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Coating nanoparticles with tunable surfactants facilitates control over the protein corona

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ABSTRACT

Nanoparticles with long blood circulation time are a prerequisite for targeted drug delivery. To make the nanoparticles invisible for phagocytizing cells, functional moieties on the particle surface are believed to be necessary to attract specific so-called 'stealth' proteins forming a protein 'corona'. Currently, covalent attachment of those moieties represents the only way to achieve that attraction. However, that approach requires a high synthetic effort and is difficult to control. Therefore, we present the coating of model nanoparticles with biodegradable polymeric surfactants as an alternative method. The thermodynamic parameters of the coating process can be tuned by adjusting the surfactants' block lengths and hydrophilicity. Consequently, the unspecific protein adsorption and aggregation tendency of the particles can be controlled, and stealth proteins inhibiting cell uptake are enriched on their surface. This non-covalent approach could be applied to any particle type and thus facilitates tuning the protein corona and its biological impact.

1. Introduction

The intentional future use of nanomaterials as drug carriers requires precise control of their biological 'fate' after administration. A long blood circulation time of the nanomaterials is crucial for a targeted delivery with high efficiency. A key parameter for the circulation time is the so-called protein 'corona' which forms around a nanoparticle (NP) in biological fluids like human blood plasma and consists of physically adsorbed proteins [1–3]. As previously investigated by several groups, altering the pattern of the protein corona, in other words controlling the types of proteins adsorbed to a NP, can have a crucial effect on its biological fate [4,5]. More specifically, the presence of opsonins in the protein corona marks the NP to be taken up by phagocyte cells and thus be removed from the blood stream [4,6,7]. Recently, it was shown that this phagocytosis can be reduced by the enrichment of specific stealth proteins on the NP surface rather than only suppressing unspecific protein adsorption [6,8–10]. The reduction of unspecific cellular uptake of NPs is referred to as the 'stealth effect'.

Currently, the covalent attachment of polyethylene glycol (PEG) chains to the surface of a nanoparticle (NP) – the so-called PEGylation – represents the standard procedure to obtain a stealth effect for NPs. PEGylation induces a selective protein enrichment of namely clusterin and apolipoprotein A-I in the protein corona of NPs [5,11,12]. Due to their different protein corona composition, the PEGylated NPs exhibit a significantly decreased unspecific cellular uptake. However, since PEG is non-biodegradable, accumulation as well as uncontrolled oxidative degradation of PEG into toxic products has been reported [13,14]. Therefore, the use of biodegradable polymers that do not involve the danger of accumulation are favorable. Recently, the biodegradable poly(phosphoester)s were identified to induce a similar stealth effect as PEG, while preventing bioaccumulation due to eventual degradation [6]. Within the field of biocompatible polymers, PPEs occupy an outstanding position due to their tremendous

Abbreviations: NP, nanoparticle; PEG, polyethylene glycol; PPEs, poly(-phosphoester)s; SDS, sodium dodecyl sulfate; ITC, isothermal titration calorimetry; HSA, human serum albumin; DLS, dynamic light scattering; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; ACF, autocorrelation function; PBS, phosphate-buffered saline; PEEP, poly(ethyl ethylene phosphate); PMEP, poly(methyl ethylene phosphate); PEBP, poly(2-ethylbutyl phosphate); PDI, polydispersity index.

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chemical versatility provided by the pentavalency of the phosphorus center and the numerous synthetic accesses. By variation of the binding motifs, the polymer backbone and the pendant chain a precise tailoring of their properties concerning hydrophilicity/hydrophobicity and degradation behavior can be achieved [15–17]. Thus, the PPEylation, i.e. the covalent attachment of PPEs, is favorable for the modification of NPs. To prepare a PPEylated surface, the polymer chains are linked to the nanoparticle surface via the formation of covalent bonds after synthesis of the NPs and the polymers.

However, this covalent attachment requires a high synthetic effort and the process is difficult to control. For example, the NP surface needs to feature certain functionalities where PPE chains can be attached so that several synthesis steps are necessary. Therefore, we propose a non-covalent coating of nanoparticles with functional polymeric surfactants. Surfactants are widely used for the synthesis and stabilization of colloids in solution and thus are present anyway on the surface of most nanoparticle samples [18–20]. The application of polymeric surfactants only requires physical adsorption, so the step of attaching the polymer chains covalently to the surface of the NP (as for PPEylation) can be omitted. With this technique, a surface functionalization similar to PPEylation is possible, while the preparative effort will be significantly reduced.

In our study, we use nonionic poly(phosphoester)-surfactants developed in our group for nanoparticle coating. The PPE-surfactants consist of blocks with different hydro- and lipophilicity and provide steric stabilization of the nanoparticles. Their lipophilic block can adsorb to the nanoparticle surface while their hydrophilic part reaches out into the aqueous phase. The length of the different blocks can be varied and their hydrophilicity can be adjusted through variation of the side chain [15, 21]. This enables the design of specifically tailored surfactants with desired adsorption properties. Further, the controlled synthesis by anionic polymerization and the potential biodegradability as well as low toxicity of PPEs make them promising candidates for biomedical applications and the replacement of PEG [21].

The aim of this work is to illustrate the potential of applying novel PPE-surfactants adsorbed on model polystyrene (PS) nanoparticles to enable the easy tuning of the surfactant/surface properties and simultaneously obtaining a stealth behavior of the material. The binding affinity of different surfactants to PS-NPs and the stoichiometry of the coating process were determined by isothermal titration calorimetry (ITC). Further, NPs coated with the stoichiometric amount of surfactant were titrated with plasma and HSA respectively to investigate the effect of the surfactant-coating on protein adsorption. Dynamic light scattering (DLS) measurements were conducted to assess the question of surfactant detachment resulting in aggregate formation in plasma. In addition, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out to determine the protein pattern and thus the enrichment of the stealth proteins clusterin and apolipoprotein A-I. Finally, the effect on cell uptake in macrophages was determined.

2. Methods

2.1. Nanoparticle synthesis and characterization

Model polystyrene NPs were prepared by radical polymerization in miniemulsion as previously described [18,22]. Briefly, sodium dodecyl sulfate (SDS) was dissolved in deionized water. 2,2′-azobis(2-methylbutyronitrile) and hexadecane were dissolved in purified styrene. The two phases were then combined, stirred for 1 h at room temperature and homogenized by ultrasonication. The polymerization was carried out for 16 h at 72 °C. Afterwards, PS-NPs were dialyzed (MWCO 12000 g mol^{-1}) against deionized water for purification. For further details, see Supplementary Information.

2.2. Surfactant synthesis and characterization

Two surfactants (C_{18}-$PEEP_{21}$ and C_{18}-$PEEP_{78}$) were synthesized by ring-opening polymerization of 2-ethoxy-1,3,2-dioxaphospholane-2-oxide using 1-octadecanole as initiator and 1,5,7-triazabicyclo[4.4.0]dec-5-ene as catalyst. For details see Supplementary Information. Two diblock copolymer surfactants were synthesized in an organocatalytic one-pot sequential ring-opening polymerization using 2-(benzyloxy)ethan-1-ol as initiator according to a literature procedure [23]. The obtained polymers have been characterized by ^{1}H, ^{13}C and ^{31}P NMR spectroscopy as well as by size exclusion chromatography (SEC). For details see Supplementary Information.

2.3. Isothermal titration calorimetry (ITC)

ITC experiments were performed with a NanoITC Low Volume from TA Instruments (Eschborn, Germany). The effective cell volume is 170 μL and the temperature was set to 25 °C during all the experiments. A stirring rate of 350 rpm was chosen for the experiments. To correct the data by the heat of dilution, the titrant was titrated into water and the resulting heats were subtracted from each titration of NPs with the corresponding titrant. The obtained data of heat vs. molar ratio were analyzed with an independent binding model [24] using the software NanoAnalyze, version 3.5.0 by TA Instruments (for details on the model see Supplementary Information).

Details on all other involved characterization methods and cell experiments can be found in the supporting information.

3. Results

Polystyrene NPs prestabilized with sodium dodecyl sulfate (SDS) were used as a model system to study the interaction with PPE-surfactants and plasma proteins. The NPs were prepared via miniemulsion polymerization as described previously [18] and dialyzed against deionized water for 24 h for purification. Characterization with regards to size and charge was performed by dynamic light scattering and zeta potential measurements. A hydrodynamic diameter of ~ 108 nm and a narrow size distribution (PDI = 0.028, see Supplemental Fig. S1) as well as a zeta potential of −49 mV were obtained. The negative zeta potential indicates that even after dialysis there is still a certain small amount of SDS remaining in the dispersion which is necessary to ensure colloidal stability.

For surface modification of the NPs, two different classes of PPE-surfactants were studied. Surfactants L1 and L2 are the PPE-analog of Lutensol®, a widely used commercial surfactant, as the only difference in structure is the replacement of the PEG block in Lutensol® by a PEEP-block (Fig. 1 and structure S1). In these Lutensol®-analog surfactants, the hydrophobic part was not varied. In the diblock-copolymer surfactants B1 and B2, the hydrophobic as well as the hydrophilic part can be varied by adjusting the length of the respective block (Fig. 1, structure S2 and S3).

To characterize the thermodynamics of surfactant adsorption, isothermal titration calorimetry (ITC) can be applied to measure the enthalpy changes arising from the interaction between two different components directly, which makes it a very useful tool in this study. The thermodynamic parameters of surfactant adsorption on polystyrene NPs, such as association constant K_a, binding