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Hydroxyl density affects the interaction of fibrinogen with silica nanoparticles at physiological concentration

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ABSTRACT

An increasing interest in the interaction between blood serum proteins and nanoparticles has emerged over the last years. In fact, this process plays a key role in the biological response to nanoparticles. The behavior of proteins at the biofluid/material interface is driven by the physico-chemical properties of the surface. However, much research is still needed to gain insight into the process at a molecular level.

In this study, the effect of silanol density on the interaction of fibrinogen at physiological concentrations with silica nanoparticle/flat surfaces has been studied.

Silica nanoparticles and silica wafers were modified and characterized to obtain a set of samples with different silanols density. The interaction with fibrinogen has been studied by evaluating the extent of coverage (bicinchoninic acid assay) and the irreversibility of adsorption (shift of the ζ potential). To clarify the molecular mechanism of fibrinogen/surface interactions, confocal micro-Raman spectroscopy (nanoparticles) and atomic force microscopy (wafers) were used. Finally the effect of fibrinogen on the agglomeration of nanoparticles has been evaluated by Flow Particle Image Analysis.

The data reported here show that a minimal variation in the state of the silica surface modifies the adsorption behavior of fibrinogen, which appears mediated by a competition between protein/protein and protein/surface interactions. By comparing the data obtained on nanoparticles and silicon-supported silica layers, we found that hydrophilicity increases the tendency of fibrinogen molecules to interact with the surface rather than with other molecules, thus inhibiting fibrinogen self-assembly.

This study contributes to the knowledge of the processes occurring at the surface/biological fluids interface, needed for the design of new biocompatible materials.

1. Introduction

Engineered nanoparticles (NPs) are considered to hold great promise in medicine. Due to their small size, NPs may efficiently cross the physiological barriers, thus possibly allowing more efficient and specific drug accumulation at the target site [1,2]. Functionalized organic NPs like liposomes, solid-lipid NPs (SLN), or polymeric NPs have been developed in recent years for drug delivery or imaging. Inorganic nanoparticles such as gold, iron oxides, silica or carbon nanotubes have been recently proposed for both delivery of therapeutic agents and as diagnostic tools [3–5].

Hemocompatibility is obviously of high relevance for nanovectors. Moreover, since NPs may enter into the bloodstream by translocation from the lung [6], the interaction with blood components is of high relevance also for the hazard assessment of occupationally and environmentally relevant NPs.

Plasma proteins are known to play an important role in the biological response to materials [7]. After implantation, biomaterials are rapidly covered by a dynamic layer of proteins which composition is regulated by the affinity of each protein for the surface. The formation of the layer is a competitive processes among thousands different proteins. A competitive displacement of early adsorbed proteins by other proteins with stronger affinities for the surface occurs and this exchange is commonly referred to as the "Vroman effect" [8]. The formation of a protein layer is generally unfavorable and could potentially lead to major complications, including inflammation, thrombus formation, and microbial infections. These effects are likely mediated by surface-driven conformational changes which lead the exposure of cryptic domains which, in turn, initiate adverse reactions [9]. The occurrence and the extent of these processes are thought to be related to the surface chemistry of the materials [9,10]. Properties like surface hydrophilicity, roughness, charge have been shown to modulate the adsorption

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of the different proteins on the surface and, consequently, the biocompatibility of the biomaterial [11].

The interactions with proteins play an important role also in the biocompatibility of NPs [12]. In plasma, a dynamic layer of proteins is rapidly formed [13–16]. Such a layer is composed of a core of strongly bound proteins and an outer layer of fast exchanging molecules [13,17]. Unfolding or stabilization may occur for those proteins irreversibly adsorbed at the surface [18].

In the case of NPs, the curvature of the surface needs to be considered among the determinants of the protein/NPs interaction [18–20]. Therefore, data obtained on flat surfaces should not be directly transposed to nanoparticles.

Although the understanding of the protein-surface interactions is of significant interest, the description of these processes at a molecular level is still at the early stages.

Fibrinogen (FG), one of the most abundant plasma proteins, is a flexible elongated ($47.5 \times 9 \times 6$ nm) [21] protein (340 kDa). Its structure is characterized by two identical subunits composed by three polypeptide chains. The subunits are organized in one central domain (E) and two domains (D) linked together by triple stranded α-elica coiled coils. Two further peripheral domains (αC) interact with the central domain E.

This protein has a net negative charge at physiological pH, having an isoelectric point (pI) of 5.5. However, the highest concentration of negatively charged residues resides on the E and D domains, while the αC domains, which are rich in arginine and lysine residues, are positively charged [21].

FG is a poly-functional protein. It plays a key role in blood clotting, being the precursor of fibrin. At the same time, FG participates, with fibronectin, in the stabilization of thrombus by promoting platelet aggregation through a site (RGD) involved in the interaction with integrin $\alpha_{IIb}\beta_3$ [22]. One site, located in the D domain, is responsible for the recruitment of macrophages and leukocytes through the Mac-receptor [23,24].

Conformational changes mediated by the surface of implantable medical devices are known to activate FG, leading to the exposure to cryptic sites [9]. A similar effect was recently reported to occur with coated gold NPs [25].

FG rapidly adsorbs onto inorganic oxide NPs [26–28]. However, differences in affinity and degree of unfolding have been recently reported depending upon the chemical nature of the NPs [28,29].

Amorphous silica nanopowders are employed in many different industrial fields: as additives in food and cosmetics, as reinforcing fillers for silicon and rubber, as carriers and free flow agents for many pharmaceutical and food applications, for toners or fire extinguisher powders [30].

The substantial covalence and flexibility of the Si–O bond in silica accounts for the variety of crystalline polymorphs and for the large number of amorphous silica forms. This is also reflected in the variety of surface functionalities that may be found at a silica surface: silanols (SiOH) and siloxanes (Si–O–Si), which govern hydrophilicity and hydrophobicity. The hydrophilicity of silica increases with the number of silanols, or silicon bonded hydroxyl groups, capable of forming hydrogen bonds with physical water molecules [31–33].

This study aims to elucidate the molecular mechanisms of adsorption of FG in a range of concentrations (0.5–10 g/L) including the physiological one (around 1.8–3.5 g/L) on tailored silica NPs showing different hydrophilic/hydrophobic surfaces, i.e. possessing a different ratio of surface silanols/siloxanes. A set of four surface-tailored nanostructured amorphous silica NPs have been prepared in order to assess how surface hydrophilicity drives FG adsorption. The extent of the protein coverage and the irreversibility of the adsorption process were monitored by combining adsorption curves and shift of ζ potential respectively. Silica-induced conformational changes of FG tertiary structure were investigated by Raman spectroscopy. To further investigate the silica–protein interaction, two Si/SiO_2 wafers, with high and low hydrophobic surface, have been prepared and the interaction of the hydrophilic/hydrophobic surfaces with fibrinogen investigated by means of atomic force microscopy (AFM).

2. Materials and methods

2.1. Silica nanoparticles

A hydrophilic fumed silica (Aerosil OX 50, Evonik) was used as starting material. This sample is obtained by pyrolysis of $SiCl_4$. The silica surface was cleaned to remove possible adsorbed species by a mild thermal treatment (200 °C outgassing) (S_p). The powder was then modified to produce a set of silica nanoparticles with different hydrophilicity by using the following treatments: (1) the pristine powder was stirred overnight in a 1 M solution of hydrochloric acid. ($S_{hi\text{-}1}$); (2) the pristine powder was placed into a reactor for hydrothermal synthesis with inner Teflon lining (Parr Instrument, Moline, IL – USA. Model 4748 large capacity acid digestion vessel 125 ml) loaded with ultrapure water. The bomb was heated at 240 °C for 4 h, slowly cooled to room temperature ($S_{hi\text{-}2}$); (3) the pristine powder was inserted into a quartz tube and connected to a vacuum line (residual pressure = 1×10^{-6} Torr; 1 Torr = 133.33 Pa) equipped with a liquid–N_2 cold trap and then heated at 1000 °C for 6 h (S_{ho}).

2.2. Silica substrates

Silica substrates were prepared from cleaned silicon wafers. The samples were placed in an oven and then the temperature was increased from 25 °C to 1000 °C, for 90 min, in a slow flow of air. Afterwards, the silicon wafers were kept, for 25 min, in a flow of pure oxygen, characterized by a constant vapor tension. Finally, the temperature was decreased from 1000 °C to 25 °C in a slow flow of air (SW-1).

Part of the processed silica wafer was treated to increase the hydrophilicity by exposing it to air plasma for 10 min (RF power = 25 W; water vapor pressure = 1.1 Torr) (SW-2).

2.3. Surface area measurements

The surface area of the particles was measured by means of the Brunauer, Emmett, and Teller (BET) method based on N_2 adsorption at 77 K (Micrometrics ASAP 2020).

2.4. FT-IR spectroscopy

For the FTIR measurements (Bruker IFS28; resolution = 2 cm^{-1}; DTGS detector), the silica powders, pressed into self-supporting pellets, were placed into a quartz IR cell equipped with KBr windows. Adsorbed molecular water was removed at room temperature (RT) by connecting the cell with the sample to a conventional vacuum line (residual pressure = 1×10^{-6} Torr, 1 Torr = 133.33 Pa) equipped with a liquid–N_2 cold-trap.

2.5. Contact angle measurement

To characterize the surface of the two types of silicon wafers, contact angle measurements were carried out with a drop-shape analyzer (Krüss Instruments, Germany), equipped with a charge-coupled device camera and an automatic dosing system for the liquid. Only water was employed as liquid for the analysis using the sessile droplet method.