Silk Fibroin/Gold Nanocrystals: A New Example of Biopolymer-based Nanocomposites

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Abstract: The dispersion of nanoparticles in ordered polymer nanostructures can provide control over particle location and orientation, and pave the way for tailored nanomaterials that have enhanced mechanical, electrical, or optical properties. Here we used silk fibroin, a natural biopolymer, to embed gold NCs (nanocrystals), so as to obtain well-ordered structures such as nanowires and self-assembled triangular nanocomposites. Monodisperse gold NCs synthesized in organic media are mixed to silk fibroin and the obtained nanocomposites are characterized by UV-visible spectroscopy, TEM (transmission electron microscopy), FE-SEM (field emission scanning electron microscopy), AFM (atomic force microscopy) and infrared spectroscopy. The optical properties study of gold NCs and silk-gold nanocomposites shows that the surface plasmon band is blue shifted compared to gold NCs. The size and shape of NCs gold superlattices can be well controlled by the presence of silk fibroin giving nanowires and also self-assembled triangular nanocomposites as characterized by TEM, FE-SEM and AFM. The strong interaction between gold NCs and silk fibroin is also revealed by the conformation change of silk protein in presence of gold NCs, as shown by FTIR analysis. The formation of such ordered nanocomposites (gold NCs/silk fibroin) will provide new nanoplasmonic devices.

Key words: Hybrid nanomaterial, self-assembly, superlattice, protein.

1. Introduction

Tailored interaction between biomolecules and monolayer-protected NPs (nanoparticles) can be utilized to generate highly organized assemblies with a large structural diversity [1]. These hybrid superstructures combine tunable NP features with conformational flexibility of the biomolecules and, provide a potential route to build nanoscale devices. Proteins such as fibroin have the advantage of a high degree of stiffness and greater stability than DNA, largely used as a ligand for tuning NPs assemblies. In addition fibroin has an average chemical stability as demonstrated by its resistance to proteases and protein denaturants [2].

Silk fibroin is a high-molecular-weight protein consisting of a heavy (390 kDa) and a light (28 kDa) chain linked by a single disulfide bond [3]. The heavy chain accounts for up to 85% wt of the degummed silk

fibers. The heavy chain contains highly repetitive hydrophobic domains comprising GAGAGS and GA(V)GAGY amino acids forming the crystalline regions interspersed with more hydrophilic amorphous regions. In the insect gland, the native fibroin in solution undergoes a structural transition from random coil to crystalline-pleated sheets in solid silk fibers [4]. For our study we used regenerated silk fibroin from the degummed silk cocoons. Based on the properties of self-assembling, regenerated silk fibroin has been processed into a variety of material formats, such as films, electrospun fibers, three-dimensional porous scaffolds, microspheres and hydrogels, mainly for tissue engineering and cell/drug delivery applications [5]. Adsorption of this hydrophobic protein onto hydrophobic nanoparticles should be driven mainly by Van der Waals interactions [6]. Due to their low size distribution the methylated-functionalized Au NCs self organize spontaneously in 2D and 3D SLs (superlattices). The

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most frequent SL structure is FCC (face centered cubic). The morphology and structure of the SLs is known to depend on the nature of the ligands [7], their solvation [8] and on the nanoparticle size [9]. The silk fibroin bounded to the methylated-functionalized monodisperse Au NCs should act as an interparticle spacer and then should affect the self-organization of the nanoparticles resulting in different SLs morphologies via a different assembly process.

2. Materials and Methods

2.1 Chemicals

All products and solvents were used as purchased without further purification. This includes toluene (> 98%, Riedel de Haën), acetone (VWR chemicals), chloro triphenylphosphine gold (I) (98%, STREM), and 1-dodecanethiol and tert-butylamine borane complex (97%, Aldrich). The water used was purified with a Millipore system (18.2 MΩ). Salts such as LiBr (lithium bromide) and Na₂CO₃ (sodium carbonate) were purchased from Sigma-Aldrich.

2.2 Syntheses of Au Nanocrystals

Au NCs were obtained by the reduction of a gold phosphine precursor [10]. Briefly, we first dissolved 0.25 mmol of PPh₃AuCl in 25 mL of toluene, and then 500 μL of dodecanethiol was added to the solution. The metal precursor was reduced by using a solution of tert-butylamine borane complex in toluene (5 mmol dissolved in 2 mL of toluene). During the synthesis, the temperature was fixed at 100 °C. At the end of the reduction, the solution was cooled to room temperature and washed with acetone to eliminate byproducts. Finally, the Au NCs were dispersed in toluene. The average NCs diameter was 4.6 nm with 8% in size distribution as determined from TEM (transmission electron microscopy) measurements (Fig. 1).

2.3 SF (Silk Fibroin)

Lyophilized silk fibroin from Bombyx Mori

silkworm cocoons was prepared as described elsewhere [11]. Briefly the degummed SF fibers were prepared by immersing fresh cocoons in 23 mmol Na₂CO₃ aqueous solutions at 100 °C for 2 hours to remove sericin. The degummed silk was then washed with hot deionized water and dried at room temperature. The SF solution was prepared by dissolving degummed SF fibers in aqueous solution containing LiBr at 9.3 mol for 4 hours at 65 °C. The aqueous SF solution was dialyzed 5 times against deionized water using a dialysis tubing (Pierce, France) with a 3.5 kDa molecular weight cutoff. The concentration of the final silk fibroin solution was checked before lyophilisation and found to be about 2-3 wt%. Finally, the lyophilized fibroin was dissolved in toluene at a concentration of 0.5 mg/mL under gentle stirring over night.

2.4 Nanocomposites Synthesis

The nanocomposites were prepared by mixing 2 mL of the Au NCs solution at a final concentration of 4 mm with 2 mL of fibroin solution and centrifuged at 2,000 rpm for 5 minutes.

2.5 UV-Visible Spectroscopy

All absorbance measurements were performed using a Varian Cary 5,000 spectrophotometer. The concentration of Au nanocrystal solution was measured by UV-visible spectroscopy at $\lambda = 520$ nm using an extinction coefficient of 3×10^6 mol⁻¹·L·cm⁻¹. For the analysis of the nanocomposites or of Au nanocrystal, the samples were prepared by evaporation of 15 μL of the toluene solution at 2 mM in Au NCs on a 1 cm² piece of carbon/glass substrates [12].

2.6 FTIR Spectroscopy

Micro-infrared spectroscopy analyses were performed in reflection mode using a Bruker Equinox 55 spectrometer coupled to an IRscope II microscope equipped with an X35 objective. A liquid nitrogen-cooled

Fig. 1 Size distribution of the prepared Au nanocrystals calculated on 3,603 particles using Image J software.

MCT (Mercury Cadmium Telluride) detector was used, and a background on pure gold surface was collected before each sample's spectra. The 128 scans were accumulated between 4,000 and 600 cm^{-1} with a 4 cm^{-1} resolution for a window dimension of 20 $\mu\text{m} \times 20 \mu\text{m}$.

2.7 FE-SEM (Field Emission Scanning Electron Microscopy)

The FE-SEM imaging was performed with a Hitachi Su-70 instrument at an acceleration voltage of 5 kV and a working distance of about 8 mm. The carbon-glass was used as substrates.

2.8 AFM (Atomic Force Microscopy) Microscopy

For morphological characterization, an Agilent microprobe (5100 System) was used. It was operated

in non-contact mode under ambient conditions, using NSC cantilevers with resonant frequency of 118 kHz and spring constant of 5 N/m (Mikromasch, Germany). Image analysis was performed using Gwyddion software. The samples were investigated on carbon-glass supports or on highly oriented pyrolytic graphite (HOPG, Mikromasch, Germany).

2.9 TEM (Transmission Electron Microscopy)

TEM samples were prepared by depositing a drop (10 μL) of Au NCs or nanocomposites solution (2×10^{-3} M) on an amorphous carbon coated TEM grid maintained by an anti-capillary tweezers. The solvent (toluene) evaporation takes place in a glove box under nitrogen flux, at ambient temperature. The samples were then analyzed by a JEOL JEM 1011 electron microscope with an accelerating voltage of 100 kV.

3. Optical Response

The optical responses of Au NCs dispersed in solution or deposited on an amorphous carbon substrate were monitored. The UV-visible spectra of the solution displayed a strong SPR (surface plasmon resonance) band around 520 ± 1 nm caused by SPR of individual NCs, while the deposited NCs displayed a SPR band red shifted to 565 nm, due to an increased dipolar coupling (Fig. 2) [13]. Compared to the deposited Au NCs, the $\lambda_{\max}$ of the solid nanocomposites was blue-shifted at 555 nm, and the absorbance is slightly decreased for equivalent amount of Au NCs, indicating a physi-sorption between the fibroin molecules of high hydrophobicity onto the alkylated NCs. Since the refractive index of the protein, around 1.5 [14], is closed to those of the capping agent used with a refractive index of 1.45 [10], no significant effect is expected due to the change of the molecular surroundings of the Au NCs [13]. However, the use of proteins as spacer between metallic nanoparticles has been developed to tune the interparticle distance [15]. The maximum position of the LSPR band being mainly determined by interparticle distance for a given NCs size [16], the formation of hybrid system (Au NCs/silk fibroin) appears thus to be at the origin of the observed blue shift.

4. Morphology Studies

The deposit of the Au NCs by evaporation of toluene is controlled by FE-SEM (Figs. 3A and 3B). A spontaneous organization of the Au NCs (4.6 nm in diameter) Ps on amorphous carbon substrates into well-ordered multilayers films is observed in agreement with previous results [9]. In contrast when Au NCs at the same concentration were incubated with the silk fibroin, two main types of assemblies are observed with small and large fibrils and individual well-faceted triangular nanocomposites (Au NCs/silk fibroin) as shown in Figs. 3C and 3D. From the image collected by FESEM (Fig. 3C), fibrillar assemblies

indicated by arrows have an average width of 66.5 ± 16.7 nm as determined from 30 fibrils. These fibrillar forms have length varying from 1 to exceeding 10 μm , and height in the range of 15-20 nm from AFM analysis. Au NCs recovered the latter and are also inserted in the protein matrix forming nanowires as shown by the TEM image (Fig. 3E).

Larger fibrils having apparent diameter of 200 nm are also observed but in a less extent than the aforementioned nanowires (Fig. 3D). These larger fibrils are decorated by the nanowires as shown by FESEM image (Fig. 3D) and AFM images (Figs. 4A and 4B). Most intriguing is the formation of well-faceted triangular nanocomposites as observed in FESEM (Fig. 3D) and AFM (Fig. 4A). The hybrid nanocomposites with 0.5 to 2 μm long edges have height comprised between 300 nm (Fig. 4D) and exceeding 1 μm as measured from tilted SEM images (data not shown). The surface of the nanohybrids is recovered by fibroin assembled in fibrillar form and

Fig. 2 UV-Visible spectra of Au NCs in toluene at a 2 mM concentration using a 1 mm optical path quartz cuvette (plain line), of the solid Au NCs (bold line) and the fibroin-Au nanocomposites (dashed line) on carbon substrates at equivalent Au NCs concentration.

Fig. 3 FE-SEM images for the deposit of Au NCs alone at low resolution (A), at high resolution (B) and for the deposit of Fibroin-Au NCs at low resolution (C), at high resolution (D). For sake of clarity the inset in (C) corresponds to the FFT treatment of the image. Detailed TEM image (E) of the fibrils presents in the detailed FFT image in (C).

spaced by nanocrystals as depicted in FESEM image (Fig. 3D) and AFM phase image (Fig. 4B). Their well-defined shape suggests long-range order.

AFM was used to confirm the FE-SEM observations. The formation of both large fibrils of different widths and superimposed triangular nanocomposites are shown in Fig. 4A while the morphology of the fibroin alone deposited on hydrophobic substrates such as HOPG is shown in Fig. 4C.

Deposition of fibroin in low concentration in toluene yielded formation of globular shapes with height of 7.9 ± 2.6 nm and width of 140 ± 28 nm. Concerning the spatial conformation of native SF molecules only partial data are available in the literature. AFM studies were performed either on directly natural silk fibers [17] or on deposit of regenerated silk fibroin on mica or aminated silica slides [18, 19]. From the study of Huang et al [19], the fibroin deposited on aminated-surfaces revealed a transformation from rodlike aggregations 100-200 nm long to small globules 50 nm in diameter as the solution concentration decreased. A more recent study

reports dimensions of single fibroin molecule in globular shape with diameters in the range of 20-25 nm with an apparent height of around 1.2 nm from deposition of low protein concentrations onto a mica substrate [18].

5. Fibroin Conformational Change

The FTIR absorbance spectra of silk fibroin alone and of the deposit of the mixture fibroin-Au NCs are shown in Fig. 5.

The pure protein spectrum exhibits the main Amide A, Amide I, II and III bands appearing at 3280, 1650, 1530 and 1234 cm^{-1} , respectively. The corresponding attributions of the IR bands are the stretching NH mode, stretching CO, bending NH and more complex bending NH and stretching CC mode of the peptide CONH groups. The most intense Amide I band is sensitive to the protein conformation [20]. This band is centered at 1,650 cm^{-1} for the pure fibroin indicating a random conformation [21]. Regenerated silk fibroin in solution or frozen at low temperature is generally in a random coil structure as reported previously [11]. The Amide I band is shifted at 1,641 cm^{-1} when the

Fig. 4 AFM topographical image of the fibroin-Au NCs (A) and corresponding AFM tapping phase (B) showing 2 types of assemblies. AFM topographical image of the pure fibroin deposited on HOPG (C). Profiles (D) describing fibrils and triangular assemblies are from topographical image (A).

Fig. 5 IR spectra of the pure fibroin film (plain line) and the fibroin-gold nanocomposites (bold line). Infrared bands assigned to alkanethiol are marked by an asterisk.

protein is in presence of Au NCs as stated by the presence of the CH stretching bands at 2,919 and 2,850 cm^{-1} and the corresponding bending modes at 1,461 and 1,378 cm^{-1} . The shift of 9 cm^{-1} of the most sensitive Amide band is significant of a conformational change of the silk fibroin. The strong interaction with the Au NCs induces a conformational change of the fibroin by rearranging its hydrophobic domains towards the NCs surface [22]. It should be noticed that the IR spectrum is an average of the different morphologies fibers and hybrid nanocomposites and the fibroin could adopt different conformation depending on the morphology of the assemblies.

6. Conclusion

The hybrid assembly of silk fibroin and Au nanocrystals yields to interesting morphologies such as nanowires, larger fibrils and well-faceted triangular nanocomposites. These different structures are obtained by the strong interaction of the hydrophobic fibroin with the Au NCs as demonstrated by a blue-shift of the maximum position of the LSPR band and a protein conformational change. The high tendency of the fibroin to self-assemble modifies the organization of the Au NCs and interestingly gives rise to new hybrid well-ordered nanocomposites. Right now, we are studying the plasmonic and structural properties of such new assemblies differing by their hierarchical ordering.

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