show that the protein source plays a key role in the formation of various protein profiles in the hard corona layer. The composition of the corona may depend on both the protein sources and the biophysicochemical properties of the nanoparticles; therefore, the *in vivo* biological identity and fate of the nanoparticles may be dramatically different from *in vitro* results.

In addition, we show that reliable, reproducible, and precise interpretation of cellular nanoparticle uptakes and the corresponding toxicities can be achieved by a deep understanding of both the composition of the corona and the "cell observer." Furthermore, we show that consideration of the protein corona alone is not adequate for precise prediction of cellular nanoparticle uptakes and the corresponding toxicities.

Highly monodisperse superparamagnetic iron oxide nanoparticles (SPIONs) were chosen as model NPs in this study because of their unique biomedical applications and their commercial availability for future biological studies and applications. SPIONs, unlike other nanoparticles, can be targeted to a desired site or heated in the presence of an externally applied AC magnetic field because of their inducible magnetization [16,17]. In addition, SPIONs have been recognized as a promising type of nanoparticle, not only for their excellent biocompatibility but also for their multiple applications, which can significantly decrease patient compliance issues associated with other therapeutic approaches [18–21]. SPIONs have been extensively employed for both *in vitro* and *in vivo* biomedical applications, such as magnetic resonance imaging (MRI) contrast enhancement [22], tissue-specific release of therapeutic agents [23], hyperthermia [17], transfection [24], cell/biomolecular separation [24], and targeted drug delivery [19].

2. Experimental section

2.1. Materials

Analytical-grade iron salts (iron chloride) and sodium hydroxide (NaOH) were purchased from Merck Inc. and were used without further purification. Dextran with an average molecular weight of 5000, dimethylsulfoxide, sodium periodate, potassium cyanide, and ammonium persulfate were procured from Sigma–Aldrich.

2.2. Preparation of the carboxyl-dextran

The carboxylated dextran was prepared according to a previously reported procedure [25]. The hydroxyl groups in the dextran were oxidized to aldehyde groups by sodium periodate [26]. Briefly, sodium periodate was dissolved in deoxygenated deionized (DI) water and introduced to the dextran solution (4 g in 30 mL of deoxygenated DI water). The obtained solution was homogenized for 2 h at room temperature, followed by dialyzing with a membrane bag with a 1000 molecular weight cutoff for 4 days. The obtained solution was allowed to react with potassium cyanide to prepare intermediate cyanohydrins. Finally, carboxylic acid groups were created on the terminal units of the dextran by hydrolysis of the intermediate cyanohydrins. The prepared carboxylated dextran was lyophilized and stored at −80 °C.

2.3. Preparation of the aminodextran

The aminodextran was prepared according to a previously reported procedure [27]. Briefly, 10 g of dextran were dissolved in 75 mL of deoxygenated DI water containing 2.5 g of sodium hydroxide and 0.2 g of sodium borohydride at a pH of 11, obtained by dropwise addition of 2.5 N NaOH and 2 mL of allyl bromide and a temperature of approximately 50 °C. Acetic acid was used to neutralize the pH of the solution. The solution was then incubated at 4 °C for 2 h. The top organic layer was removed, and 100 mL of fresh deoxygenated DI water was added. The solution was then dialyzed using a membrane bag with a 50,000 molecular weight cutoff for 24 h. To prepare aminodextran conjugate, the dialyzed mixture was allowed to react with 7.5 g of aminoalkyl thiol compound in 30 mL dimethylsulfoxide, where 0.1 g of ammonium persulfate was used as an initiator. After 3 h 75 ml of fresh deoxygenated DI water was added to the reactor, the pH was adjusted using sodium hydroxide, and the product was diluted with 140 mL sodium acetate buffer (0.02 mol/L, pH 4). In order to ensure the complete removal of excess materials, the obtained aminodextran conjugate was dialyzed using a membrane bag with a 50,000 molecular weight cutoff for 24 h. The prepared aminodextran conjugate was lyophilized and stored at −80 °C.

2.4. Synthesis of ultra-small SPIONs with various surface characteristics

In order to be certain of the deoxygenation of the DI water, the solutions were bubbled with a neutral gas, argon, for a period of 30 min. The iron salts, $FeCl_3$ and $FeCl_2$, were dissolved in separate beakers containing the mixture of deoxygenated DI water and HCl (i.e. total molarity of HCl in DI water was1.5); the obtained iron salt solutions were blended together by adjusting to a molar fraction of 2 (Fe^{3+}/Fe^{2+}). The three types of dextran—carboxylated dextran, plain dextran, and amino-conjugated dextran—were each dissolved in deoxygenated DI water. The various dextran types were mixed with the iron salt solutions and introduced into a three-neck flask equipped with a homogenizer stirring at 10,000 rpm. Each type of dextran produced SPIONs with a different surface coating: carboxylated dextran produced a negatively charged surface coating; plain dextran produced a neutral surface coating; and aminodextran produced a positively charged surface coating. To obtain single-coated nanoparticles, the dextran/iron mass ratio was fixed at two [28].

The SPIONs were formed by dropwise addition of the base medium, NaOH, to the prepared dextran and iron salts mixture with vigorous stirring under argon protection. To achieve highly monodisperse nanoparticles, it was necessary to decrease mass transfer, which allows nanoparticles to combine and build larger polycrystalline nanoparticles. To decrease mass transfer, the reactor was transferred to an ultrasonic bath (100 W) to create turbulent flow [29]. After an hour, the black suspension was placed in a strong magnetic field gradient produced by a permanent Nd-Fe-B magnet (with a cylindrical shape, diameter of 4 cm and height of 3 cm), which exhibits superior magnetic properties, and the dextran-coated SPIONs were collected. The supernatant was completely removed, and the coated SPIONs were redispersed in DI water several times. In order to be certain about the removal of excess ammonia, iron cations, and free dextran macromolecules, the obtained ferrofluid was dialyzed using a membrane bag with a 50,000 molecular weight cut-off for 24 h. The obtained ferrofluid was kept at 4 °C for protein-interaction experiments. To obtain bare particles, the same procedure was applied without using dextran. The prepared SPIONs with various surface chemistries are shown in Fig. S1 of SI.

2.5. Preparation of larger, double-coated SPIONs with various surface characteristics

The three types of dextran were dissolved in DI water at the same concentrations, and various synthesized small dextran-coated SPIONs were individually added to the reactor under vigorous agitation. After an hour, the double-coated SPIONs (i.e., a few SPIONs dispersed on a dextran bead) were collected with a strong