Significance of cell “observer” and protein source in nanobiosciences

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\bf{A B S T R A C T}

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 \textit{in vitro} cell/tissue studies to those appropriate for \textit{in vivo} studies. These changes in the hard corona have deep implications for \textit{in vitro}–\textit{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.

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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 \cite{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 \cite{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 \cite{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 \cite{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” \cite{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 hydroxide, 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 various dextran types 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 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 materials, the obtained aminodextran conjugate was dialyzed using a membrane bag with a 50,000 molecular weight cutoff for 4 days. To prepare aminodextran conjugate, the dialyzed mixture was allowed to react with potassium cyanide to lyophilize 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, FeCl3 and FeCl2, were dissolved in separate beakers containing the mixture of deoxygenated DI water and HCl (i.e. total molarity of HCl in DI water was 1.5); the obtained iron salt solutions were blended together by adjusting to a molar fraction of 2 (Fe3+/Fe2+). 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 cutoff 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
magnet. The supernatant was completely removed, and the double-coated SPIONs were redispersed in DI water several times. In order to ensure the complete removal of excess ammonia 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.

2.6. Interaction of SPIONs with various physicochemical properties with different protein sources

When nanoparticles are in a biological fluid, they can associate with a range of biomolecules (e.g., proteins and lipids) leading to an interface organization called “corona”. There are two components, a “hard” and “soft” corona with long and short exchange times between the adsorbed biomolecules and the nanoparticle surface respectively [30–33]. Lundqvist et al. [30] studied the long-lived (“hard”) protein corona formed from human plasma for a range of polystyrene nanoparticles that differ in surface properties and size.

To elucidate the key role of protein sources, we investigated the effects of two different protein sources, fetal bovine serum (FBS) and human plasma (HP), on the composition and protein thickness of hard and soft coronas formed at the surface of SPIONs with various sizes and surface chemistries.

2.6.1. Hard corona

The interactions of all SPIONs with both fetal bovine serum (FBS) and human plasma serum (HP) in both the hard corona and in situ states were probed. Human plasma was obtained from 10 to 15 volunteers following the HUPO BBB guidelines [34]. Typically, 100 µL of particles (with a concentration of 100 µg/mL) were mixed with 900 µL of FBS or HP, followed by incubation at 37 °C for 1 h. The 1-h incubation time was chosen because previous reports confirmed that the protein corona is formed in a relatively stable manner over a period of 1 h [5]. After the incubation time, SPIONs with hard coronas (SPIONs-SC) were obtained by centrifugation. The standard procedure consists of three washing steps before resuspension of the final pellet to the desired concentration. The washing process is designed to remove the excess (unbound or loosely bound) proteins. The hard corona samples were centrifuged (at 15 °C, 20,000 rpm, 50 min) to pellet the SPION–protein complexes, and the supernatant was carefully removed. The pellet was resuspended in a small amount of PBS (500 µL) and centrifuged again (at 15 °C, 20,000 rpm, 30 min) to pellet the SPION–protein complexes. Before the final washing step, the samples were transferred into a low-protein attachment Eppendorf tube, followed by the final centrifugation, and the obtained SPIONs-HCs were stored.

2.6.2. Soft corona

The interactions of all SPIONs with FBS and HP in in situ states were probed. Typically, 100 µL of particles (with a concentration of 100 µg/mL) were mixed with 900 µL of FBS, followed by incubation at 37 °C for 1 h. After incubation, protein absorption at the SPI- ON surface was probed using the differential centrifugal sedimentation (DCS) method.

2.7. Cell culture and treatments

Various cell lines from different origins (e.g., immune cells, pancreas, and cervix) were used for the uptake and cytotoxicity assay. Panc-1 (i.e., human pancreatic carcinoma) and Capan-2 (i.e., human pancreas adenocarcinoma) cells were kindly donated by Dr. Daizy Flamez (Free University of Brussels, Experimental Medicine Laboratory, Belgium). Panc-1 cells were cultured in pyruvate-free DMEM culture medium (Lonza, Verviers, Belgium) supplemented with 10% fetal bovine serum and nonessential amino acids (both from Invitrogen) and penicillin/streptomycin (Lonza). Capan-2 cells were cultured in advanced RPMI-1640 culture medium supplemented with 10% fetal bovine serum and glutamax (both from Invitrogen) and penicillin/streptomycin (Lonza). Jurkat (human T cell lymphoblast-like) cells (gift from Prof. Oberdan Leo, Free University of Brussels, IBMM, Belgium) were cultured at a concentration of less than 1 x 10^6 cells/mL in RPMI-1640 culture medium (Sigma–Aldrich, Bornem, Belgium) supplemented with 10% heat-inactivated newborn calf serum and antibiotic–antimycotic (both from Invitrogen). Hela (human cervical adenocarcinoma) cells were cultured in MEM culture medium supplemented with 10% fetal calf serum, glutamax, antibiotic–antimycotic, nonessential amino acids, and sodium pyruvate (all from Invitrogen).

It is noteworthy to mention that the predetermined cell lines were selected in this study because they are not phagocytes, and therefore, their behavior would be restricted to differences in cell surface charges and particular composition (i.e. negative surface for Capan-2 and Panc-1 in addition to the mucin presence; surface rich in fucose (not charged) for HeLa cells; surface charged positively for Jurkat T cells).

2.8. Characterization methods

2.8.1. Materials and protein corona composition

The biophysicochemical properties of the various SPIONs were characterized as follows. The morphologies of various SPIONs were analyzed by a TEM operating at 200 kV. To prepare samples for TEM, a drop of the suspension was placed on a copper grid and dried. DCS experiments were performed with a CPS Disc Centrifuge DC24000. The analyzer measures particle size distributions using centrifugal sedimentation within an optically clear spinning disc filled with sucrose fluid, which has a gradient of 8–24% sucrose in PBS at 22,000 rpm. An iron concentration of 100 µg/mL was selected as the optimal amount of SPIONs for DCS. Dynamic light scattering (DLS) measurements were performed with a Malvern PCS-4700 instrument equipped with a 256-channel correlator. The 488.0-nm line of a Coherent Innova-70 Ar ion laser was used to determine the hydrodynamic diameters of the SPIONs.
as the incident beam. The laser power used was 250 mW. The scattering angles $\theta$ ranged between 40° and 140°. The temperature was maintained at 25 °C with an external circulator. Zeta potential determination was performed using a Malvern Zetasizer 3000HSa. Data analysis was performed according to standard procedures and interpreted through a cumulant expansion of the field autocorrelation function to the second order. To obtain a distribution of decay rates, a constrained regularization method, CONTIN, was used to invert the experimental data.

In order to define the protein profiles of hard coronas formed on the surface of various SPIONs, 1D SDS–PAGE was employed. For this process, the SPIONs-HCs were resuspended in 40 µL of fresh PBS, followed by the addition of 20 µL loading buffer containing 10% DTT.

### 2.8.2 Reactive oxygen species (ROS) assays

For visualization by confocal microscopy, the Panc-1 cells were seeded on cover slips before incubation with various compounds.
The cells were incubated for 24 h with 55.845 μg iron/mL (1 mM iron) added to the culture medium. Control cells were not incubated with SPIONs. The cells were then washed three times with ice-cold PBS and incubated for 1 h with 10 mM 5-[(and)-6]-chloromethyl-2,7-dichlorodihydrofluorescein diacetate acetyl ester (H₂DCFDA, Invitrogen, Merelbeke, Belgium) in PBS at 37 °C. The cells were subsequently washed three times with ice-cold PBS, fixed with 2% paraformaldehyde for 15 min at room temperature, and the cell-coated cover slips were mounted on microscope slides with 2% paraformaldehyde for 15 min at room temperature, and the cell-coated cover slips were mounted on microscope slides using Vectashield mounting medium with DAPI (Vector Labcon, Brussels, Belgium) [35]. The method of ROS labeling was slightly modified for Jurkat cells, which grow in suspension.

3. Results

3.1. Characterization of nanomaterials

The scheme for the preparation of SPIONs with various surface chemistries is shown in Fig. S1 of Supporting information (SI). Fig. S2 of SI shows TEM images of both single- and double-coated SPIONs. According to the figure, NPs were synthesized with a narrow size distribution; as expected, double-coated SPIONs have bigger sizes in comparison with single-coated SPIONs. DLS and zeta potential methods were performed for all samples, and the results are summarized in Table 1. According to the data in Table 1, the DLS results are highly dependent on the surface chemistry of the SPIONs. More specifically, the hydrodynamic size is minimized for the negative particles and maximized for the positive ones; thus, one can conclude that colloidal clustering of particles, the immobilized number of ions and the water layer at the surface of the SPIONs are highly dependent on the surface charge and size of the nanoparticles. These results should be considered in descriptions of protein absorption phenomena.

3.2. Hard corona and in situ composition

All samples (see Table 1) were studied by DCS under various media, including PBS buffer, DMEM medium supplemented with FBS, and DMEM medium supplemented with HP. DCS is recognized as one of the very few techniques that can be applied to complex biological systems without the need for either fluorescent labels or extreme nanoparticle dilution [5]. DCS was applied to the dextran-coated SPIONs with various sizes and charges (Fig. 1). Dextran-coated SPIONs, with negative

2.8.3. Lysosome labeling

Panc-1 cells were labeled with the Image-iT™ LIVE lysosomal and nuclear labeling kit (Molecular Probes, Invitrogen), which provides a red fluorescent LysoTracker® Red DND-99 dye for lysosome staining and a blue fluorescent Hoechst 33342 dye for staining the nucleus. The cells were seeded on cover slips and were incubated (37 °C, 24 h) with SPIONs added to the culture medium at a concentration of 55.845 μg/mL (1 mM of iron). Control cells were not incubated with SPIONs. After rinsing the cells with Hank's Buffered Salt Solution (HBSS), they were labeled with the Image-iT™ LIVE lysosomal and nuclear labeling kit according to the supplier's instructions. Briefly, the cells were incubated for 5 min with 2 μg/mL of Hoechst 33342 solution, followed by a 1-min incubation with 100 nM of LysoTracker Red DND-99®. The cells were rinsed two times with HBSS after each dye. The living cells were finally mounted in HBSS on microscope slides and observed on a DM2000 Leica microscope (Leica Microsystems, Groot Bijgaarden, Belgium); images were acquired with a Leica DFC 290 camera. The microscope images were finally analyzed using the ImageJ software, as described above.
and plain charges, were highly stable in water and in PBS because of the presence of strict repulsion forces; however, the small aggregations were detected in positive particles (see peak wide shoulders in Fig. 1); the formation of these small aggregations may be attributed to the fact that PBS contains strongly negatively charged ions, which caused the formation of salt bridges from these multivalent ions with the amine functional groups on the positively charged SPION surfaces [36].
The results showed a significant effect of the protein source and the surface chemistry of the SPIONs, rather than the size of the SPIONs, on the thickness of the protein corona. Particles with different sizes and charges illustrate various potentials for the absorption of protein coronas. In order to define the precise size of the protein-SPION complex, using DCS results, the exact shape and internal density distribution of each aggregate should be understood. A lack of understanding of these parameters is recognized as a limitation of complex biological media. Although a core–shell method was proposed to solve this problem [5], this proposed method contains several assumptions which may not be valid for our small dextran coated particles. In this case, we report the achieved main DCS peaks for description of the SPION-protein interactions.
DCS data shows that the interactions of negative and plain single- and double-coated SPIONs with the FBS protein source resulted in the formation of particles coated with protein coronas, whereas the interactions of both single- and double-coated SPIONs with the HP proteins, in the absence of free excess plasma, resulted in small aggregations of the SPIONs (see DCS results and TEM images for positive particles in Fig. 1c and d and Table 2). More specifically, when plain and negative SPIONs are added to the various protein mediums, the nominal SPION DCS peaks shift to smaller sizes, confirming the formation of protein corona at the surface of SPIONs (because the particles with protein corona have lower density compared to the bare particle itself). However, interaction of particles with HP in the absence of excess plasma show larger nomination peaks, confirming the formation of SPIONs stable clus-
ters. In comparison with the HP hard corona results, it seems that the severe drag- and shear-forces of the centrifugation process used to evaluate the hard corona played a key role in the formation of the small aggregations of negative and plain SPIONs; there is no trace of small clustering of the SPIONs in situ. Quite remarkably, interactions of positive double coated-SPIONs with FBS and HP in the absence of excess plasma and in situ show smaller nomination peaks, confirming the fact that the small aggregation of particle in PBS were removed after interaction with protein sources; in contrast, the large aggregated of positive single coated-SPIONs with HP in the absence of excess plasma were observed.

SDS–PAGE results (see Fig. 2) show the formation of different protein patterns caused by the various protein sources and surface chemistries of the nanoparticles; slightly different distributions of the aggregate species occurred. The differences in DCS curves from various FBS- and HP-hard-corona-SPION samples confirmed this claim. However, slight differences are apparent between the same particle types from different batches, confirming that there are
slight differences between the protein corona compositions of the SPION clusters. The biological implications of these observations are considerable.

DLS experiments on the various protein-SPION complexes were performed as a function of the scattering vector $q$. The results (see Table 3) are completely in agreement with the DCS and 1D SDS–PAGE data on the hard protein coating of the various SPIONs after 1 h of incubation in FBS and HP. Quiet remarkably, the gel results for positive particles are in contradiction with the DCS and DLS results; this happened due to the fact that positive parti-

Fig. 3. (continued)
cles faced on intense aggregations during centrifugation process; thus, one can conclude that the centrifugal process would not be suitable for hard corona evaluations of positively charged SPIONs.

3.2.1. Remarks on the hard corona and in situ investigations

We have been able to obtain a complete structural characterization of SPION–protein complexes not only for SPIONs with various sizes and surface chemistries but also for different protein sources. The data revealed significant differences of the hard corona protein profiles caused by the absorbance of both various protein sources and different SPIONs. Moreover, the surface charge of the particles is clearly very important for establishing a deeper understanding of what living organisms “see” in mixtures of nanoparticles and biological fluids. In addition to the physicochemical properties of the

![Fig. 3. (continued)](continued)
particles, the sources of proteins (e.g., FBS and HP) are crucial for decoration of the protein corona. The stability level of the hard corona is also dependent on the biophysicochemical properties of the SPIONs and the type of proteins in the biocolloidal medium.

We observed that the values of the zeta potential for negative, plain and positive SPION-hard corona complexes were reduced in comparison with the values of the zeta potential in a PBS buffer. This reduction may be caused by their various dispersion stabilization mechanisms, which are different from those (such as charge and steric hindrance) typical for non-protein-coated SPIONs. SPION stability is defined by the specific protein layer characteristics, reminiscent of the mechanisms by which thousands of different proteins in plasma are colloidally stable, despite their crowded environment; the same results have been observed for other vari-

Fig. 3. (continued)
eties of nanoparticles (e.g., surface-carboxylated and surface-sulfonated polystyrene and silica) [5].

We have also been able to show the importance of the protein sources in determining what the cell “sees” in bionanosciences. Hard-corona protein profiles, in the absence and presence of excess proteins, of the same nanoparticles were significantly different for interactions with various protein sources; therefore, in order to obtain significant improvement in the prediction of the in vivo fate of nanoparticles, the exact in vivo protein source of the desired species (e.g., animal or human) should be examined in vitro.

### 3.3. Cell uptakes and toxicity

In order to check the effect of cell “observer” on the uptake and toxicity of the NPs, different cell lines (i.e., Capan-2, Panc-1, Hela, and Jurkat cells) were treated with the same amount of various SPION preparations. The lysosomes and intracellular ROS were visualized by fluorescent microscopy (Fig. 3) and the results of their semi-quantitative analysis are shown in Fig. 4. The results suggest that both physicochemical properties of NPs and the cell “observer” effect have significant influence on NPs uptake and their correspondent toxicities.

In culture conditions, NPs aggregations were observed in the case of SPION preparations with positive or negative surface charges, but not in the case of plain SPION; however, the SPION agglomerations in the same culture conditions were not reproducible from one experiment to another. The ROS were generally positively correlated with lysosome formation for almost all the types of SPION preparations, with the exception of S Plain and D Positive NPs. A relatively positive correlation ($r^2 = 0.574$) between the lysosome formation and ROS production was found in the case of HeLa cells, while Jurkat ($r^2 = -0.390$) and Capan-2 ($r^2 = -0.548$) cells have shown a negative relationship between these two parameters; no significant correlation could be identified in the case of Panc-1 cells ($r^2 = 0.001$). All the NPs enter the cells by phagocytosis, but their fate depends on the digestion system of the cell (i.e. lysosomes), where the low pH promotes their dissolution. This may provoke the increase of the osmotic pressure and the eventual lysosome burst with the release of the iron atoms inside the cytoplasm leading to subsequent toxic events related to the ROS production [37]. So, we expect that ROS production might be positively correlated with lysosome content subsequent to the NPs uptake by the cells. However, this could be only being proven only in the case of HeLa cells.

For the other cells, either the NPs were not efficiently digested subsequent to their uptake or the defense mechanisms of the cells have limited the ROS production. The different uptake of NPs by each cell line depends on the cell membrane charge and on the degree of NPs agglomeration. The pancreatic cancer cell lines contain high concentrations of the sialic acid in their membrane glycoproteins, which is responsible for the negative charges on the cell surface [38]. HeLa cells also contain sialic acid on the cell membrane.
but probably at lower levels [39], whereas Jurkat cells have low contents of sialic acid on their surface [40]. This means that pancreatic cancer cell lines may be able to incorporate positively charged NPs as opposed to Jurkat cells. However, the cell uptake of S-positive SPION (as expressed by the lysosome content) was the highest in Panc-1 cells, followed by HeLa, Jurkat, and then by Capan-2 cells. This means that Capan-2 cell may have some other particularity of the cell membrane that modifies the NPs phagocytosis. One of this may be related to the differences between the composition of plasma membrane of various cell lines, which is usually quite different and heterogeneous, both laterally (lipid rafts, microdomains) and in the inner or outer leaflet. This heterogeneity influences not only the interaction of water-soluble proteins and peptides with the cell membrane [41] but even that of water-soluble NPs which impose an additional physical factor in the form of surface curvature. The possible mechanisms that validate the observed cellular response relate to the various detoxification strategies that any particular cell can utilize in response to nanoparticles. The uptake and defence mechanism could be considerably different according to the cell type. Thus, what the cell "sees", when it is faced with nanoparticles, is most likely dependent on the cell type and the interacted protein sources.

Based on the aforementioned results, we conclude that due to their "vision", cells have various ways to handle the NPs; although more investigations should be done to further explore the various responses, we suggest that the preferred route that an individual cell takes constitutes a mapping response just like a "fingerprinting" of the humans.

4. Conclusion

As interest grows in the principles governing bionanointeractions, one can expect the quest for the fundamental interacting unit in biological media to accelerate. In this article, we have shown the key role of protein sources in the formation of the associated protein corona. Moreover, the nature of the proteins in the corona is determined by the local chemical properties of the nanoparticles. Even for a fixed nanoparticle material type, the size of the nanoparticle and its surface modifications are able to entirely change the nature of the biologically active proteins in the corona and thereby also change the cellular uptake and toxicity. We also probed the cell “observer” effect and found that it had a great impact on nanoscale systems. The uptake and toxicity of the same amount of each type of nanoparticle on each cell type were probed, and the results indicated that each cell type responded differently to the nanoparticles. We speculate that significant differences in the composition of cell membranes are responsible for these different uptake and toxicity results. There remains, of course, the fundamental question regarding the plasma membrane compositions of various cell lines and to what degree they are able to control the uptake and toxicity of nanoparticles. The answer to such questions will likely determine the future roadmap of nanomedicine, nanosafety, and perhaps nanoscience itself.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jcis.2012.10.005.

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