A facile route to diatoms decorated with gold nanoparticles and their optical properties

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This paper presents a facile route for the decoration of biogenic silica from diatoms with gold nanoparticles (AuNPs) with dimensions from 10 to 40 nm. The AuNP decoration on the diatoms is performed through initial attachment of colloidal AuNPs (2 nm) followed by enhancement of the size of the AuNPs by mild reduction of tetrachloroauric (III) acid from solution. The samples were characterised by transmission electron microscopy (TEM), energy-dispersive X-ray analysis, Fourier transform infrared spectroscopy and light spectroscopy in the ultraviolet and visible (UV/Vis) range. The size of the isolated AuNPs on unmodified acid-cleaned diatoms was 10 nm according to TEM micrograph analysis. The measured UV/Vis spectrum showed an absorption peak at 525 nm, which corresponds well to the optical properties of AuNPs of the same size. The extent of decoration with AuNPs of the diatoms was significantly improved after functionalisation with 3-aminopropyltriethoxysilane of the biogenic silica. The absorption peak in the visible range was redshifted to 590 nm due to enlargement of the AuNPs up to 40 nm and clustering of the AuNPs. The AuNP decoration on the hierarchical and complex structured biogenic silica of diatoms might be interesting for biodiagnostic and surface-enhanced Raman spectroscopic applications.

1. Introduction

The design and fabrication of complex and hierarchically inorganic architectures with specific functionalities is a rich field of current research.^{1–4} A feasible and appealing solution for the problem of generating complex, hierarchically structured materials involves chemical synthesis or deposition of inorganic phases at locally confined sites on predefined material architectures. This is known as the biotemplating approach and defines a general fabrication pathway for complex inorganic materials.⁴ A wide variety of complex inorganic materials which cannot be generated by any other method are accessible by biotemplating.^{1,4} Biotemplating involves inorganic phase formation in the presence of a biological structure, which might be basically organic (wood⁵), inorganic (diatoms^{6,7}) or even a combination of both.^{8,9}

The biotemplating approach is of particular interest for the controlled patterning of inorganic nanoparticles.² Noble metal particles¹⁰ – that is, gold nanoparticles (AuNPs) – are of interest in various applications in diagnostics or advanced optical applications such as localised surface plasmon resonance (LSPR) and surface-enhanced Raman spectroscopy (SERS¹¹). When a continuous noble metal particle decoration on a surface interacts with light, resonant surface electromagnetic waves result in the absorption of the incident light, which is known as surface plasmon resonance.¹² Nanoparticulate noble metal materials are of specific interest here because of their strong LSPR.¹³ This electromagnetic potential is derived from the collective oscillation of conduction electrons, called surface plasmons, which were activated by incident light.¹⁴ This effect is highly sensitive to the size, shape and material composition of the nanostructure.¹⁵ The material properties of the surrounding medium of the noble metal nanoparticles also determine the resonance

frequency of the nanoparticle. The frequency of the LSPR experiences a redshift (lower frequency) with an increase in the dielectric constant of the substrate.^{16,17} The fabrication of a multilayer nanomaterial composed of organic, inorganic and metallic components with interesting optical properties was recently reported.^{18,19} Cellulose nanocrystals were used as a template structure and coated with silica. Gold (Au) and gold–silver nanoparticles from solution were attached to the surface of the cellulose/silica composite by a wet chemical process. By tuning the noble metal particle composition between pure gold and gold–silver, the plasmon resonance could be tuned between 423 and 550 nm. The materials could also be applied as network substrates for SERS. In this context, it would be desirable to use biogenic silica structures directly, which would omit the silica deposition step on a complex structured biotemplate. Biogenic silica is formed by diatoms.²⁰ These microalgae are composed of two complex shaped shells (frustules) with hierarchical patterns from the nano- to the micro-scale. The frustules consist of amorphous porous silica with structural features that are difficult to reproduce synthetically. Therefore, diatoms (*Coscinodiscus wailesii* and *Eucampia zodiacus*) were used as templates for the decoration with noble metal nanoparticles.^{21,22} Attachment can be achieved through layer-by-layer deposition and covalent linkage.²³ The fabricated noble metal nanoparticle diatom silica material showed a particular enhancement of SERS intensities. In another approach, the periodic pore pattern of diatom frustules (*Coscinodiscus wailesii*) was exploited for SERS.²⁴ AuNPs were self-assembled on the diatom templates and inside the frustule pores. An enhancement in the SERS field on the order of 10⁸ was observed. However, a direct correlation of the LSPR and AuNPs on diatom frustules has not been described so far. The present work outlines a simple route for the deposition of AuNPs on diatom

frustules (*Thalassiosira pseudonana*). The optical properties of the AuNP-decorated diatoms were investigated.

2. Experimental section

2.1 Materials and methods

Suspensions of the acid-cleaned diatoms (*T. pseudonana*; obtained from the Kröger Group, B Cube – Centre for Molecular Bioengineering, Dresden, Germany) were carefully centrifuged and washed several times with ethanol pro analysi (Roth, Karlsruhe, Germany). Part of the diatom suspension in ethanol was treated with 3-aminopropyltriethoxysilane (APTES, Fluka, Steinheim, Germany) at 90°C for 1 h under reflux and subsequently centrifuged and washed with ethanol pro analysi. The pristine and the APTES-functionalised diatoms were then mixed with a gold sol which was synthesised separately with the reducing agent tetrakis (hydroxymethyl)phosphonium chloride (Sigma-Aldrich, Steinheim, Germany) according to a procedure published by Duff *et al.*²⁵ in 1993. After being stirred for 24 h, the mixture was centrifuged and washed with double-distilled water (H₂O) several times until the non-attached nanoparticles were removed from the suspension. In the next step, a gold coating solution composed of tetrachloroauric (III) acid (HAuCl₄)/potassium carbonate (K₂CO₃) (50 ml water, 0.013 g potassium carbonate, 0.75 ml tetrachloroauric (III) acid (25 mM, Merck, Ismaning, Germany)) was prepared and subsequently reduced by adding 50 µl formaldehyde (37 wt% in water) (Sigma-Aldrich, Steinheim, Germany). 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (1.0 mM Sigma-Aldrich, Steinheim, Germany) was added as a buffer.

2.2 Characterisation

For characterisation using a transmission electron microscope (TEM), a drop (10 µl) of the AuNP-decorated diatom suspension in ethanol was placed on a copper grid coated with a 20 nm carbon film (Plano, Wetzlar, Germany) and dried for 24 h. The TEM investigations were conducted with a Philips CM30 TEM (Eindhoven, the Netherlands) operated at 300 kV.

Energy-dispersive X-ray analysis (EDX; Röntec, Germany) was performed on a Zeiss DSM 910A scanning electron microscope (SEM) operated at 10 kV.

Optical properties of the AuNP-decorated diatom suspensions in water were measured with an ultraviolet and visible (UV/Vis) spectrometer (J&M, Tidas, Germany) between 300 and 700 nm.

Infrared spectra of the samples were obtained in attenuated total reflectance geometry with a Fourier transform infrared spectrometer (FTIR; Nicolet 380, Thermo Fisher Scientific, USA) in the wave number region of 4000–500 cm⁻¹.

3. Results and discussion

The general scheme for the fabrication of AuNP-decorated NPs is given in Figure 1. First, the diatoms (*T. pseudonana*) are treated and cleaned with dilute acids to remove organic residues (proteins) and to ensure a hydroxyl group (silanol)-terminated silica surface of the frustule. AuNPs are attached and self-assembled from gold solution either on the acid-cleaned or on the frustules functionalised with APTES.

The TEM investigation of the pristine acid-cleaned diatoms shows a typical spherical appearance with a diameter of 2 µm. The delicate porous architecture of the diatom including functional elements such as the rimoportulae is clearly visible (Figure 2(a)). The silica of the frustules forms a nanostructured complex network. After treating with the decorating gold solution, AuNPs with sizes between 10 and 20 nm were found on the silica of the frustules (Figure 2(b)). The observed diameters of the AuNPs correspond well to those of AuNPs found on silica-coated cellulose nanocrystals.¹⁸ The AuNPs are randomly distributed on the frustule, moderately covering the diatom surface. The presence of gold was confirmed by EDX measurements in the SEM. A closed continuous film is not observed.

However, there seems to be a slight preference for the frustule edge, where several AuNPs are also connected to each other (Figure 2(d)). The attachment of AuNPs to the APTES-functionalised diatom is obviously very strong as can be seen at a higher magnification in the TEM (Figure 2(e)). The thickness of the silica at the rimoportula walls is only a few nanometres. Even at such low wall thickness, a distinct attachment of AuNP is observed. The selected area electron diffraction shows a well-resolved ring pattern where all relevant rings could be assigned to nanosized and nanocrystalline gold (Figure 2(f)).

Figure 1. Decoration of acid-cleaned diatoms (*T. pseudonana*) and of diatoms functionalised with APTES with AuNPs

Figure 2. TEM micrographs of (a) a pristine acid-cleaned diatom (*T. pseudonana*) and (b) after deposition of AuNPs; (c) an APTES-functionalised diatom decorated with AuNPs; (d) AuNP clusters on the frustule edge and (e) the decorated nozzle; (f) selected area electron diffraction of nanocrystalline AuNPs on the decorated diatom in (c)

The functionalisation with APTES generally improves attachment of the slightly negatively charged AuNPs since the terminal amino function provides a positively charged substrate surface.¹⁸ The silane groups of APTES covalently bind to the silica surface. The modification of the diatoms with APTES was confirmed by FTIR analysis through the appearance of APTES-derived peaks in the spectra at 1130 and 762 cm^{-1} (Figure 3).

The freshly prepared reference AuNPs without any templates exhibited a maximum in the UV/Vis absorption spectrum at 520 nm (Figure 4, a). This value indicates an AuNP size of 10–20 nm.²⁶ The observed red colour of the AuNP-modified diatom suspensions was judged based only on a subjective perception. The visual appearance of the AuNPs decorated on APTES-functionalised diatom silica was brownish. As stated earlier, the LSPR strongly depends on the size, shape and dielectric environment of the AuNPs.^{17,18,26} Since the prepared AuNPs from solution and after attachment to the frustule are predominately spherical in appearance, the influence of shape is negligible. The UV/Vis spectrum for AuNP-unmodified diatoms with individual AuNPs shows a peak at 525 nm (Figure 4, b). This is the same value as observed for the as-prepared AuNPs from solution. This means that the dielectric property of the silica has only a limited effect on the LSPR, when the particle is directly point-connected to the silica surface.¹⁷ The UV/Vis spectrum for the AuNP decorated on APTES-functionalised diatoms yielded AuNPs with diameters from 10 to 40 nm, which should result in a shift to about 530 nm.²⁷ However, the observed

peak for the AuNP-decorated APTES-functionalised diatoms was shifted to 600 nm (Figure 4, c). This peak shift would correspond to a particle size of around 100 nm.²⁶ Such a high shift with a

Figure 3. FTIR spectrum of diatoms functionalised with APTES (a) compared to that of pure APTES (b)

Figure 4. UV/Vis spectra of as-prepared AuNPs (a), AuNPs decorated on acid-cleaned diatom silica (b) and AuNPs decorated on an APTES-functionalised diatom silica (c)

given particle size can occur only when the particles form interconnected dimers or clusters with a certain aspect ratio.²⁸ Since the AuNPs on the APTES-functionalised diatoms partly appear in clusters with dimensions around 50 nm, the observed peak shift is derived from a longitudinal frequency part in the LSPR.²⁶ The observed frequency shift in the UV/Vis spectrum is consequently related to the dielectric environment and the shape of the AuNPs in combination with a distinct cluster formation. Furthermore, the peak is extensively broadened, which is due to the AuNP particle size distribution from 10 to 40 nm.

As stated earlier, LSPR is observed on the AuNPs resulting from the collective oscillation of the conduction electrons only when the diameter of the nanoparticle d_{AuNP} is much smaller than the wavelength of the light λ (d_{AuNP} 10–20 nm $\ll$ $\lambda \approx$ 520 nm). For AuNPs, the resonant excitation of the excitons by the incoming photons at visible wavelengths is responsible for the observed colour. The colour is dependent on the size, solvent, temperature and possibly attached ligands. The dielectric silica of the diatoms, to which the AuNPs are attached, might affect LSPR.²⁹ Furthermore, the resonant wavelength can be significantly influenced by the interparticle distance.³⁰ As a consequence, the vicinity of other nanoparticles might result in the shift of the LSPR – for example, the aggregation of nanoparticles induces in a significant redshifting from around 520 to around 650 nm.^{31,32} Using gold nanorods (diameter 10 nm, length 90 nm) results in a shift of the LSPR peak to about 1000 nm.³³ The authors anticipate that the observed redshift is not derived, however, from the attachment of the AuNPs to the nanoparticulate dielectric diatom silica. Coupling between the particles due to the formation of

small aggregations is considered to be responsible for the redshift corroborating the authors' earlier findings.¹⁸

4. Conclusions

It could be shown that diatom silica is a suitable template for decoration with AuNPs from solution with a size of 10 nm. The attachment of the AuNPs could be significantly increased by prior functionalisation with APTES. An increased decoration with AuNPs on the biogenic diatom silica was observed, because the silica surface is now positively charged. The particle size distribution was broadened at the same time. The unmodified AuNP-decorated diatoms showed almost no shift of the absorption peak at 525 nm compared to an AuNP suspension in water with a particle size of 10 nm. The dielectric properties of the biogenic silica of the diatoms exert only a weak to negligible effect on the optical properties of the AuNPs. After functionalisation of the biogenic diatom silica with APTES, decoration with AuNPs was significantly improved. The absorption peak in the visible range was significantly redshifted to 590 nm. This shift could be related to the enlargement of the AuNPs up to a size of 40 nm size and to some observed clustering of the AuNPs. The observed LSPR from the AuNPs on a hierarchical and complex structured biogenic silica template (diatoms) might be interesting for biodiagnostic and SERS applications.

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