Formation of different gold nanostructures by silk nanofibrils

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ABSTRACT

Metal nanostructures that have unique size- and shape-dependent electronic, optical and chemical properties gain more and more attention in modern science and technology. In this article, we show the possibility that we are able to obtain different gold nanostructures simply with the help of silk nanofibrils. We demonstrate that only by varying the pH of the reaction solution, we get gold nanoparticles, nano-icosahedrons, nanocubes, and even microplates. Particularly, we develop a practical method for the preparation of gold microplates in acid condition in the presence of silk nanofibrils, which is impossible by using other forms of silk protein. We attribute the role of silk nanofibrils in the formation of gold nanostructure to their reduction ability from several specific amino acid residues, and the suitable structural anisotropic features to sustain the crystal growth after the reduction process. Although the main purpose of this article is to demonstrate that silk nanofibrils are able to mediate the formation of different gold nanostructure, we show the potential applications of these resulting gold nanostructures, such as surface-enhanced Raman scattering (SERS) and photothermal transformation effect, as same as those produced by other methods. In conclusion, we present in this communication a facile and green synthesis route to prepare various gold nanostructures with silk nanofibrils by simply varying pH in the reaction system, which has remarkable advantages in future biomedical applications.

1. Introduction

Metal nanostructures have been attracted considerable attention due to their unique size- and shape-dependent electronic, optical and chemical properties, and show great potential in different fields of modern science and technology, such as electronics, optics, biosensor, catalysis, electrochemistry, and biomedicine [1-7]. The development of facile and effective synthetic methods to precisely control over the size and shape is essential for the fabrication of metal nanostructures with desired properties. The synthesis of gold (Au) nanostructures with well-defined shapes appears to be one of the most popular research subjects over the past decade. Au nanostructures have some unique properties, such as surface-enhanced localized surface plasmon resonance (LSPR) and biocompatibility, so in recent years they are considered to be an ideal platform for chemical and biological sensing, as well as other biomedical applications [8-10]. For instance, the optical resonance features of Au nanostructures in the near-infrared region are useful for biomedical applications because soft tissues are only transparent in this range [11,12]. Consequently, there is a great deal of interest in the development of Au nanostructures for photothermal therapy, because they are able to convert the absorbed photons into thermal energy to kill the tumor cells [13-16].

Many techniques have been exploited to prepare Au nanostructures including polylol synthesis [17], seed mediated synthesis [18,19], template, surfactant or polymer assisted synthesis [20-22], electrochemical synthesis [23], and photochemical synthesis [24]. However, most of them have one or more drawbacks, such as complicated multistep synthetic procedure, high energy consume, use of non-environmentally friendly chemicals, or need of additional shape controlling agents. Concerns for the environmental impact on the use of organic solvents and the application of final nanomaterials in biological applications have motivated the search for the more environmentally benign alternatives to chemical synthesis. Past decade has witnessed an increased emphasis on the topic of “green” chemistry and chemical processes, and the development of reliable and eco-friendly metal nanostructure is an important step in the field of nanotechnology [25-27]. There has been several “green” methods developed to generate Au plates (with both micro- and nanoscale dimensions) by using amylloid fibrils [28-30], chitosan [31], bovine serum albumin [32], and fungi [33]. In addition, extracts from lemongrass [34], brown seaweed [35], grapefruit [36], and filamentous fungus [37] have also been employed to grow Au plates. However, these extracts are inherently mixtures of biomacromolecules, so it is difficult to identify the particular species to be responsible for the growth of Au plates.

Bombyx mori silk fibroin is one of the most thoroughly characterized, abundant, inexpensive, and sustainable non-bioactive protein for practical use. The repeating motif of GAGAGS, is widely accepted as the

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http://dx.doi.org/10.1016/j.msec.2016.03.113
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building block for β-sheet crystalites in the B. mori silk [38], and tyrosine residue with phenolic hydroxyl groups in the polypeptide chain are thought to be able to serve as a mild reducing agent [39,40]. As an appealing biomacromolecule, silk fibroin possesses unique sequence-specific self-assembly behavior and substrate recognition property, acting as a diverse biotemplate for the synthesis of inorganic nanomaterials with controllable morphology and polymorphs. For instance, we successfully synthesized hierarchical three-dimensional copper oxide nanostructures and hematite nanostructures using silk fibroin as template [41–43]. We also used silk fibroin to in situ reduce silver ions to prepare a silk fibroin-silver nanoparticle composite with effective antibacterial and biofilm-disrupting properties [44].

Recently, we found that silk nanofibrils not only are able to serve as a stabilizing agent against the aggregation of Au nanoparticles but also have the ability as a reducing agent to directly prepare Au nanostructures. In this article, we demonstrate a facile and green synthesis route, i.e., only by using one simple material, silk nanofibrils, we are able to obtain different Au nanostructures (nanoparticles, nanocubes, nano-icosahedrons, and even microplates) in an aqueous solution at room temperature.

2. Materials and methods

2.1. Preparation of regenerated silk fibroin (RSF) solution and silk nanofibrils

RSF aqueous solution was prepared from B. mori silkworm cocoons following a conventional procedure as described in our previous works [45,46]. In brief, raw B. mori silkworm silks were degummed twice with 0.5 wt% NaHCO₃ solution at 100 °C for 30 min and then washed with distilled water and allowed to air dry at room temperature. The degummed B. mori silk fibers were dissolved in 9.3 mol/L LiBr aqueous solution at 60 °C. After dialysis against deionized water in a Visking dialysis tube (MWCO: 10 kDa) for 3 days at room temperature, the solution was filtered and resulting SF solution was about 5 wt%.

The approach to generate the RSF nanofibrils is also described in our previous work [47]. Briefly, the RSF solution was diluted to 0.14 wt% and adjusted pH to 9.5 with 0.5 mol/L NaOH aqueous solution. Afterwards, ethanol was added to the RSF solution in which the final ethanol concentration was 7.0 vol%. Finally, the mixture was incubated at room temperature for one week until the appearance of RSF nanofibrils. We chose 0.14 wt% RSF concentration to prepare the nanofibrils because we found the most uniform silk nanofibrils were obtained at such a concentration.

2.2. Synthesis of Au nanostructures

Au nanostructures were prepared in the aqueous solutions by varying the concentration of HAuCl₄ and the pH value. The concentration of HAuCl₄ were in range of 0.3 to 10 mmol/L. Typically, certain amount of HAuCl₄ was added to 5 mL 0.14 wt% silk nanofibrils solution to an over-all Au(III) concentration of 10 mmol/L. The pH of the reaction system was adjusted by adding 1 mol/L HCl or NaOH solution. Then, the mixture was gently stirred with a magnetic stirrer, and the reaction was carried out at room temperature for 3 days.

2.3. SERS measurements

Au microplates were formed according to the method shown above at pH of 0.7, silk nanofibrils concentration of 0.14 wt%, and HAuCl₄ concentration of 10 mmol/L. Then, the resulting solution was vacuum-filtrated through a filtration membrane (pore size: 0.2 μm) to form an Au microplates/silk nanofibrils composite film. Such a film was used as the matrix for SERS measurement. Raman spectroscopy was performed with a Renishaw inVia Reflex Raman spectrometer, using 785 nm wavelength of a He-Ne laser with the energy of 6 mW. The samples for SERS studies were prepared by dripping 1 mL of 1 mmol/L rhodamine B onto the Au microplates/silk nanofibrils composite film. Two-dimentional (2D) Raman microscopic imaging was used to characterize the SERS of Au microplates in the composite. The experiment was carried out with a 100× objective lens and date collection was taken at 10 s acquisition time between 800 and 2000 cm⁻¹. A 40 μm square composite film was scanned and generate the 2D Raman image by choosing the characteristic peak of rhodamine B located at 1360 cm⁻¹.

2.4. Photothermal transformation measurements

Au nanoparticles were formed according to the method shown above at pH of 9, silk nanofibrils concentration of 0.14 wt%, and HAuCl₄ concentration from 0.3 to 4 mmol/L. Before the photothermal transformation measurements, we adjusted the pH of Au nanoparticles/silk nanofibrils solution to neutral with 1 mol/L HCl. The photothermal transformation measurements of Au nanoparticles/silk nanofibrils solutions were obtained with a FLIR E40 equipment running on FLIR tools system, in conjunction with the 808 nm laser. Au nanoparticles/silk nanofibrils solution samples with different Au concentration were placed in a series of specimen bottles irradiated by an 808 nm laser (3 W/cm²). The temperature signals were recorded in 5 min and analyzed with FLIR tools systems.

2.5. Other characterizations

The optical absorption spectra of the Au nanostructures in aqueous medium were recorded with a Hitachi UV-2910 UV–vis spectrophotometer in the wavelength region of 350–700 nm in quartz glass cuvettes of 10 mm optical path length. The particle size and morphology of silk nanofibrils and Au nanostructures were studied using a Hitachi S-4800 field-emission scanning electron microscope (FESEM) at 20 kV and a Hitachi H-600 transmission electron microscope (TEM) at 75 kV. Atomic force microscopy (AFM) images were acquired with a Bruker Multimode 8 system in the tapping mode under air atmosphere. Powder X-ray diffraction (XRD) experiments were carried out with CuKα radiation using a Bruker D4 X-ray diffractometer, employing a scanning rate of 0.02°/s in the 2θ range from 10° to 90°.

3. Results and discussion

3.1. Preparation of various Au nanostructures

As presented in our previous work, the silk nanofibrils we prepared were up to 1 μm long in contour length and 3–4 nm in diameter (Fig. 1A and B), and β-sheet was the dominant conformation [47,48]. The silk nanofibrils was stably in aqueous medium and was opalescent as shown in Fig. A1A. It is well known that RSF solution is very instable that shows a strong tendency to form hydrogel or aggregate under environmental conditions like low pH, high temperature, shear force, and alcohol treatment [49,50]. When we directly added HAuCl₄ into RSF aqueous solution, the silk protein aggregated. However, if we added HAuCl₄ into the silk nanofibrils solution, it was still stable without any precipitation because it was already evidenced that the silk fibroin has been self-assembled into β-sheet in silk nanofibrils [48].

We found Au nanoparticles were formed when we adjusted the pH value of the HAuCl₄/silk nanofibrils solution higher than 7. TEM image (Fig. 1C) shows the formation of well-dispersed Au nanoparticles with an average size of 8.0 nm (inset is an HRTEM image). In the meantime, the synthesized Au nanoparticles are spherical without other nanostructures. UV–vis extinction spectroscopy is often used for the characterization of the optical properties of the Au nanoparticles. Fig. 1D shows that Au nanoparticles exhibit an intense surface plasmon resonance band at about 530 nm (there is no absorption peak from 350 to
750 nm for the pristine silk nanofibrils), which confirms the spherical morphology of the nanoparticles [31]. In the meantime, we find the color of solution changes from pale yellow to vivid ruby red, which is also a strong evidence of the formation of Au nanoparticles (Fig. A.1). Increasing the concentration of HAuCl4 in the reaction system, the characteristic peak of synthesized Au nanoparticles becomes broader and is slightly red-shifted due to the formation of larger nanoparticles [16], as shown in Fig. 1D. In the meantime, the color of the Au nanoparticles/silk nanofibrils solution also changes to darker (Fig. A.1). All these phenomena demonstrate that silk nanofibrils are able to reduce HAuCl4 and form Au nanoparticles in alkaline condition, and further stabilize the resulting Au nanoparticles. However, it is not too surprising for us, as there has been already reported that the Au nanoparticles can be synthesized by bovine serum albumin at alkaline pH [51].

Thanks to the stability of silk nanofibrils solution in a wide pH range, we are able to adjust the pH of HAuCl4/silk nanofibrils solution lower than 7, in which it is difficult for RSF solution (silk fibroin normally precipitates). We found various forms of Au nanostructures can be obtained in acidic condition. When pH was adjusted to 4, polyhedron Au nanoparticles were obtained, dominantly icosahedral in shape (Fig. 2A and B). These Au icosahedral nanoparticles have a uniform size about 500 nm. To our knowledge, this is the first report on the synthesis of icosahedral Au nanoparticles in aqueous medium by reduction and regulation of a protein. If we further decreased the pH of the reaction solution to 1, the product became cubic nanoparticles or microparticles (Fig. 2C).

Large Au microplates were appeared when the pH of the reaction solution was set lower than 1 (pH = 0.7). After the formation of Au microplates, the color of the reaction solution turns to golden yellow (Fig. 3A), which is different from the ruby red of Au nanoparticles (Fig. A.1). Fig. 3B is the optical microscopic image, which shows the formation of Au microplates (up to 20 μm in size). The TEM images shown in Fig. 3C further confirm the formation of the microplates, which mainly consist of hexagonal shapes together with some triangular and truncated triangular shapes. The SAED pattern (Fig. 3D) of an Au microplate was acquired by focusing the electron beam on the microplate lying flat on the TEM grid. From the diffraction spot pattern, it indicates a single crystalline nature of Au microplates with an atomically flat surface and a preferential growth direction along the Au (1 1 1) facet [37, 51–53]. The crystalline nature of the Au microplates is further confirmed by the XRD pattern shown in Fig. 3E. Five sharp diffraction peaks are observed that assigned to the (1 1 1), (2 0 0), (2 2 0), (3 1 1), and (2 2 2) facets of Au [54]. It is found that the (1 1 1) diffraction peak at about 38.3° is much stronger than the other diffraction peaks, indicating the predominance of (1 1 1) facet on the microplates [55], which is consistent with the SAED result. Moreover, both AFM and SEM images indicate that there are silk nanofibrils attached on the surface of Au microplates (Fig. A.2).

More interestingly, if we decreased the concentration of silk nanofibrils to one tenth of “normal” concentration, i.e., to 0.014 wt%, we were not able to get large Au microplates, but only found Au nanoparticles tightly grew on the silk nanofibrils (Fig. A.3). The average size of Au nanoparticles on silk nanofibrils is about 8 nm, thus the “apparent” diameter of Au nanoparticles-loaded silk nanofibrils increases to 20 nm compared to 4 nm of original silk nanofibrils.

In conclusion, we are able to obtain several Au nanostructures (nanoparticles, nano-icosahedrons, nanocubes, and microplates) using silk nanofibrils as reducing and regulating agent by only adjusting the pH of the reaction solution.

3.2. Discussion on formation mechanism of Au nanostructures

We find Au nanoparticles are easily synthesized when the reaction solution is in basic pH. It is not surprised because there are already some reports showing that the pH of the reaction solution play a crucial role in the biosynthesis of Au nanoparticles. For instance, some other proteins like BSA and lactoferrin can also serve as reductant to produce Au nanoparticles with the addition of NaOH. The reason is due to the reduction rate of AuCl4− is much faster under basic condition than those under neutral and acidic conditions. It has been confirmed that Tyr or custom peptides containing Tyr residues are able to reduce Au(III) ions through their phenolic groups, and their reduction capability is greatly improved by increasing the reaction pH [56,57].

In the acidic pH, it is reported that the acidic amino acid residues with carboxyl groups may also reduce Au(III) ions, and is favorable for
the anisotropic growth of Au nanoparticles [37]. Therefore, we suppose that different charges on the silk fibroin molecular chains at different pH values may affect the strength of interaction between the silk fibroin chains and Au, leading to the formation of different Au nanostructures. In the meantime, we explored the effect of the concentration of silk nanofibrils on the formation of Au nanostructure. We found the concentration of silk nanofibrils is a key factor for the growth of Au microplates. We demonstrate above that when the concentration of the silk nanofibrils was 0.14 wt%, large Au microplates are formed in acid solution. However, when we decrease the concentration to 1/10 of that (i.e., 0.014 wt%), only Au nanoparticles along the silk nanofibrils can be found. Therefore, we propose a possible mechanism for the formation of Au nanostructures at acidic condition as follows.

The silk nanofibrils in acidic aqueous solution is cationic, which are able to adsorb AuCl4− ions electrostatically, and subsequently reduce Au(III) to Au(0). The reduced Au(0) on the silk nanofibrils nucleates and forms Au nanoparticles. Afterwards, silk nanofibrils serve as a soft template to control the growth rate of various facets of Au nanoparticles by selectively adsorbing onto the crystallographic planes of Au, resulting in the formation of different Au nanostructures. It is known that the selective ‘face-blocking’ and the reduction rate of the metal ions govern the growth kinetics [54]. The former involves the selective adsorption of species onto specific crystallographic facets, hindering the access to these facets of the metal atoms that are being deposited, and on contrary, promoting the relative growth rates of other facets. The latter determines the speed at which the metal atoms are deposited. When Au nanoparticles are formed, the silk nanofibrils selectively adsorb on their surface due to the strong affinity of Au atoms towards nitrogen sites in protein, and forms a shell around the Au nanoparticles. However, the adsorption ability of silk nanofibrils to various Au crystallographic facets is different, hence resulting in a different growth kinetics of different facets. The pH of reaction solution may influence the adsorption ability of silk nanofibrils to crystallographic facets of Au nanoparticles. When the pH of reaction solution is 4, silk nanofibrils are less protonated, so few silk nanofibrils are able to adsorb on Au nanoparticles. Therefore, the growth of crystallographic facets is slightly restricted, so Au icosahedrons can be obtained. When the pH of reaction solution is lower than 1, the silk nanofibrils are highly protonated, so they are tightly adsorbed on Au nanoparticles. Thus, they act as capping agents by preferentially covering the low surface energy (1 1 1) facet of Au nanoparticles, preventing subsequent Au atom deposition on it [35]. As a result, other facets grow more quickly than (1 1 1) facet, forming large Au microplates. In addition, the content of the silk nanofibrils is also important to regulate the nanostructure of Au. If the concentration of the silk nanofibrils is low (for example, 0.014 wt% as shown above), they cannot effectively adsorb on Au nanoparticles to hinder the growth of one specific facet. Meanwhile, the less silk nanofibrils means less reduction ability, which leads less Au(0) to be reduced for the growth of large microplates.

As a summary, we believe that the electrostatic interactions between AuCl4− and silk nanofibrils in the acidic condition lead them to interact closely, and then the specific amino acid moieties in the silk nanofibrils reduce the Au ions into solid Au nanoparticles. In the early stage, most of the formed Au nanoparticles adhere on the silk nanofibrils. As soon as the Au nanoparticles grow in size beyond the typical dimensions of a single silk nanofibril, more silk nanofibrils are needed to cooperatively assist for the further the growth of the Au nanoparticles. They bring more reduced Au atoms as “raw material” and adsorb on the specific facet of the formed Au nanoparticles to hinder the growth of such a facet. In addition, the relatively slow reduction rate of Au ions by silk nanofibrils in acid solution may also promote the formation of large anisotropic Au nanostructures.

3.3. Potential applications of Au nanostructures/silk nanofibrils composites on SERS and photothermal transformation

There has been several reports to show that Au nanostructures are able to work as a promising SERS platform for the detection of Raman-sensitive analytes [8,9,58,59], so we also study the SERS effect of Au nanostructures made by our method. Another advantage of our research is that the silk nanofibrils in the reaction system can serve as a matrix material simultaneously. Fig. 4A shows a homogeneous Au microplates/silk nanofibrils composite film (5 cm in diameter) can be easily prepared by vacuum-assisted filtration [47]. As in many previous
properties of Au nanoparticles have been actively studied as a promising sensor as a SERS matrix. In the meantime, the unique physicochemical properties exhibit tunable SERS activity, which is promising for the application of SERS based sensors and optical imaging [63,64]. However, most of the synthesis methods reported use poor biocompatible or even toxic chemicals, which prevent them from the practical application in biomedical field, but Au microplates synthesized by silk nanofibrils do not have such a problem and may have great potential in future applications.

Another potential application of Au nanostructures in biomedical field is for photothermal therapy. Photothermal therapy of tumors based on the near infrared laser is a minimally or non-invasive therapeutic modality. In previous studies, the good biocompatibility of Au nanoparticles themselves [8–10] and Au nanoparticle/protein composites, including Au nanoparticle/silk protein composites [5,65,66] has been confirmed. On the other hand, the unique physicochemical properties of Au nanoparticles have been actively studied as a promising and versatile platform for cancer therapy diagnosis. Due to the tunable localized surface plasmon resonance, Au nanoparticles are able to become highly localized heat sources when irradiated with a laser through the photothermal effect [67–69].

The Au nanoparticles/silk nanofibrils composite solution show a concentration-dependent photothermal transformation behavior as shown in Fig. 4C, while the pristine silk nanofibrils solution shows a negligible temperature increase. It has been reported that the concentration of Au nanoparticles is critical to temperature increment [5]. In our case, Au nanoparticles/silk nanofibrils composite solution with 4 mmol/L Au nanoparticle concentration shows the highest temperature increment. It is able to increase 15 °C in first 50 s from 30 to 45 °C, and up to 60 °C after 300 s irradiation at a power density of 3.0 W/cm² (Fig. 4C and D). We know a minimum temperature enhancement of 5 °C is the critical temperature required for cancer hyperthermia therapy [70]. As shown in Fig. 4D, the temperature of Au nanoparticles/silk nanofibrils composite solution is able to increase swiftly to 42 °C within 30 s, and to 46 °C after 50 s of irradiation. Therefore, the high temperature of liquid medium heated by Au nanoparticles/silk nanofibrils composite (inset of Fig. 4D) is enough to cause a sufficient tumor hyperthermia and restrict its malignant proliferation.

We should point out that the main purpose and contribution of this research is to find an easy and environmentally friendly way to prepare different gold nanostructures simply with the help of silk nanofibrils, so we just very briefly and preliminarily show the possible application of the resulting Au nanostructures on SERS and photothermal therapy. We just want to say the Au nanostructures obtained from our method may have the same application potential as those obtained by other reported methods, and we believe both the SERS and photothermal effect of Au nanostructure-based composites could be significantly increased.
if we improve the preparation process of such materials in future research.

4. Conclusions

In this article, we present our preliminary findings that several different Au nanostructures can be obtained by simply modifying the pH in the reaction system, Au nanoparticles, nanocubes, nano-icosahedrons and microplates can be obtained successfully. Particularly, it is the first time to obtain large Au microplates with the help of a cheap, abundant, and sustainable silk protein. We also prove that Au nanostructures obtained by our method hold the same properties as those prepared by other reported methods, for example, Au microplates/silk nanofibers composite can be potentially applied as a substrate for SERS and Au nanoparticles/silk nanofibers composite shows the tunable property of photothermal transformation. This work demonstrates that silk nanofibers serve as both bioreductant and biotemplate in the formation of an organic-inorganic nanocomposite, and add the advantage of increasing biocompatibility of the final products for biomedical applications.

Acknowledgments

This work is supported by the National Natural Science Foundation of China (Nos. 21274028, 21574023 and 21574024).

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.msec.2016.03.113.

References


