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Research Paper

# Comparative examination of adsorption of serum proteins on HSA- and PLGA-based nanoparticles using SDS–PAGE and LC–MS

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ABSTRACT

The behavior of nanosized drug carrier systems under cell culture conditions and therefore also the destiny in the body are highly influenced by the protein corona, which is formed upon entering a biological environment. Some of the adsorbed proteins, named opsonins, lead to a shortened plasma circulation half-life of the nanoparticles. Others are attributed to promote the transport of nanoparticles into other compartments of the body, just to mention two examples. Hence, detailed knowledge concerning the composition of the protein corona is of great importance. The aim of this work was to investigate the influence of the nanoparticle starting material and the surface modification on the composition of the adsorbed serum proteins in a cell culture environment. Therefore, positively charged nanoparticles based on the biodegradable polymer poly(DL-lactide-co-glycolide) (PLGA) stabilized with didodecyldimethylammonium bromide (DMAB) and negatively charged nanoparticles based on human serum albumin (HSA) were prepared and modified with hydrophilic polymers. By incubating the nanoparticles with fetal bovine serum (FBS) the adsorption of serum proteins on the colloidal system was investigated. Using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) a semi-quantitative analysis of the protein corona was performed and after enzymatic in-solution-digestion the adsorbed proteins were identified using high resolution LC–MS. Our study accentuates the influence of the core material, surface charge, and surface modification on the amount and nature of the adsorbed proteins. The combination of SDS–PAGE and LC–MS turns out to be a simple and reliable method to investigate the protein corona of nanoparticles.



## 1. Introduction

Biodegradable nanoparticles are promising drug carrier systems for the treatment of many serious diseases, particularly cancer. Numerous advantages may accompany with the use of nanocarriers such as enhanced bioavailability, controlled release, and reduced toxicity [1]. A special feature of nano sized carrier systems is their large surface-to-volume-ratio resulting in a high surface energy [2]. In order to reduce this energy, nanoparticles dispersed in a biological medium interact with biomolecules [3]. Hence, after application into a biological environment nanoparticles are immediately covered by proteins [4]. This protein corona changes the original properties of the particles giving them a biological identity which affects the physiological response namely biodistribution, kinetics, and toxicity [5]. Finally, it is not the nanoparticle itself but the nanoparticle-protein-complex that interacts with cells [6]. Thus, thorough understanding of the relationship between the physicochemical properties of the prepared drug carrier system, the resulting protein corona, and the physiological response is essential in the research and development of colloidal drug carrier systems [5].

Protein adsorption on the surface of nanoparticles is a dynamic process. The composition of the protein corona changes continuously [7]. In a first time frame, proteins with a high plasma concentration adsorb onto the surface of the nanoparticles. In a second step, these proteins can be replaced by proteins having a higher binding affinity [4,8]. This phenomenon of competitive adsorption depending on incubation time, protein concentration, and affinity of adsorption is called "Vroman-effect" [9,10]. Typically, the abundant proteins albumin, immunoglobulin G and fibrinogen adsorb in the early phase and are then replaced within seconds by apolipoproteins and complement factors [5]. Proteins, that adsorb with high affinity and show slight tendency to desorb, form the so-called hard corona. However, the soft corona is composed of loosely bound proteins,

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which tend to desorb [11]. The long-lasting hard corona seems to be of greater relevance for physiological processes [5].

The composition of the protein corona is strongly influenced by the physical and chemical properties of the particle system such as size, shape, hydrophobicity, and surface charge [10]. It is well-known that the adsorption of proteins rises with increasing hydrophobicity of the nanoparticle surface [12]. Furthermore, the surface charge is an important factor for protein adsorption. With growing charge density, the protein adsorption increases [13]. Basic proteins with pI > 5.5 prefer negatively charged surfaces while acid proteins with pI < 5.5 favor nanoparticles with mainly positive charge [5].

As well as the nanoparticle's properties affect the protein corona, the protein corona influences the destiny of the particle system in the body [10]. Besides the changes in particle size and charge, the protein corona affects the biodistribution, cellular uptake or cytotoxicity of the nanoparticles. Adsorption of some blood proteins named opsonins on the particle surface facilitates the recognition by macrophages of the reticuloendothelial system (RES), such as Kupffer cells in the liver [14]. Hence, nanoparticles are taken up and removed by the RES via phagocytosis resulting in a shortened plasma circulation half-life. Some common opsonins are immunoglobulins, complement proteins, and fibronectin [15]. Besides, some adsorbed proteins protect the nanoparticles and prevent their phagocytosis. These proteins are called dysopsonins. One of the most famous dysopsonins is albumin [16]. Furthermore, proteins can regulate cellular uptake. Apolipoprotein E is known to support the transport of nanoparticles through the blood–brain barrier using lipoprotein receptors on the surface of capillary endothelial cells [17].

In this work we compared the protein composition of several colloidal systems. The investigated nanoparticles differed in their starting materials, surface charge and modifications. Therefore, nanoparticles based on the biodegradable polymer poly(DL-lactide-co-glycolide) (PLGA) stabilized with didodecyldimethylammonium bromide (DMAB) were prepared (PLGA-DMAB-NP). These particles with positive surface charge are ideal for gene transfection and an intracellular drug delivery [18,19]. The promising increased cellular uptake is based on the electrostatic interaction between the cationic surface and the negatively charged lipid membrane [20]. A modification of the PLGA-DMAB-NP was performed by altering the surface using the hydrophilic polymer polyethylene glycol (PEG). For uncoated PLGA-NP protein adsorption results were already published [21]. The second colloidal system was based on human serum albumin (HSA). The use of HSA as starting material is associated with a number of benefits. Its biodegradable and biocompatible properties and the ability to bind a plurality of drugs, make HSA an ideal starting material for a colloidal drug carrier system. In contrast to the PLGA-DMAB-NP, HSA nanoparticles (HSA-NP) exhibit a negative surface charge. Surface modification of HSA-NP was performed using PEG reagents as well, which allows a covalent reaction with primary amino groups of the HSA nanoparticles. We analyzed the previously presented different nanoparticle systems concerning protein adsorption. Hence, fetal bovine serum (FBS), which is a common component of cell culture media, was added to the nanoparticle suspensions and the adsorbed proteins were determined. Using a combination of SDS–PAGE and high resolution LC–MS, a semi-quantitative and a qualitative analysis of the different protein coronas was possible.

## 2. Materials and methods

### 2.1. Reagents

The biodegradable polymers poly(DL-lactide-co-glycolide) (PLGA, Resomer® RG 502H; molecular weight: 12,000 g/mol [22]) and poly(DL-lactide-co-glycolide)-co-polyethylene glycol diblock (PEG-PLGA, Resomer® RGP d50155, molecular weight: 39,000 g/mol [23,24]) were purchased from Evonik Industries (Darmstadt, Germany). Human serum albumin (HSA, lyophilized powder, purity ⩾ 96%), glutaraldehyde (25% solution in water, grade I), poly(vinyl alcohol) (PVA, 30–70 kDa), didodecyldimethylammonium bromide (DMAB), DL-dithiothreitol (DTT), and iodoacetamide were obtained from Sigma (Steinheim, Germany). The amino reactive poly(ethylene glycol) (PEG) N-hydroxysuccinimide (NHS) ester, methoxy PEG succinimidyl hexanoat (m-PEG-SHA, 5 kDa), was purchased from JenKem Technology USA Inc. (Allen, Texas, USA). The active ester was kept under argon and stored at −20 °C. 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES) and fetal bovine serum (FBS) superior were obtained from Biochrom AG (Berlin, Germany). Roti®-Load 1 (8% (w/v) SDS, 20% (v/v) β-mercaptoethanol, 40% (v/v) glycerin, 0.015% (w/v) bromophenol blue) and all other substances for the performance of the sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) were purchased from Carl Roth GmbH & Co. KG (Karlsruhe, Germany). Trypsin (sequencing grade) for enzymatic digestion was delivered by Promega Corporation (Madison, USA). Urea was obtained from Merck KGaA (Darmstadt, Germany). All other chemicals were obtained from Roth (Karlsruhe, Germany).

### 2.2. Preparation of HSA nanoparticles

HSA nanoparticles were prepared by a desolvation technique as previously described [25]. HSA was dissolved in a concentration of 50 mg/mL in purified water and the pH was adjusted to 8.3. A volume of 1.0 mL of the HSA solution was transformed into nanoparticles by the continuous addition of 4.0 mL ethanol 96% (v/v) at a defined rate of 1.0 mL/min under constant stirring (550 rpm). Particle crosslinking (40%) was induced by addition of 11.6 µL 8% (v/v) glutaraldehyde in water. The crosslinking process was performed under stirring (220 rpm) of the suspension overnight at room temperature. The resulting nanoparticles were purified by three cycles of differential centrifugation (16,000g, 20 min) and redispersion of the pellet with purified water to the original volume. The resulting nanoparticle yield was determined gravimetrically and the nanoparticles were adjusted to 10 mg/mL using HEPES buffer pH 8.0.

### 2.3. Modification of HSA nanoparticles with PEG

The methoxy poly(ethylene glycol) succinimidyl hexanoat (m-PEG-SHA) was dissolved in purified water in a concentration of 50 mg/mL. An aliquot (62.5 µL) of the PEG solution was immediately mixed with 250 µL HSA nanoparticle suspension (10 mg/mL) in HEPES buffer. The sample was incubated for 2 h at 22 °C under constant shaking (Eppendorf Thermomixer comfort, Eppendorf AG, Hamburg, Germany). The resulting PEGylated HSA nanoparticles (HSA-PEG-NP) were purified by three cycles of centrifugation (16,000g, 20 min) and redispersion of the pellet with purified water. In addition, a blank sample was treated in the same way. For this purpose, 250 µL HSA nanoparticle suspension (10 mg/mL) was incubated with 62.5 µL purified water instead of m-PEG-SHA and purified as described (HSA-NP).

### 2.4. Preparation of DMAB-stabilized PLGA nanoparticles

Nanoparticles were prepared by an emulsification-diffusion method [18]. Briefly, 100 mg PLGA (Resomer® RG 502H) was dissolved in 2.5 mL ethyl acetate. The organic phase was added to a 5 mL aqueous solution containing 10 mg/mL DMAB and emulsified using a high-speed homogenizer at 15,000 rpm for 5 min. The resulting o/w emulsion was slowly poured into 5 mL purified



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