Colloids and Surfaces B: Biointerfaces 170 (2018) 179–186

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Modulation of serum albumin protein corona for exploring cellular behaviors of fattigation-platform nanoparticles

Van Hong Nguyen[a,1], Nilesh M. Meghani[b,1], Hardik H. Amin[b], Thao T.D. Tran[c,d], Phuong H.L. Tran[e], Chulhun Park[b], Beom-Jin Lee[b,*]

[a] Pharmaceutical Engineering Laboratory, Biomedical Engineering Department, International University, Vietnam National University, Ho Chi Minh City, 70000, Vietnam
[b] College of Pharmacy and Institute of Pharmaceutical Science and Technology, Ajou University, Suwon 16499, Republic of Korea
[c] Department for Management of Science and Technology Development, Ton Duc Thang University, Ho Chi Minh City, Vietnam
[d] Faculty of Pharmacy, Ton Duc Thang University, Ho Chi Minh City, Vietnam
[e] Deakin University, Geelong Australia, School of Medicine

ARTICLE INFO

Keyword:
Bovine serum albumin
Cellular uptake
Gelatin-oleic nanoparticles
Lung cancer cells
Protein-nanoparticle interaction
Protein corona
Albumin-precoated nanoparticles

ABSTRACT

Albumin is the most abundant protein in blood, and is the most frequently identified protein in the protein corona of nanoparticles (NPs). Thus, albumin plays an important role in modulating NPs' physicochemical properties and bioavailability. In this study, the effect of bovine serum albumin (BSA) on gelatin-oleic nanoparticles' (GONs) physicochemical properties and cellular uptake were evaluated. Coumarin-6 was used as indicator to track the cellular uptake of GONs. The binding of BSA onto the GON surface increased the size, slightly reduced the negative net charge of the GON, and improved GON stability. The presence of BSA in cell culture media reduced the cellular uptake of BSA-uncoated GONs on human embryonic kidney cells 293 (HEK 293) and human adenocarcinoma alveolar basal epithelial cells (A549) in the media without FBS addition. Pre-coated BSA corona decreased cellular uptake of GONs in A549 cells in the media, with and without supplemented with 10% fetal bovine serum (FBS) but drastically increased cellular uptake on HEK 293 cells. BSA could be used to modulate protein corona as an endogenous ligand in NP design simply by mixing or incubating BSA with NPs before in vivo administration to inhibit or induce cellular uptake in specific cell types.

1. Introduction

Three main strategies for cancer treatment are surgical removal of tumor, radiotherapy and chemotherapy. Recently, research on micro and nanoparticles targeted drug delivery systems have shown tremendous progress and enhanced hope to eradicate the tumor in vivo as well as in clinical studies [1,2]. In order to increase the success rate to treat the cancer by the nanoparticles, rigorous in vitro and in vivo evaluation studies are highly desired. After in vivo administration, nanoparticles (NPs) are affected by biological environments such as the pH, blood composition, temperature, and especially, proteins. Proteins adsorbed onto the NPs' surface will create the bio-identity of the NPs, possibly changing their effectiveness. In the initial stage, proteins at high concentrations are adsorbed onto the surface; however, over time, they are replaced by higher affinity proteins, via Vroman's effect [3,4]. The protein-NP complexes, not the NPs alone, control the circulation, allocation, biocompatibility, and bioavailability of the NPs. The adsorbed protein corona on each NP is unique, since they are the combined results of numerous factors, such as the protein composition and concentration, media pH, temperature, and fluidic states; and the physicochemical properties of the NPs, such as their size, shape, net charge, and surface chemistry [5–7]. Therefore, the designed formation of an adsorbed protein layer for NPs can alter biological outcomes [8,9].

Among a large number of blood proteins, albumin is the most abundant serum protein and is a prominent protein in the protein corona of various NPs. The binding of albumin increases the NPs' size and neutralizes their charge [10,11]. Furthermore, albumin is also well known as a "dysopsonin", which inhibits complement activation, reduces phagocytosis, and improves the blood circulation time for nanomaterials [12–14]. The addition of bovine serum albumin (BSA) at 5 mg/mL has shown to reduce the internalization of polystyrene NPs to 51% compared with that without BSA [15]. At the concentration of 3 mg/mL micelles prepared from hyaluronic-oleic conjugations, the cellular uptake of cancer cell line HCT-116 of these micelles decreased 80% with the presence of albumin at 50 mg/mL [16]. Moreover,

* Corresponding author.
E-mail address: bjl@ajou.ac.kr (B.-J. Lee).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.colsurfb.2018.05.060
Received 23 January 2018; Received in revised form 3 April 2018; Accepted 26 May 2018
Available online 28 May 2018

inserting the albumin-binding domain into recombinant adenoviruses has protected them from rapid elimination out of the body [17].

We have pioneered fattigation-platform based nanoparticles which are amphiphilic in nature and have shown greater efficacy and biocompatibility in recent years. The term “fattigation” is defined as the covalent conjugation between natural proteins (gelatin, transferrin, serum albumin etc.) and fatty acids (e.g. oleic acid, stearic acid etc.) to form new amphiphilic biomaterials which retain the biocompatible characteristic of each material involved in the conjugation process [18–20]. Due to the amphiphilic nature and availability of abundant −COOH and $-NH_2$ groups of proteins and fatty acids, fattigation-platform nanoparticles have been applied for enhanced solubility of poorly water soluble drugs, higher bioavailability, targeting cancer cells and for theranostic applications by combining GON with magnetic nanoparticles (MNP) [21–23]. In this study, BSA decoration of the surfaces of GONs was tested as an easy method (simple incubation) to explore the effects on the cellular uptake of GONs. We evaluated the BSA-induced modifications of the physicochemical properties of the GONs, and its effect on the cellular uptake of GONs by HEK 293 and A549 cells, using coumarin-6 as the fluorescent label for GONs. Furthermore, BSA pre-coated GONs were prepared by simply mixing them with a BSA solution and were characterized about BSA’s “dysopsonin” functions on both cell lines in media with and without fetal bovine serum (FBS), a common protein mixture used in cell culture. The variety of the internalization of the intact GONs and pre-coated GONs on treated cells was illustrated in Fig. 1 and elucidated through our study.

2. Materials & methods

2.1. Materials

Gelatin type A bloom 300 (50–100 kDa), EDC (1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride), NHS (N-hydroxysuccinimide), oleic acid, pyrene, sodium dodecyl sulfate (SDS), 2,4,6-trinitrobenzenesulfonic acid (TNBS), and dimethylsulfoxide (DMSO) (NMR grade), 4′,6-diamidino-2-phenylindole (DAPI) and coumarin-6 from Sigma-Aldrich Co. (St. Louis, MO, USA). Ethanol (HPLC grade) from Fisher Scientific Korea (Seoul, Republic of Korea). The dialysis membrane was from Sigma-Aldrich Co. The phosphate buffer saline (PBS), Dulbecco's Modified Eagle Medium (DMEM), Roswell Park Memorial Institute (RPMI) 1640 medium, fetal bovine serum (FBS), and penicillin G sodium from Gibco, Life Technologies (California, USA).

2.2. Preparation of GONs and coumarin 6-loaded GONs

2.2.1. Gelatin-oleic conjugate (GOC) synthesis

GOC was synthesized using the method detailed in our previous study, with some modifications [18]. Briefly, 1 g of gelatin type A and 1 mL of oleic acid were added separately in 20 mL of 60% ethanol, with the addition of 1 M NaOH to completely dissolve them. EDC and NHS were added to the oleic acid solution to activate the acid group. The activated oleic acid solution was then mixed with the gelatin solution, followed by a 12 h incubation. The resultant solution was purified by dialysis against water for 48 h.

2.2.2. GON preparation

GOC (5 mg/mL) in 60% ethanol was added drop wise into water to generate GONs at 37 °C, with stirring at 500 rpm for 4 h to evaporate the ethanol in the mixture. After centrifugation at 12000 rpm (2x), for 15 min, the GONs were collected at a concentration of 3 mg/mL in water and stored at 4 °C.

2.2.3. Preparation of coumarin 6-loaded GONs

GONs (4 mg) were incubated with 20 µg of coumarin 6 for 6 h at 4 °C. The coumarin 6-loaded GONs were collected by centrifugation at 12000 *g* for 10 min, followed by redispersal in phosphate buffered saline (PBS) for the cellular uptake experiment.

2.2.4. Preparation of BSA pre-coated GONs

BSA adsorption onto the coumarin 6- loaded GONs was performed by incubating 4 mg GONs with 10 mg/mL BSA at 4 °C for 1 h, followed by centrifugation at 12000 rpm, 15 min to collect the BSA pre-coated GONs. The amount of BSA adsorbed onto GONs’ surfaces was evaluated using UV spectroscopy [24].

2.3. Physicochemical characterization of the GONs

2.3.1. Size and zeta potential characterization

GONs were incubated with different BSA concentrations (0.1, 0.3, and 0.5 mg/mL) in PBS for 1 h at 37 °C. The average particle size, size distribution (polydispersion index, PDI), and zeta potential of the GONs and GON-protein complexes were measured using an electrophoretic light scattering (ELS) spectrometer (Otsuka, Japan).

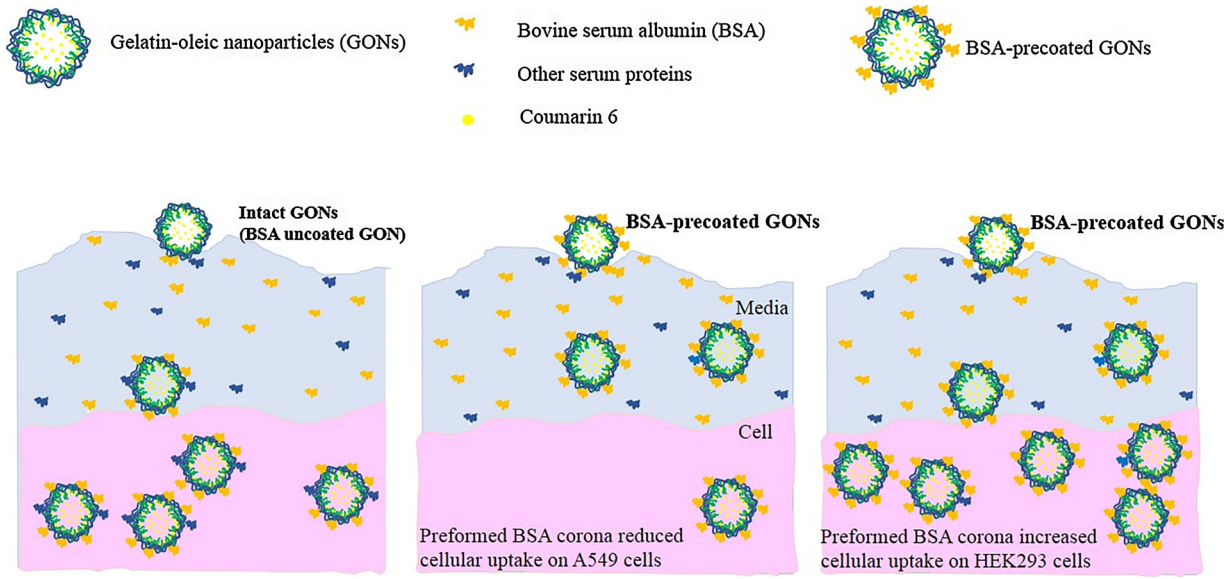

Fig. 1. Schematic mechanism of cellular uptake of NPs in presence of BSA protein corona for A549 and HEK 293 cells.