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Preformed albumin corona, a protective coating for nanoparticles based drug delivery system

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ABSTRACT

The non-specific interaction between nanoparticles (NPs) and plasma proteins occurs immediately after NPs enter the blood, resulting in the formation of the protein corona that thereafter replaces the original NPs and becomes what the organs and cells really see. Consequently, the in vivo fate of NPs and the biological responses to the NPs are changed. This is one substantial reason for the two main problems of the NPs based drug delivery system, i.e. nanotoxicity and rapid clearance of NPs from the blood after intravenous injection. Here, we demonstrate the successful application of the preformed albumin corona in inhibiting the plasma proteins adsorption and decreasing the complement activation, and ultimately in prolonging the blood circulation time and reducing the toxicity of the polymeric PHBHHx NPs. Since the interaction of proteins with various nano-materials and/or -particles is ubiquitous, pre-forming albumin corona has a great potential to be a versatile strategy for optimizing the NPs based drug delivery system.

1. Introduction

The rapid development of nanotechnology offers nanoparticles (NPs) a great application potential in various fields, like drug delivery and materials [1,2]. It is believed that NPs will become a leading vehicle for disease diagnosis and therapy in the future. In the past decades, the research reports on NPs application in drug delivery have been well documented [3–9]. Nevertheless, big challenges, like the non-specific proteins adsorption, short circulation time and toxicity, still exist and limit the practical use of NPs.

It is well known that the small size of NPs confers it some beneficial properties, like the improved solubility, the lower dose and the enhanced bioavailability [10,11]. But that is also the small size that confers NPs a very large surface-to-volume ratio and thus leads to some undesired results after NPs enter the body. It has been demonstrated in lots of research reports that NPs will be coated by hundreds if not thousands of plasma proteins immediately after their entry into the blood, forming the so-called "protein corona" [12–18]. The protein corona, undoubtedly, will mask the original properties of NPs and become the substance our organs and cells really see. Consequently, the in vivo fate of NPs and the biological responses are altered. This is probably the main reason why the NPs show good results in vitro but become worse or even non-effective in vivo in many cases. Among the identified composition of the protein corona, there are two proteins also called opsonins (i.e. immunoglobulin (IgG) and complement) that play important roles in opsonization [19,20]. Their adsorption onto the NPs surface will promote the uptake of NPs by the mononuclear–phagocyte system since there are their corresponding receptors expressed on the phagocyte surface. That is one substantial cause for the rapid clearance of NPs from the blood circulation after intravenous (i.v.) injection. In addition, the conformation and function of certain proteins are changed following adsorption onto the NPs, finally resulting in the toxicity [12,21–23].The rapid clearance and toxicity have been the obstacles for the wide and practical use of NPs.

It is not hard to see according to the description above that the uncontrollable plasma proteins adsorption is the underlying reason for the rapid clearance and toxicity of NPs (Scheme 1A). Therefore,

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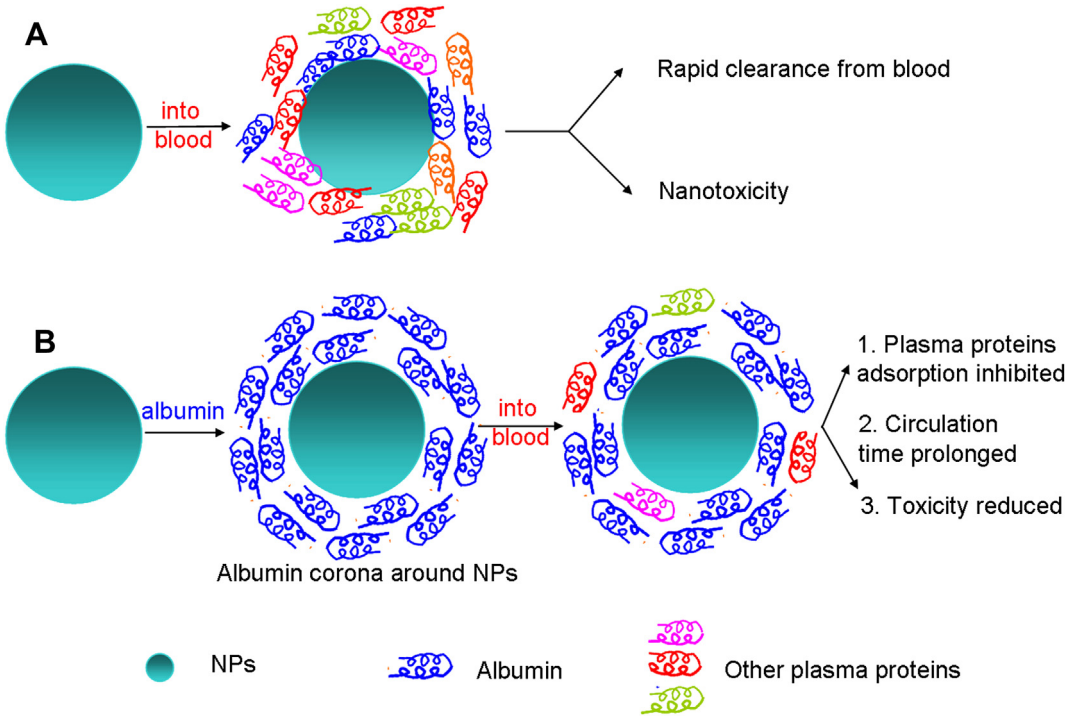

Scheme 1. Schematics of the biological responses to NPs in the absence or presence of albumin corona. A) Various plasma proteins are adsorbed onto the naked NPs immediately after its entry into the blood resulting in the rapid clearance and toxicity. B) The preformed albumin corona around NPs is able to inhibit the plasma proteins adsorption and thus prolong the circulation time and reduce the toxicity of NPs.

inhibition of plasma proteins adsorption onto the NPs may be an effective strategy to solve the above problems. Although this is a big challenge since the NPs–proteins interaction is complicated and unavoidable, opportunities still exist if such interaction can be utilized properly. In recent years, the NPs–proteins interaction has become a research hotspot and lots of related papers can be retrieved [16,24,25], but few of them focus on how to utilize such interaction to optimize the NPs based drug delivery systems. In order to achieve this, one optimal protein is needed to interact with NPs and form a pure protein corona in advance. Albumin, the most abundant protein in the blood, plays important roles in maintenance of plasma colloid osmotic pressure, transportation and detoxification, and has been developed as a drug carrier due to its biocompatibility, non-toxicity, ready availability and long circulation time [26]. In addition, albumin is an always-found component in the plasma protein corona surrounding NPs [18–20,27,28].

Therefore, inspired by the non-specific interaction of NPs with proteins and the great potential of albumin in drug delivery, we attempt to prepare the NPs–albumin complex via forming the pure albumin corona surrounding NPs in the absence of competitive adsorption from other proteins. The preformed albumin corona, serving as a protective coating for NPs, is able to inhibit the plasma proteins adsorption, prolong the circulation time and reduce the toxicity (Scheme 1B).

2. Materials and methods

2.1. Materials

Poloxamer188 (F68) was kindly donated by BASF (China) Co. Ltd. (Shanghai, China). Sodium deoxycholate (DOC-Na) and bovine serum albumin (BSA) were supplied by Amresco (Ohio, USA). Poly-3-hydroxybutyrate-co-3-hydroxyhexanoate (PHBHHx, M_w = 175,000) containing 14 mol% of R-3-hydroxyhexanoate (HHx) was kindly donated by Lukang Group (Shandong, China). Coumarin-6 (C6) was purchased from Sigma (St. Louis, USA). All other chemical reagents were of analytical grade or better.

2.2. Animals

The healthy male Sprague–Dawley rats (200–260 g) were purchased from Laboratory Animal Centre of Sichuan University (Chengdu, China). All the animal experiments were approved by the Institutional Animal Care and Ethic Committee of Sichuan University. The rats were housed in cages (5 rats per cage) under the controlled conditions (~25 °C, ~55% air humidity) with free access to rat food and tap water. Prior to use they were acclimatized for at least 7 days.

2.3. Preparation of PHBHHx nanoparticles

In this work, a biopolymer PHBHHx was utilized to prepare NPs according to our previous work [4]. Briefly, PHBHHx was dissolved in chloroform as an organic phase. An aqueous phase containing 0.5% (*v*/*v*) of F68 and 0.5% (*v*/*v*) of DOC-Na was mixed with the above organic phase at a volume ratio of 20:1, followed by sonication for 30 s. The PHBHHx NPs suspension was obtained by evaporating the resulting emulsion at room temperature (RT) for 20 min. When preparing coumarin-6 loaded PHBHHx NPs (C6–NPs), the C6 and PHBHHx were co-dissolved in chloroform, and the following procedures were the same as above.

2.4. Physicochemical characterization of PHBHHx NPs

The particle size, size distribution and zeta potential (ZP) of PHBHHx NPs were measured using dynamic light scattering (DLS) and electrophoretic light scattering (ELS) technologies, respectively, in the instrument of Zetasizer Nano ZS90 (Malvern Instruments Ltd., U.K.). The particle size was presented by intensity distribution, and the size distribution was evaluated by polydispersity index (PDI).

The morphology of NPs was observed by the scanning electron microscopy (SEM, INSPECT F, FEI, Netherlands) and the atomic force microscopy (AFM, SPM9600, Shimadzu, Japan). The freshly prepared NPs were diluted with distilled water by 300–500 folds, one drop of which was placed on the mica sheets. After air-drying, 1) the NPs loaded mica sheets were subjected to AFM directly; 2) the mica sheets were coated with gold before SEM.

2.5. Formation and characterization of NPs–BSA complex

In this present work, the bovine serum albumin (BSA) was used to form the albumin corona. The BSA corona around PHBHHx NPs was formed by incubating the NPs with BSA solution (20 mg/ml in physical saline) at 4, 37 °C or room temperature (RT). At fixed time intervals, the formed NPs–BSA complex was separated by centrifugation (16 krcf, 5 min). The non-adsorbed BSA left in the supernatant was quantified by fluorescent spectrometry (Ex: 280 nm; Em: 332 nm) [29], and the BSA