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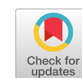
Comprehensive study on biocorona formation on functionalized selenium nanoparticle and its biological implications



Debolina Chakraborty [b], Pooja Chauhan [c], Sruthi Ann Alex [a], Savita Chaudhary [c], K.R. Ethiraj [b,*], N. Chandrasekaran [a], Amitava Mukherjee [a,**]

[a] *Centre for Nanobiotechnology, VIT, Vellore, India*
[b] *School of Advanced sciences, VIT, Vellore, India*
[c] *Department of Chemistry and Centre for Advanced Studies in Chemistry, Panjab University, Chandigarh, India*

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ABSTRACT

Selenium nanoparticles paved its way into the field of medicine because of its lowered toxicity and excellent biological properties. Interaction between these nanoparticles and serum proteins leads to the formation of protein corona (hard and soft) on the nanoparticle surface modulating its biological behaviour and fate. In the present study, we have emphasized on the biocorona formation of most abundant serum proteins i.e. human serum albumin, immunoglobulin-G, and transferrin on functionalized selenium nanoparticles (CTAB, SDS, and brij-58). Changes in protein adsorption pattern, mean hydrodynamic size, and zeta potential of differently functionalized selenium nanoparticles, following protein coronation were analysed for various incubation periods. Quantification of hard corona and soft corona for individual protein and for protein mixture was also done. The biological impact of protein coronation on selenium nanoparticles was evaluated by the cellular uptake and cell viability studies. Finally, modulation in radical scavenging property of selenium nanoparticles before and after protein corona formation was studied through DPPH assay.

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1. Introduction

To understand the critical role played by nanoparticles in human health, several nanoparticles (NPs) such as gold, silver, silica, iron–platinum (FePt) NP, and carbon dots have been widely used for several in vivo studies [1–5]. Nanoparticle susceptibility to interact with a vast array of biomolecules such as lipids, nucleic acids, proteins, and also with several metabolites and immunogenic cells can be attributed to the fact that they have large surface-area-to-mass-ratio [6, 7]. Of specific importance is the interaction of plasma proteins with the nanoparticles, which forms a protein layer known as "protein corona" on the nanoparticle surface. The identity of the nanoparticle changes due to the presence of protein corona which in turn modulates the nanoparticle fate and several biological behaviours such as toxicity, uptake, and immunogenicity [8]. Several forces such as Van der Waals, hydrogen bonds, and electrostatic interactions help protein corona formation [6], and the corona thus formed is differentiated into — hard and soft. Hard corona being more stable and tightly bound to the nanoparticle surface is composed of high-affinity proteins, whereas the soft corona layer is loosely bound, more dynamic in nature, and is composed of low-affinity proteins [9].

Among 3700 proteins present in plasma that form the protein corona on the nanoparticle surface, of particular importance is the high-affinity proteins that have a longer resilience time and replace the other low-abundance proteins in the blood [10]. In this regard, protein corona formation on varied nanoparticles is generally focused to human serum albumin (HSA), immunoglobulin-G (IgG), transferrin, and apolipoprotein-A [11]. Lennart et al. (2014) have demonstrated that corona formed by pure HSA and chemically modified HSA on DHLA- quantum dots can reduce its effective membrane binding and cellular uptake [12]. In another study, HSA, IgG, and transferrin corona formation on citrate-coated and silica-coated silver nanoparticles (AgNPs) of different size were studied, wherein the nature of the protein corona determined cellular uptake in HEK cells [13]. A similar study of the above-mentioned protein coronas were done on differently functionalized gold nanorods wherein a similar reduction in the cellular uptake and toxicity was observed [11]. Protein corona studies of single walled-CNTs (SWCNTs) with bovine fibrinogen (BFG), bovine serum albumin (BSA), transferrin, and gamma globulins showed varied cellular cytotoxicity depending on the layering of proteins and the type of protein adsorbed [14]. Zhou et al. (2009) explored the variation in serum protein adsorption pattern of albumins, apolipoproteins, IgM, IgG etc. on different metal oxide NPs (ZnO, SiO_2, TiO_2, synthetic TiO_2 nanorods

* Correspondence to: K.R. Ethiraj, School of Advanced sciences, VIT, Vellore 632014, India.
** Correspondence to: A. Mukherjee, Centre for Nanobiotechnology, VIT, Vellore 632014, India.
E-mail addresses: krethiraj@vit.ac.in (K.R. Ethiraj), amitav@vit.ac.in (A. Mukherjee).

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and nanotubes) based on their agglomeration sizes and shapes [15]. While several studies conducted on various metallic, inorganic and polymeric nanoparticles have given us an idea about the modulatory effects that corona can have on the nanoparticle, the effect of corona formation on nano selenium, alteration in its bioactivity, cellular uptake, and toxicity still remains unexplored.

Selenium is an essential trace-element required for proper functioning of various selenoenzymes. Elemental selenium is said to play a major role in cancer prevention, stimulation of immune response, and prevention of rheumatoid arthritis [16, 17], and the chemical nature and its effective dose determines its bioreactivity and bioavailability. In this regard, elemental nano selenium (SeNPs) tested in vivo has proved to be more efficient in terms of upregulating selenoenzymes along with lowered toxicity as compared to other seleno-compounds such as selenomethionine (SMet) and sodium selenite [18]. A study conducted by Ling et al. (2011) showed that SeNPs inhibited the growth of prostate cancer LNCaP cells by caspase-mediated pathway [18]. In another study, it has been shown to act as a protective agent by attenuating the reproductive toxicity during cisplatin chemotherapy [19]. A wide range of in vivo applications of SeNPs for various theranostic and drug-delivery applications makes it necessary to study the effect of protein coronation on such particles.

In the current study, we have emphasized on the biocorona formation of the most abundant serum proteins i.e. HSA, IgG, transferrin and their mixture on differently functionalized SeNPs. For SeNP functionalization, cationic (CTAB), anionic (SDS), and non-ionic (brij-58) surfactants were used. To the best of our knowledge, this is the first-ever work that explores the modulatory effects of surface functionalization of SeNPs on the protein corona formation of the three most abundant serum proteins. A time-based analysis for adsorption of different proteins and quantification of soft and hard corona along with the changes in mean hydrodynamic diameter and surface charge of SeNPs post-protein coronation were done. For deeper insights, changes in the biological behaviour of the native SeNPs after corona formation of individual proteins and their mixture were studied by measuring the cellular uptake, cytotoxicity and anti-oxidant potential.

2. Materials and methods

2.1. Chemicals

Human serum albumin, immunoglobulin-G, transferrin, cetyltrimethylammonium bromide (CTAB), sodium dodecyl sulphate (SDS), brij-58, and selenious acid were procured from Sigma Aldrich (India). Ascorbic acid was purchased from SD Fine Chemicals Ltd. (India). All the glassware were cleaned thoroughly with aqua regia solution (HCl: $HNO_3 = 3:1$) followed by rinsing with milli-Q water (18.2 Ω) and dried in a hot-air oven. 2,2-diphenyl-1-picrylhydrazyl (DPPH), Dulbecco's modified Eagle's Medium (DMEM), fetal bovine serum (FBS), antibiotic and antimycotic solution, 10× phosphate buffered saline, trypsin, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), and dimethyl sulphoxide were brought from Himedia laboratories, India, for performing the in vitro studies and measurement of antioxidant potential.

2.2. Synthesis of functionalized SeNPs

Selenious acid was used as a predecessor for the synthesis of selenium nanoparticles. Firstly, 0.03 M of selenious acid was dispersed in 25 mL of water. Then, 0.5% aqueous solution of $NaBH_4$ was added to the above solution drop by drop. The finally prepared mixture was orange red in colour. This mixture was allowed to stand for 24 h at room temperature. The final solution was dried and collected for further analysis. The synthesis of surfactant-coated selenium nanoparticles was performed similar to bare nanoparticles; the only difference is the addition of 0.05 M aqueous surfactant solution of Brij-58, SDS, and CTAB in 0.03 M selenious acid and 0.5% aqueous solution of $NaBH_4$ in 25 mL of distilled water [20–24].

2.3. Analysis of hard and soft corona on functionalized SeNPs

All the protein solutions (HSA, IgG, and transferrin) were freshly prepared in phosphate buffer (10 mM, pH – 7.4), and the functionalized SeNPs were sonicated using a probe sonicator (30 min). A lower concentration of SeNPs was taken as compared to the high physiological concentration of serum protein in order to simulate conditions presented during several biological applications. SeNPs (10 ppm) and protein solution with fixed final concentration of 40, 12, and 3 mg/mL for HSA, IgG, and transferrin, respectively, were mixed together in a volume ratio of 1:1 and incubated for 4 h at 37 °C [13]. Following this, each sample mixture was centrifuged four times at 9000 rpm for 10 min. The supernatant was collected, and the pellet was resuspended in phosphate buffer each time. The resuspended sample was then mixed with 2× lammeli buffer in a volume ratio of 1:4 and incubated at 90 °C for 5 min and centrifuged again [11]. The amount of unbound protein in the supernatant was quantified after first centrifugation step and used for analysing the varied adsorption pattern of proteins on different SeNPs. Supernatants obtained from the two subsequent centrifugation were quantified for soft corona, and after treatment with 2× lammeli buffer and another round of centrifugation for hard corona. The protein content in the supernatant collected after every centrifugation step was quantified by adding Bradford reagent and reading the absorbance at 595 nm. The functionalized SeNPs without any protein solution was treated as the control.

2.4. Cellular uptake study of coronated SeNPs

MDA-MB-231(Human mammary gland carcinoma) cell lines were brought from National Cell Centre Science (NCCS), Pune, India. Confluent cells were obtained by maintaining under sterile conditions in 25 cm^2 culture flasks, in 5% CO_2 incubator at 37 °C. Further for cell culture, DMEM medium containing 10% FBS and 1% antibiotic and antimycotic solution was used. Trypsinised cells were mounted on a haemocytometer, after which viable cells in the growth phase were counted using tryphan blue dye exclusion method. The cells were further allowed to reach confluency by seeding it on a 6-well plate at a cell concentration of 4×10^5 cells per well [25]. A 24-h treatment of the cells with phosphate buffer (control), functionalized SeNPs, and coronated functionalized SeNPs was done. The cells were rinsed with PBS to remove any unbound particles and trypsined. For cellular uptake analysis, trysined cells were acid digested, and the concentration of selenium was analysed using AAS at an analytical wavelength of 196 nm [26, 27].

2.5. Cytotoxicity assessment of coronated SeNPs

Trypsinized viable cells counted by tryphan blue dye exclusion method were seeded on 96-well plate at a cell concentration of 1×10^4 cells per well [11]. A 24-h treatment of the cells with phosphate buffer (control), functionalized SeNPs, and functionalized SeNPs with protein corona was done. The effect of the respective treatments on cell viability was assessed by MTT assay. The cells were further treated with MTT (5 mg/ mL) and incubated for 4 h in CO_2 incubator for the development of purple-coloured formazan product. The intensity of the purple colour formazan formed was read at 590 nm using an ELISA plate reader after solubilising it with DMSO (50 μL).

2.6. Antioxidant assay

2.6.1. DPPH assay

Radical scavenging activity of SeNPs before and after corona formation was evaluated by the method developed by Anna et al. (2011) using DPPH assay [28]. 1 mM DPPH was freshly made in methanol

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