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Amino acid-based anti-fouling functionalization of silica nanoparticles using divinyl sulfone ☆

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ABSTRACT

Natural amino acids are zwitterionic molecules and the good biocompatibility promises them potential candidates as anti-fouling materials. Here, we developed a new method to functionalize silica nanoparticles with a natural amino acid-based anti-fouling layer. Amino acids were covalently immobilized on 3-aminopropyltriethoxysilane modified silica nanoparticles using divinyl sulfone through a two-step reaction in aqueous solution at room temperature. The progress was monitored with NMR, X-ray photoelectron spectroscopy (XPS), transmission electron microscope (TEM) and zeta potential measurements. A library of amino acids was screened and the nonspecific protein adsorption of bovine serum albumin (BSA) and fetal bovine serum (FBS) were investigated using dynamic light scattering method. The results showed that cysteine, lysine and arginine functionalized silica nanoparticles can effectively resist protein adsorption due to the zwitterionic structure. Among them, lysine functionalized silica nanoparticles had the best anti-fouling performance, which showed hydrodynamic diameter increases of only 10% after incubated in BSA solution and 20% after incubated in FBS solution for 24 h. The neat aqueous modification process can conveniently create a thin zwitterionic layer on silica particles, and it has a great potential in biomolecule immobilization and biofunctional surface preparation.

Statement of Significance

Zwitterionic polymer is an outstanding class of anti-fouling material; but the difficulty in synthesis is challenging its spread utilization. In this study, we developed a new method to create an amino acid-based zwitterionic layer on APTES functionalized silica nanoparticles through a two-step reaction in aqueous solution at room temperature. The surface chemistry was monitored with NMR, XPS, TEM and zeta potential measurements. With this method, a library of amino acid conjugated-silica nanoparticles was synthesized and their anti-fouling performance was evaluated using dynamic light scattering method. The results showed that the cysteine, lysine and arginine conjugated nanoparticles all can effectively resist nonspecific protein adsorption. Among them, lysine conjugated nanoparticles show the best anti-fouling performance, which showed hydrodynamic diameter increases of only 10% after incubated in BSA solution and 20% after incubated in FBS solution for 24 hours. These results indicates that the anti-fouling silica nanoparticles are of great potential in many biomedical applications, especially biosensing and diagnose imaging. The modification reactions in aqueous solution at room temperature are easily conducted in laboratory, indicating high potential in the functionalization of silica particles/surfaces with other biomolecules.

1. Introduction

Silica (Si) nanoparticles (NPs) have attracted extensive attention due to their wide spread applications in biomedical fields including biosensing [1–3], drug delivery [4–6] and diagnostics imaging

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[7–9], because they are easily available from commercial sources with a wide size range. However, Si particle surfaces possess a high negative charge at physiological pH [10,11], which leads to rapid nonspecific protein adsorption [12]. Nonspecific protein adsorption, which would lead to NP aggregation and deactivation of the functional sites [13], is one of the major challenges to many biomedical applications.

To reduce nonspecific adsorption of biomacromolecules in complex biological systems, several materials [14,15] have been employed to create the anti-fouling capability on the NP surfaces. Among these anti-fouling materials, poly(ethylene glycol) (PEG) [16,17] has been widely used because the repeated ethoxy units can form compact hydration layer through hydrogen bond, which would provide efficient energy and steric barrier to protect protein adsorption. The commercially-availability of a variety of PEG derivatives with different functional groups makes it possible to meet the needs of surface modifications. However, recent studies found that PEG suffers from slow degradation in physiological conditions [18,19], and immune system may produce PEG-specific antibodies [20].

Zwitterionic materials [15,21–25] are a new class of anti-fouling material that has demonstrated to be efficient due to the formation of a strong hydration layer through electrostatic interaction. Zwitterionic polymers [26,27] have been used to stabilize Si NPs, providing excellent resistance to nonspecific protein adsorption. However, high molecular-weight polymer coatings lead to an increase in the hydrodynamic size of NPs [28], which may negatively impact their biomedical applications. In addition, the difficulty in the synthesis of zwitterionic polymers is challenging their utilization. Zwitterionic silane chemistry [13,29] is a straightforward strategy to produce zwitterionic surface and has been proved to be successful in anti-fouling without significantly increasing the particle size; however, strict anhydrous condition and inert gas protection were required during the silane synthesis, which is challenging the large scale NP synthesis.

Amino acids are common zwitterionic molecules in biological system and have been used as anti-fouling coating [30–32]. The conjugation of cysteine [33] on Si NPs was reported to be a successful method to create anti-fouling coating. However, few studies were reported on creating anti-fouling layers on Si NPs with other amino acids. A versatile and more efficient method for amino acid coupling is urgently needed to extend the utilization of amino acid as anti-fouling materials.

Vinyl sulfones have been widely used in bioconjugation [34–39] for its pH-dependent reactivity towards versatile nucleophiles in aqueous solutions at room temperature. It can react with mercapto group in neutral aqueous solutions while reacts with amine and hydroxyl group under basic conditions [40]. The versatile reactivity makes it a potential coupling agent for amino acid due to the abundant amine group in amino acid. And it is easy to create a vinyl sulfone-active surface on Si NP for the well-established silane coupling agents can introduce nearly any reactive functional groups (e.g., thiol, amino, epoxy, azide group and so forth) [33,41–44] that are required. With vinyl sulfone-functional groups on the surface, we speculate to conjugate amino acid on the Si NPs.

The aim of this study is to investigate the applicability of divinyl sulfone as a coupling agent to immobilize amino acids on the surface of Si NPs, and effectiveness of the resulting anti-fouling property based on the zwitterionic nature of amino acids. Herein, we prepared a series of amino acid functionalized Si NPs with a new method of conjugating amino acid onto 3-aminopropyltriethoxysilane (APTES) modified Si NPs. The step-wise modification was confirmed by ^{1}H NMR, X-ray photoelectron spectroscopy (XPS), transmission electron microscope (TEM) and zeta potential measurements. A library of amino acid conjugated NPs was prepared and the anti-fouling ability was screened. Then, the colloidal stability of the NPs in salt solutions was examined. Finally, the protein resistant ability in complex media was assessed in serum solution.

2. Materials and methods

2.1. Materials

LUDOX AS-40 (40 wt% colloidal silica in water) was purchased from Sigma-Aldrich (St. Louis, MO, USA). 3-Aminopropyltriethoxysilane (APTES, 98%), L-lysine (98%), L-cysteine (99%), L-arginine (98%), sodium phosphate monobasic (99%) and sodium chloride (99.5%) were purchased from J&K Scientific Ltd. (Beijing, China). Divinyl sulfone (DVS, 98%) was purchased from Geleixiya Chemical Ltd. (Sichuan, China). [4-(2-Hydroxyethyl)-1-piperazine] ethanesulfonic acid (HEPES, 99%) and bovine serum albumin (BSA, 98%) were purchased from Melonepharma Technology Ltd. (Dalian, China). Fetal bovine serum (FBS) was purchased from Pan Biotech GmbH. (Aidenbach, Germany). Anhydrous ethanol was ACS grade from Tedia Company Inc. (Fairfield, OH, USA). Ultrapure Millipore water with 18.2 MΩ·cm resistivity was used in all experiments.

2.2. APTES modification of Si NPs

An amount of 5 mL LUDOX AS-40 Si NPs was dispersed in 100 mL of 95% ethanol by ultrasonication and the pH was adjusted to 4.0 by addition of 1 M nitric acid. 1 mL of APTES was added to the mixture with vigorous stirring, and the reaction was allowed to proceed for 24 h at room temperature. Then the APTES modified Si NPs (APTES-Si NPs) were collected by centrifugation at 11,000 rpm for 30 min and purified through triplet washes with 30 mL of ethanol.

2.3. DVS Modification

1 g of APTES-Si NPs was dispersed in 50 mL of HEPES buffer (pH 9.5, 10 mM) containing 10% (v/v) acetone by ultrasonication. 2 mL of DVS were added and the mixture was stirred for 12 h at room temperature. Then the DVS modified Si NPs (VS-Si NPs) were collected by centrifugation at 11,000 rpm for 30 min and purified through triplet washes with 30 mL of ethanol.

2.4. Amino Acid functionalization

182 mg of L-lysine was dissolved in 20 mL of HEPES buffer to prepare lysine solution (pH 9.5, 50 mM). 200 mg of VS-Si NPs were dispersed in the lysine solution by ultrasonication, and the mixture was stirred for 12 h at room temperature. Then lysine functionalized Si NPs (Lys-Si NPs) were collected by centrifugation at 11,000 rpm for 30 min and purified through triplet washes with 10 mL of water.

Arginine, glycine and phenylalanine conjugated Si NPs (Arg-Si NPs, Gly-Si NPs and Phe-Si NPs) were prepared by dispersion of 200 mg of VS-Si NPs in arginine solution (pH 9.5, 50 mM), glycine solution (pH 9.5, 50 mM) and phenylalanine solution (pH 10, 50 mM) for 12 h. Cysteine conjugated Si NPs (Cys-Si NPs) were prepared by dispersion of 200 mg of VS-Si NPs in cysteine solution (pH 7.5, 10 mM) for 12 h. A neutral solution was used for the conjugation of cysteine to control the reaction site, because thiol groups can react with vinyl sulfone groups in neutral conditions while amine groups cannot [40]. The reaction and purification procedures were the same as those used for the preparation of Lys-Si NPs.

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