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Implications of protein corona on physico-chemical and biological properties of magnetic nanoparticles

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ABSTRACT

Interaction of serum proteins and nanoparticles leads to a nanoparticle–protein complex formation that defines the rational strategy for a clinically relevant formulation for drug delivery, hyperthermia, and magnetic resonance imaging (MRI) applications in cancer nanomedicine. Given this perspective, we have examined the pattern of human serum protein corona formation with our recently engineered magnetic nanoparticles (MNPs). The alteration in particle size, zeta potential, hemotoxicity, cellular uptake/cancer cells targeting potential, and MRI properties of the MNPs after formation of human serum (HS) protein corona were studied. Our results indicated no significant change in particle size of our MNPs upon incubation with 0.5–50 wt/v% human serum, while zeta potential of MNPs turned negative due to human serum adsorption. When incubated with an increased serum and particle concentration, apolipoprotein E was adsorbed on the surface of MNPs apart from serum albumin and transferrin. However, there was no significant primary or secondary structural alterations observed in serum proteins through Fourier transform infrared spectroscopy, X-ray diffraction, and circular dichroism. Hemolysis assay suggests almost no hemolysis at the tested concentrations (up to 1 mg/mL) for MNPs compared to the sodium dodecyl sulfate (positive control). Additionally, improved internalization and uptake of MNPs by C4-2B and Panc-1 cancer cells were observed upon incubation with human serum (HS). After serum protein adsorption to the surface of MNPs, the close vicinity within T_1 (~1.33–1.73 s) and T_2 (~12.35–13.43 ms) relaxation times suggest our MNPs retained inherent MRI potential even after biomolecular protein adsorption. All these superior clinical parameters potentially enable clinical translation and use of this formulation for next generation nanomedicine for drug delivery, cancer-targeting, imaging and theranostic applications.

1. Introduction

Recent advances in nanomedicine have prompted the use of various nanoparticle (NP) formulations for basic and clinical cancer therapeutics. Although 92,000 studies involving NPs are present in the literature, very few NP formulation(s) are in human clinical trials or involve human use. The primary hurdle is a lack of clear indicative synthesis and chemical modification approaches for *in vivo* human applications. Additionally, there is a remarkable gap and limited understanding of the physico-chemical properties of NPs in the physiological system [1]. Physiological conditions influence the interaction of biological systems with NPs, which can describe the fate and biosafety of NPs [2–4]. This information particularly reveals the sustained circulation phenomenon of NPs or possible clearance mechanism by the immune system. Thus, clinical translation and success of NPs will depend on key interactions with human proteins [2,3,5].

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Magnetic nanoparticles (MNPs) have been widely used for theranostic applications due to their multi-functionality, i.e., drug delivery, cell labeling, hyperthermia and magnetic resonance imaging (MRI) properties [6,7]. However, most of the MNPs are rapidly engulfed due to their aggregation by the mononuclear phagocyte system, which quickly processes them for clearing and degrading upon intravenous administration. This process not only lowers the therapeutic dose at a disease site but also induces inflammation, hampers the host defense mechanism, and sheds MNPs to un-targeted areas in the body [8–10]. These events eventually lead to accumulation at un-targeted areas and impose possible toxicity concerns [11]. Thus, synthesis of highly stable and dispersible MNPs for systemic administration is highly desirable [6,12].

Custom made, multi-layered, and multi-functional MNPs can improve the potential impact of treatment and diagnosis at the tumor site [13–15]. Our recent studies demonstrated that dual layered drug loaded MNP formulations have shown superior anti-cancer, imaging and targeting capabilities [16–19]. Viewing the translation potential, we engineered a dual layer MNP formulation which showed improved bioavailability, biocompatibility and therapeutic potential to effectively load therapeutics in cancer cells [18]. The first cyclodextrin layer depot loaded therapeutic drugs [20,21] and the second pluronic polymer (F127) layer acted as a repelling hydrophilic polymer to enrich biological performance [22,23]. F127 polymer (outer layer, polyethylene glycol chains) coating on nanoparticles has been thought to decrease recognition by phagocytic cells of the reticulo endothelial system (RES). Additionally, binding of F127 pluronic polymer(s)/PEG chain-coated nanoparticles led to less adsorption of plasma proteins and their significant role on the protein corona has been elucidated [24]. The adsorption of blood serum protein components on MNPs and interactions with phagocytes can modify the size, aggregation state, and interfacial composition and thus offer a distinct “biological identity” [25]. In addition, a clear picture of the interaction mechanisms between dual layered coated MNPs and human serum proteins is currently missing. Identification of this nanomaterial–protein complex is crucial to understanding the uptake mechanisms, biodistribution, and clearance of nanoparticles. Therefore, the aim of this study is to identify the interaction of serum proteins and MNPs; and the subsequent influence of their complex on the inherent physico-chemical properties of MNPs, such as, their uptake by cancer cells, cyto-compatibility, and influence on clinically relevant properties for drug delivery applications.

2. Materials and methods

2.1. Materials and methods

All the chemicals and reagents used in this study were purchased from Sigma Chemical Co. (St. Louis, MO) unless otherwise stated. Magnetic nanoparticles were prepared using our previous protocol [18]. Whole human serum (HS) and red blood cells of a healthy male were purchased from Biological Specialty Corporation (Colmar, PA) and used within a month.

2.2. Human serum protein binding to MNPs

In a typical experiment, 300 μg MNPs were incubated in 0.5–50 wt./v.% HS (total volume 600 μL) for 24 h at 37 °C. The solutions were centrifuged at 12,000 rpm for 15 min (twice) to recover human serum protein bound MNPs (HS@MNPs) samples as pellet. The recovered human serum proteins bound MNPs in the pellet were denoted as HS0.5@MNPs, HS1@MNPs, HS2.5@MNPs, HS5@MNPs, HS10@MNPs, HS20@MNPs, HS30@MNPs, HS40@MNPs, and HS50@MNPs when MNPs were incubated with 0.5, 1, 2.5, 5, 10, 20, 30, 40, and 50% human serum, respectively. The HS@MNPs samples were immediately lyophilized using the Labconco Freeze Dry System (−48 °C, 133 × 10^{-3} mBar; Labconco, Kansas City, MO). The lyophilized HS@MNPs samples were stored at 4 °C until analyzed. To examine the HS protein adsorption on MNPs, we performed Fourier transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD), and thermogravimetric (TG) analyses [3].

2.2.1. XRD

The XRD spectral acquisition of vertically mounted HS@MNP samples on carbon tape was recorded using a Rigaku D/Max-B diffractometer (Rigaku Americas Corp, Woodlands, TX) with cobalt-alpha radiation at λ = 0.1546 nm operating at 40 kV and 40 mA [16–19].

2.2.2. FTIR

FTIR spectra of HS@MNPs samples were obtained using a Smiths Detection IlluminatIR FTIR microscope (Danbury, CT) equipped with a zinc selenide crystal ATR accessory. The spectra were collected from an average of 32 scans for each HS@MNP sample from 500 to 4000 cm^{-1} with a resolution of 4 cm^{-1} [16–19]. However, FTIR data was presented from 600 to 1800 cm^{-1}.

2.2.3. TGA

Thermogravimetric analysis (TGA) of HS@MNPs samples was performed using a Perkin Elmer Simultaneous Thermal Analyzer STA6000 (Waltham, PA). Weight loss of MNPs, human serum and HS@MNPs samples was recorded from 25 °C to 700 °C at a heating rate of 10 °C, under a constant nitrogen gas flow (100 mL/min) [18].

2.3. Dynamic light scattering

To determine the HS protein adsorption, protein corona formation on the surface of MNPs, and stability of MNPs, we used dynamic light scattering (DLS) [26]. For this study, HS@MNPs were dispersed in PBS/different concentrations of human serum solution, and the particle size and zeta potential (ζ) (surface charge) of MNPs or HS@MNPs were measured using a Delsa™Nano C Particle Size Analyzer (Beckman Coulter, Miami, FL). Size and distribution of samples were measured for 3 min, and the zeta potential was measured for 90 runs (9 min). The data represents an average of three independent readings.

2.4. Protein adsorption and composition

HS protein binding onto MNPs was determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis [26]. For this experiment, various concentrations of whole human serum and MNP solutions in 1× PBS were used. Briefly, Protocol 1: MNPs (300 μg) were incubated in 1, 10, and 40% human serum for 0.5, 1, 2, 3 and 24 h at 37 °C; Protocol 2: 30, 60, 120, 180, and 300 μg MNPs were incubated in 10% human serum for 24 h at 37 °C; Protocol 3: 300 μg MNPs were incubated in 1, 2.5, 5, 10, 20, 30, 40, and 50% human serum for 24 h at 37 °C. In all three incubation protocols, the total volume of HS@MNPs solution was maintained at 600 μL. After completion of the incubation period, the unbound HS proteins (supernatant) and the HS protein bound MNPs (palette) were separated in the centrifugation process (twice, 12,000 rpm for 15 min). The HS@MNP samples were solubilized in an electrophoresis 2X SDS sample buffer (Santa Cruz Biotechnology, Santa Cruz, CA). The HS proteins bound on MNPs were separated and denatured by heating to 95 °C for 5 min. The bound HS protein samples were further separated by size in the 4–20% 1D SDS-PAGE in an electric field using electrophoresis at a constant voltage of 150 V for 60 min. All the samples/gels were run in triplicate. These SDS-PAGE gels were processed for SimplyBlue™ SafeStain (Coomassie® G-250 stain, Invitrogen, Carlsbad, CA), and images were scanned using a BioRad Gel Doc (BioRad, Hercules, CA) [26].

2.5. Steady-state fluorescence spectroscopy

Steady-state fluorescence spectroscopy was used to monitor the instantaneous adsorption and binding efficiency of serum protein on MNPs [19,27]. These measurements were conducted using a SpectraMax Plus 384 plate reader (Molecular Devices, Sunnyvale, CA). Intrinsic fluorescence of 1 and 2% human serum solution in 1X PBS was measured by titrating with 0–100 μg/mL MNPs. Fluorescence decay profiles were obtained by excited human serum solution at 295 nm, and the emission was set from 280 to 420 nm. Fluorescence quenching occurred due to interaction between MNPs and tryptophan units of human serum proteins. The binding constant and number of binding sites (n) were derived from the following equation:

$$\frac{Fo - F}{Fo - Fs} = \left[\frac{MNPs}{Kdiss}\right] n$$

where *Fo*, *F* and *Fs* are the fluorescence area under the curve of emission of human serum, human serum titrated with various concentrations of MNPs, and human serum saturated with MNPs, respectively. The binding constant and number of binding sites were calculated following our previous reports [19]. The final values are reported as an average of three independent measurements.

2.6. CD spectra

Circular dichroism (CD) spectroscopy was used to elucidate secondary structural changes that may have occurred in human serum proteins during their instantaneous interaction with MNPs [27]. For this study, CD measurements were performed using a Jasco 815 CD spectrometer (JASCO International Inc., Ltd. Tokyo, Japan). All the differential absorption measurements were acquired between 200 and 260 nm