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Influence of ligands in metal nanoparticle electrophoresis for the fabrication of biofunctional coatings

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ABSTRACT

Electrophoretic deposition of colloidal nanoparticles shows great promise for the fabrication of nanostructured surfaces, especially relevant for the surface modification of three dimensional medical implants. Here, the role of small and bulky, chemisorbent and physisorbent ligands on metal (gold, platinum) nanoparticle deposition dynamics are systematically investigated. To be able to compare ligand-coated to ligand-free nanoparticles, pulsed laser ablation in liquid is employed as nanoparticle fabrication method. Nanoparticles' electrophoretic properties are assessed via zeta potential measurements and nanoparticle tracking analysis, while online-UV–vis spectroscopy provides information about the deposition dynamics. Electron micrographs and contact angle measurements are employed to characterize the deposit. We show that ligand-free nanoparticles feature a high electrophoretic mobility and linear deposition kinetics, representing an excellent model material for controlled electrophoretic deposition. In contrast, the electrophoretic mobility of surface-modified nanoparticles is altered due to the surrounding ligand layer, resulting in less efficient deposition. Notably, electrophoretic mobility is not solely governed by the ligand's charge and does not correlate to the zeta potential values directly. Finally, bioactive nanotopographies with tunable wettability were created when depositing nanoparticles functionalized with cell-penetrating peptides. These peptide-nanoparticle bioconjugates have great potential to be used for mediating delivery via an implant surface such as a neural electrode.

1. Introduction

Nanostructured surfaces can be effectively created via electrophoretic deposition (EPD) of metal nanoparticles, even on substrates with complex shapes and with a wide variety of materials [1,2]. By applying an electric field to colloidal nanoparticles and utilizing the target surface as electrode of opposite charge, a solid deposit is formed over time. With regard to the nanoparticles, the process can be separated into three steps: particle migration in the electric field, accumulation in front of the surface and ultimate deposition on the surface. Since most commercially available nanoparticles derive from chemical synthesis, they contain stabilizing ligands, surfactant molecules and residual chemicals from the synthesis process. Consequently, electrophoresis is strongly affected by the nanoparticle's surface adsorbates, manifested in ligand barrier formation in front of the electrode and reduced deposition efficiencies [3].

The great potential of ligand-free, laser-generated metal nanoparticles for EPD was recently demonstrated [4–6], but critical parameters determining the electrophoretic mobility have not been studied yet. The highly pure nanoparticles are obtained by focusing a pulsed laser beam on a bulk metal target in aqueous environment [7–9]. The fabrication process allows to physically generate metal nanoparticles without the use of surfactants because of electrostatic stabilization through oxidized surface atoms [10,11] and subsequent ion adsorption [12]. Due to the high purity of these nanoparticles and resulting simplicity of the system, deposition parameters such as the colloidal stability, electric field strength and deposition time can be systematically investigated. Hence, optimal process parameters for the fabrication of three-dimensional coatings, even in liquid flow, have been determined for laser-generated metal nanoparticles [4].

In this article, the ligand influence on electrophoresis and deposition is addressed. Pulsed laser ablation in liquids (PLAL) is employed for gold colloid fabrication, because with this method it is possible to fabricate completely ligand-free nanoparticles as an absolute reference. Furthermore, these laser-generated nanoparticles are ideal starting materials for efficient ligand conjugation,

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because their bare surface makes them highly accessible for ligand adsorption [13]. In this regard, the coating of originally bare nanoparticles with ligand molecules allows the generation of colloids which are, besides the ligand layer, completely identical to the ligand-free reference nanoparticles. When comparing nanoparticles with and without ligands, ligand influences on the structure–function relationship of nanoparticles can be specifically addressed. Ultimately, the role of ligands on electrophoretic mobility and deposition can be quantitatively investigated.

Four representative ligand molecules were chosen and decorated on the nanoparticle surface to investigate the influence of ligand size and type of ligand attachment, i.e. physisorption and chemisorption (Fig. 1a). In the group of physisorbent ligands, citrate was selected as small, electrostatically stabilizing agent routinely used in chemical synthesis of metal nanoparticles [14]. Additionally, polyvinylpyrrolidone (PVP) was chosen as a commonly applied sterically stabilizing agent in colloid chemistry. Dihydrolipoic acid (DHLA) and the anionic sweet arrow peptide —CGGWVELPPPVELPPPVELPPP (SAP) were selected to study the role of chemisorbent ligands in electrophoresis. DHLA is a small, negatively charged ligand which attaches to the nanoparticle via two thiol groups [15]. SAP represents a more bulky thiol ligand, featuring a polyproline helix II, and belongs to the group of cell-penetrating peptides [16]. Although bio-application is not the primary objective of this study, this molecule was chosen as a representative ligand with biological function. In contrary to the large family of cationic cell-penetrating peptides, SAP belongs to the novel class of amphiphilic, anionic cell-penetrating peptides [17]. Hence, conjugates of anionic cell-penetrating peptides and inorganic particles were created for the first time in our study. The combination of cell-penetrating peptides and nanoparticles [18] allows the design of novel, nanoscale biomedical tools, which may be of interest for bio-imaging, cellular targeting and delivery applications [19–21].

With regard to nanoparticle transportation to the surfaces, electrophoretic mobility is supposed to be an important parameter, because it represents the general requirement for a charge-controlled deposition process. Therefore, we first characterized the electrophoretic properties of metal nanoparticles through zeta potential measurements and nanoparticle tracking analysis in dark field microscopy (Fig. 1b). Here, the ligand's effect is quantified based on its steric and electrostatic influence in reference to the bare nanoparticle. Second, the deposition process was monitored online through UV–vis spectroscopy, deriving kinetic data. The resulting nanostructured surface topography of substrates was evaluated via scanning electron microscopy and contact angle measurements. Platinum electrodes were chosen as substrates relevant for neural surgery [22,23] and coated with platinum nanoparticles carrying cell-penetrating peptides.

2. Material and methods

2.1. Nanoparticle fabrication by pulsed laser ablation in liquid

Nanoparticles were fabricated in ultrapure water by ablation with an Nd:YAG ns-laser (Rofin PowerLine E; wavelength: 1064 nm; pulse duration: 8 ns) using a self-designed 30 mL stirred batch chamber. The laser was operated at a repetition rate of 10 kHz and pulse energy of 385 μJ. The beam was focused by an F-theta lens (f = 100 mm) through a quartz window and a 3 mm liquid layer. By applying a galvanometric scanner (SCANcube10, Scanlab), the beam was scanned on the gold (99.99% Allgemeine Gold) or platinum target (99.99% Alfa Aesar) in a spiral pattern with an external diameter of 6 mm. The resulting colloid concentration was gravimetrically determined by weighing the metal target before and after laser ablation on an analytical balance (Precisa XR205SM-DR). Gold and platinum nanoparticle mass concentrations were approximately 150 μg/mL after 3 min ablation (productivity 90 mg/h).

Gold colloids were applied in most experiments, including zeta potential measurements, dark field microscopy for the determination of electrophoretic mobilities and online-UV–vis spectroscopy for the determination of deposition rates. In these experiments gold nanoparticles were preferred over platinum nanoparticles because of their stronger scattering intensity and their characteristic plasmon resonance in the UV–vis spectrum. In contrast, for evaluation of the ultimate deposits and surface topography (i.e. electrodes which were analyzed by scanning electron microscopy and contact angle measurements) platinum nanoparticles were employed. In this way, it was guaranteed that the substrate surface (silver electrode sputter-coated with platinum) was coated with nanoparticles of the same material, which represents a realistic application scenario [6].

2.2. Characterization of nanoparticle size distributions and ligand dose calculations

Size determinations of the colloids were conducted using an analytical disc centrifuge (DC 24,000, CPS Instruments) with a lower limit of detection of 4 nm. A sample volume of 0.1 mL was centrifuged at 24,000 rpm against a saccharose gradient and a PVC calibration standard (d = 238 nm). Average particle diameters are obtained by fitting the size distributions obtained from analytical disc centrifugation to logNormal distributions. The x_C value of the logNormal fit represents the mean particle diameter. The surface-weighted size distribution of colloidal gold and platinum nanoparticles is shown in Fig. 2. The mean surface-weighted diameter of gold colloids is 12 nm and 9 nm for platinum colloids.

Before ligand addition, nanoparticle mass concentrations were adjusted to 50 μg/mL. The applied ligands included PVP (Alfa Aesar, 58,000 g/mol), DHLA (Sigma) and citrate (Sigma). SAP (Ac-CGGWVELPPPVELPPPVELPPP-NH2, purity > 95%) was purchased from Genosphere Biotechnologies (Paris, France). The final ligand concentration was 10 μmol/L. Thus, an applied dosage of 10 μmol/L ligands corresponds to a ligand-to-nanoparticle ratio of 2104 ligands per gold nanoparticle (d = 12 nm) and 986 ligands per platinum nanoparticle (d = 9 nm). Ligand dosages were calculated from the following formulas based on spherical nanoparticles, because laser generated gold and platinum nanoparticles appear spherical in transmission electron microscopy:

$$N_{\mathrm{Ligands}} = \frac{c_{\mathrm{Ligands}} N_{\mathrm{Av.}}}{N_{\mathrm{NP,tot}}} = \frac{4\pi {r_{\mathrm{NP}}}^3 \rho c_{\mathrm{Ligands}} N_{\mathrm{Av.}}}{3\beta_{\mathrm{NP}}}$$

and

$$N_{\mathrm{NP,tot}} = \frac{\beta_{\mathrm{NP}}}{m_{\mathrm{NP}}} = \frac{3\beta_{\mathrm{NP}}}{4\rho\pi {r_{\mathrm{NP}}}^3}$$

where N_{Ligands} [#/NP] is the number of ligands per nanoparticle, $N_{\mathrm{Av.}}$ [1/mol] the Avogadro constant, c_{Ligands} [mol/L] the ligand concentration, $N_{\mathrm{NP,tot}}$ [1/mL] the nanoparticle number concentration, ρ [g/cm^3] the material density, r_{NP} [cm] the nanoparticle radius and β_{NP} [g/mL] is the nanoparticle mass concentration.

Further calculations on ligand concentrations necessary for monolayer formation on the nanoparticle surface and SAP-to-nanoparticle ratios for different SAP concentrations can be found in the supporting information (Tab. S1 and S2).

2.3. Nanoparticle electrophoresis on a single particle level

To analyze nanoparticle electrophoresis on a single particle level, a dark field microscopy setup (Leitz Orthoplan, Leica) usually

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