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Colloidal stability of gold nanorod solution upon exposure to excised human skin: Effect of surface chemistry and protein adsorption

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ABSTRACT

In this study, we evaluated the colloidal stability of gold nanorods (with positive, negative and neutral surface charge) in solution upon contact with excised human skin. UV–vis absorption, plasmon peak broadening index (PPBI%) and transmission electron microscope analysis were used to follow nanoparticles aggregation in solution. Our results show that positively charged gold nanorods aggregate extensively upon exposure to excised human skin compared to negatively and neutrally charged gold nanorods. Skin-induced aggregation of cationic gold nanorods was linked to the adsorption of proteins released from the dermis layer to the surface of gold nanorods. Protein adsorption significantly screen nanorod's effective surface charge and induce their aggregation. Moreover, we demonstrate that the presence of polyethylene glycol polymer on the surface of cationic gold nanorods minimize this aggregation significantly by providing steric repulsion (non-electrostatic stabilization mechanism). This work highlights the importance of evaluating the colloidal stability of nanoparticles in solution upon contact with skin, which is a "usually overlooked" parameter when studying the nanoparticle-skin interaction.

1. Introduction

Understanding the effect of shape, charge, and surface chemistry of nanoparticles on their penetration into skin and hair follicles is crucial to engineer efficient nanotherapeutics and has been the target of several recent studies. However, the colloidal stability of nanoparticle solution upon exposure to skin is usually ignored in many published reports evaluating the nano-skin interface (Table S1, Supplementary data). This overlooked parameter contributes to the current dilemma and large debate in the reported findings regarding nanoparticles penetration and distribution into skin layers. For example, a recent study conducted by Fernandes et al. (2015) has shown that positively charged gold nanorods (GNR) penetrate the skin in larger quantity in comparison to their negatively charged counterparts. These results are contrary to those drawn by Lee et al. (2013) where they indicated that skin penetration is greater for anionic GNR compared to cationic counterparts. The colloidal stability of nanoparticle solutions exposed to skin in the above two studies, as well as for the majority of published studies, was not investigated. Clearly, a systematic evaluation of the colloidal stability of nanoparticles solution upon contact to human skin is a clear need and will enrich our understanding to the nano-skin interface.

Upon contact with biological media/compartments, nanoparticles adopt new surface properties due to the formation of "protein corona" resulting in a dramatic change in their colloidal stability and biological behavior (Mahmoudi et al., 2011; Zanganeh et al., 2016). Colloidal stability of nanoparticles in various biological media is reported including: various types of cell culture media, alveolar and lysosomal fluid, synthetic lung fluid, blood, serum and plasma (Urban et al., 2016). Similarly, the physiochemical properties of nanoparticles are expected to change significantly upon exposure to skin considering its anatomical complexity and the possible interaction of nanoparticles with the diverse population of released proteins and other compounds from the exposed skin (Labouta et al., 2011; Labouta and Schneider, 2013). Recently, surfactant proteins were found in epidermis, dermis, hair follicles, sweat and sebum (Kankavi, 2006). The transcriptome analysis shows that 63% of all human proteins are expressed in the skin (Waldera-Lupa et al., 2014).

In addition to their unique optical properties and promising applications, gold nanoparticle (GNPs) are frequently used as a "model nanoparticle" to understand the nano-bio interface due to the ease of their quantification, visualization in complex biologi-

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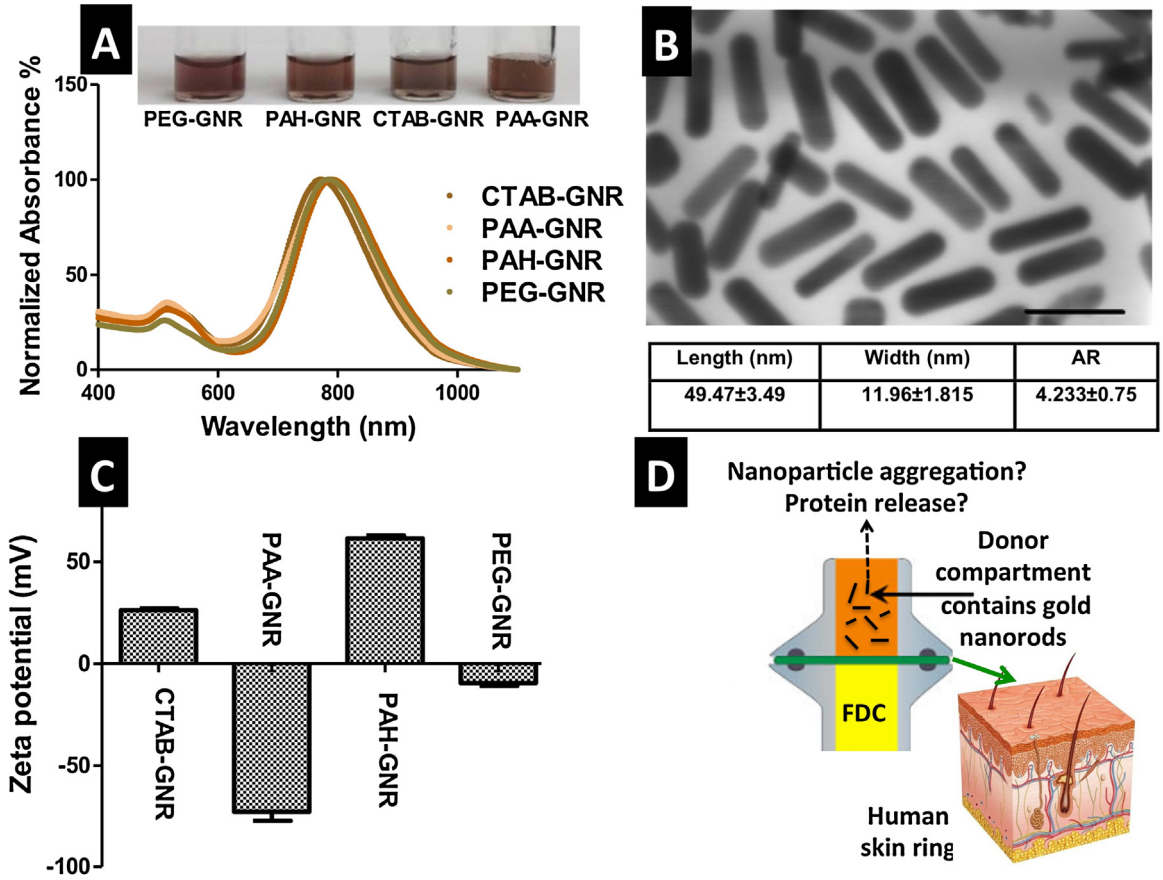

Fig. 1. Characterization of CTAB-GNR, PAA-GNR, PAH-GNR and PEG-GNR solutions. (A) UV–vis absorption spectra and optical graph of corresponding solutions as labeled. (B) TEM images (scale bar = 50 nm) and dimensions (values represent mean ± SD; $n = 150$) of CTAB-GNR. (C) Effective surface charge for purified GNR solutions as labeled. (D) Cartoon demonstrates experimental setup using FDC to evaluate GNR aggregation in solution and protein release from skin upon incubation of GNR solution with skin discs as indicated.

cal compartments and following their aggregation (Alkilany et al., 2013; Jain et al., 2008; Dykman and Khlebtsov, 2012). For example, we had reported a significant change in GNR's physiochemical properties upon exposure to cell culture media due to adsorption of serum albumin protein (Alkilany et al., 2009). Herein, we employ GNR with different effective surface charge (cationic, anionic and neutral) to evaluate the colloidal stability of nanoparticle solutions upon exposure to human skin. We also show that biomolecules (like proteins) that are released from the deeper skin layer (dermis) adsorb onto the surface of cationic GNR and play a critical role in defining the colloidal stability of nanoparticles upon contact with human skin. Finally we show that pegylation of cationic GNR prevent the nanoparticle aggregation by providing steric repulsion (non-electrostatic stabilization mechanism).

2. Materials and methods

2.1. Materials and instrumentation

Chloroauric acid ($HAuCl_4{\cdot}3H_2O$, 99.9%), sodium borohydride ($NaBH_4$, 99%), silver nitrate ($AgNO_3$, 99%), ascorbic acid (99%), poly(allylamine hydrochloride), (PAH, MW ~15,000 g/mole), poly(acrylic acid, sodium salt) (PAA, MW ~15,000 g/mole), methoxypolyethylene glycol thiol (m-PEG-SH, MW ~2000 Da), cetyltrimethylammonium bromide (CTAB, 99%), bovine serum albumin, (BSA), trypsin from porcine pancreas (lyophilized powder, 1000–2000 BAEE units/mg solid), and Bradford reagent were obtained from Sigma–Aldrich Chemicals. Cystamine dihydrochloride (97%) was obtained from Acros, UK. Sodium dodecyl sulfate (SDS) was obtained from (S.D.Fine-Chem Ltd.) and 1,4-dithiothreitol (DTT) was obtained from Biochemika. Filter membrane (Spectra/Pro cellulose membrane, MWCO 6–8000 Da, Spectrum laboratories Inc., USA) was used to filter the conditioned solution. Franz diffusion cells (FDC) with 1.0 cm^2 exposed area and flat ground joint clear glass (PermeGear Inc., Hellertown, PA, USA) was used to perform stability experiments conducted on skin. All solutions were prepared with purified 18 MΩ water and all glassware were cleaned with aqua regia and rinsed with purified 18 MΩ water before use.

UV–vis spectra of GNR were collected using UV–vis spectrophotometer (Spectrascan 80D, Biotech Eng., UK) over the range from 400 to 1100 nm. Transmission electron microscopy (TEM) images were obtained using (Versa 3D, FEI, Holland) operating at 30 kV. TEM grids were prepared for imaging by drying ~10 μL of diluted purified GNR solution on Formvar coated copper TEM grids (300 mesh, Ted Pella Inc., Redding, CA). Uranyl acetate stain (Fluka AG, Chem.) was used to stain PAH-GNR-protein sample for TEM imaging. Dynamic light scattering (DLS) and zeta potential analysis was performed on Microtraczetatrac (USA).

3. Methods

3.1. Synthesis of CTAB capped GNR (CTAB-GNR)

CTAB-GNR were synthesized according to the seed-mediated surfactant-assisted wet-chemical method (Sau and Murphy, 2004). For seed synthesis, a solution of 0.25 mM $HAuCl_4$ was prepared in 0.1 M CTAB. $NaBH_4$ (0.6 mL, 10 mM) was added to 10 mL of gold-