Hybrid Nanoparticles

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One-Step Synthesis of Collagen Hybrid Gold Nanoparticles and Formation on Egyptian-like Gold-Plated Archaeological Ivory**

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Abstract: *A one-step method is reported to synthesize hybrid gold nanoparticles (AuNPs) by reduction of $HAuCl_4$ in acetic solution in the presence of collagen (Col), dicarboxylic acid-terminated polyethylene glycol (PEG), and cetyltetrammonium bromide (CTAB) mixed with hydoxyapatite (HAP) as surfactants. Such formation process of AuNPs was shown to be responsible for purple stains naturally formed on Egyptianizing archaeological gilded ivories from 8th BC Syria. The understanding of this formation mechanism, which most likely involves a step with hybrid AuNPs, allows the establishing of an authenticity marker of ancient gold-plated ivories.*

Nanomaterials and its composites have become increasingly popular owing to their size-specific and unique properties as well as their promising breakthrough in the development of novel methods in medicine,[1] biology,[2] and for sensors.[3] The synthesis of AuNPs in aqueous solution is still the general route. The most popular method for preparing AuNPs in water uses citrate to reduce $HAuCl_4$ under boiling conditions[4] (Supporting Information, Section S1). Gold nanospheres were prepared in cetyltetrammonium bromide (CTAB).[5] Therefore, diverse approaches have been developed to reduce Au^{III} salts in water using different ligands as colloid particle stabilizers.[6] Stabilizers protect particles against aggregation and control their functional properties[6,7] but are mostly toxic. Exchange of organic molecules modified on AuNPs with polyethylene glycol (PEG) is thus performed to prepare biocompatible PEG-stabilized AuNPs.[8,9] Collagen (Col), one of the most important and abundant structural proteins in the extracellular matrix has been widely used in biomedical and biomaterial applications.[10,11] Gold NPs have been synthesized by photoreducing Col-$HAuCl_4$ complexes in solution[12] using several approaches including wet chemistry for the creation of special nanostructures. Interestingly, AuNPs have been observed on ivory, a Col-based mineralized tissue, during the study of Egyptianizing ivory carvings dating back to the end of the 9th of the beginning of the 8th century BC from the Louvre collection that originated from Arslan Tash, Syria (Figure 1).[13]

Figure 1. Optical images of the ivory carvings: a) stag (inv. no AO 11458, 125×50 mm², AI_EI_AT Louv17) and b) Horus birth (inv. no AO 11465, 82×83 mm², AI_EI_AT_Louv19), both kept in the Louvre museum, Paris.

Non-invasive micro-proton-induced X-ray Emission (PIXE) has revealed the nature of the purple stains observed on the ivory carving surface as pure gold (Figure 2a; Supporting Information, Section S2, Table S2-1). The presence of AuNPs in the purple stains on ivory was shown by VIS spectroscopy (Figure 2b; Supporting Information, Section S3, Figure S3-2) and X-ray diffraction (Figure S3-1).[14] However, the formation process is not fully understood.

Ivory is a material with a high specific surface area because of the presence of nanosized carbonated hydroxyapatite crystals (HAP) and Col that is highly functionalized by OH groups.[15] It seems a well-adapted support for AuNP

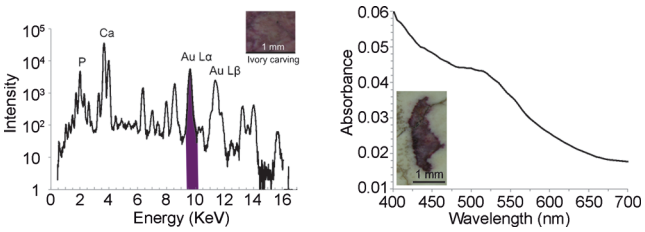

Figure 2. Purple stain analysis on the rumen of the stag from the Louvre: a) micro-PIXE spectrum indicating the presence of Au and b) Vis spectrum showing the typical absorption edge around 525 nm of AuNPs owing to plasmon resonance, giving rise to the purple color.

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growth. However, additional chemicals are needed to form and stabilize AuNPs.[16] Therefore, we intend first to synthesize hybrid AuNPs using a "simple" method with different capping materials (CTAB, Col, PEG, HAP) and study the effect of competition between capping agents on the growth process. Second, we search for a formation mechanism of AuNPs as observed as purple stains on the surface of ancient ivories.

Four routes were successfully employed using $HAuCl_4$ reduction: 1) Coating with Col in acetic acid (AA); 2) HAP was added to the synthesis of Col-Hybrid-AuNPs; 3) synthesis of Col-PEG-AuNPs in the presence of Col and PEG-diacid using sodium borohydride ($NaBH_4$) as a reducing agent; and 4) because the synthesis of Col-CTAB-AuNPs by mixing Col and CTAB solutions and then reducing $HAuCl_4$ by sodium borohydride ($NaBH_4$) was unsuccessful, which was probably due to a competition between Col and CTAB micelles, the reagent order was varied: $HAuCl_4$ reduction with $NaBH_4$ in the presence of CTAB produced Au seeds at room temperature (RT) and Col was added at the end.

As shown by UV/Vis absorption spectra AuNPs exhibit a surface plasmon band at 530 nm (Supporting Information, Section S4). The slow shift of the band position depends on the ratio of the Au salt and capping materials during the reaction processes. Collagen and PEG are used as stabilizing polymers because the dispersed solutions are due to the formation of coordination bands between Au ions and the amine or carboxylic groups, respectively. This chelation evenly dispersed Au ions, which reduced form dispersed AuNPs of uniform size.

Polarization modulation IR reflection adsorption spectroscopy (PM-IRRAS) characterized the formation of Col-Hyb-AuNPs after deposition on planar Au surface. The IR spectra of Col-AuNPs and Col-HAP-AuNPs were described previously.[17] Our results are in agreement with these findings (Figure 3, curve 1; Supporting Information, Section S5). Collagen that offers steric protection owing to the bulkiness of the protein stabilizes AuNPs, so they have free surfaces available for interaction with external species. The PM-IRRAS spectrum is modified (Figure 3, curve 2) and confirms the formation of Col-HAP-AuNPs (Figure 4).

Col-PEG-AuNPs show a characteristic IR spectrum (Figure 3, curve 3) that confirms the growth success of hybrid AuNPs. The presence of these bands clearly shows the presence of organic ligands in the PEG-AuNPs, and in particular "free" acid functions that can be later involved in covalent links with proteins or other amino groups in proteins.

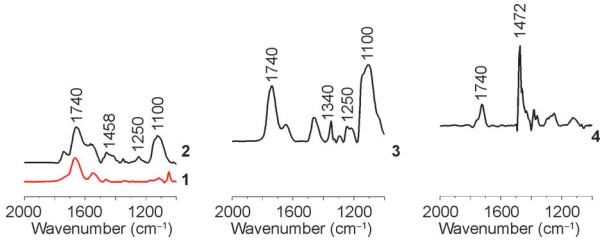

Figure 3. PM-IRRAS spectra of **1**) Col-, **2**) Col-HAP-, **3**) Col-PEG-, and **4**) Col-CTAB-AuNPs.

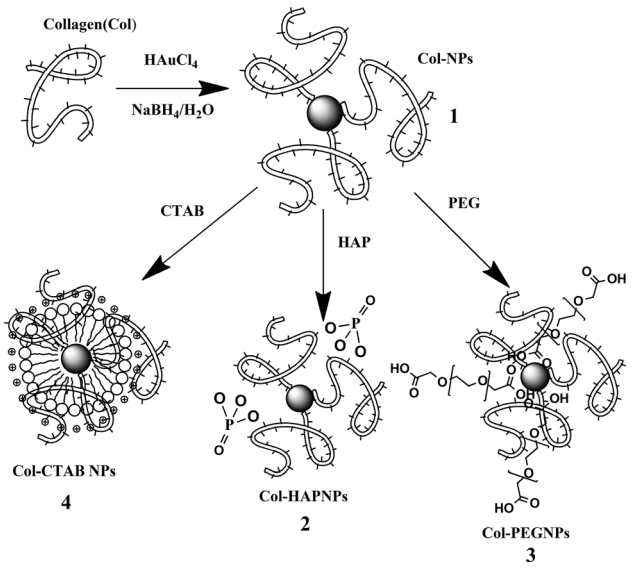

Figure 4. Representation of Col-Hyb-AuNPs.

The IR spectrum of Col-CTAB-AuNPs (Figure 3, curve 4) also confirms the formation of Col hybrid AuNPs (Figure 4).

Collagen-AuNPs (**1**) have increased stability in aqueous and biological media.[18–20] Collagen controls the shape and size of AuNPs[12] and acts as an effective template for their assembly adjusting pH, temperature, and concentration in solution[21,22] (Figure 4). When Col was introduced in the $HAuCl_4$ solution, the positively charged Col chains show strong electrostatic interaction with negatively charged $AuCl_4^-$ ions forming a Col-$AuCl_4^-$ complex that play a crucial role in the NP growth process.[17] The addiction of AA increases the reduction kinetics by Au ion complexation.[12]

Transmission electron micrographs (TEM) allowed the determination of the Col-AuNP size distribution (Figure 5-1) on 200 NPs to be of 2 ± 0.7 nm. Upon the addition of HAP solution to the Col-Au dispersion, the Ca^{2+} is trapped within the gap region near the carboxylic group, which serves as a crystal nucleation point. In other studies,[17,23] the addition of HAP to the Col-Au dispersion forms quarter-moon-like platelet structures on the basis of intertwined peptide chains.[23,24] In our case, the addiction of HAP during Col-NP growth did not modify the final shape and size of the obtained hybrid Col-HAP-AuNPs (**2**), confirming a good dispersion (Figure 5-2).

We have synthetized spherical PEG-AuNPs with uniform size distribution by a one-step synthesis using a non-toxic stabilizer.[9] Other authors have reported the AuNP growth mechanism in the presence of poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) block copolymers by varying temperature and solvent quality.[25] These block copolymers form cavities with pseudo-crown ether structure that can bind metal ions. Reduction of bound $AuCl_4^-$ ions can proceed by oxidation of the oxyethylene and oxypropylene segments by the metal center. In the present study, the AuNP formation from $AuCl_4^-$ includes the following main steps (Figure 6): 1) Formation of Col-PEG diacid mixture by electrostatic interaction; 2) initial reduction of metal ions

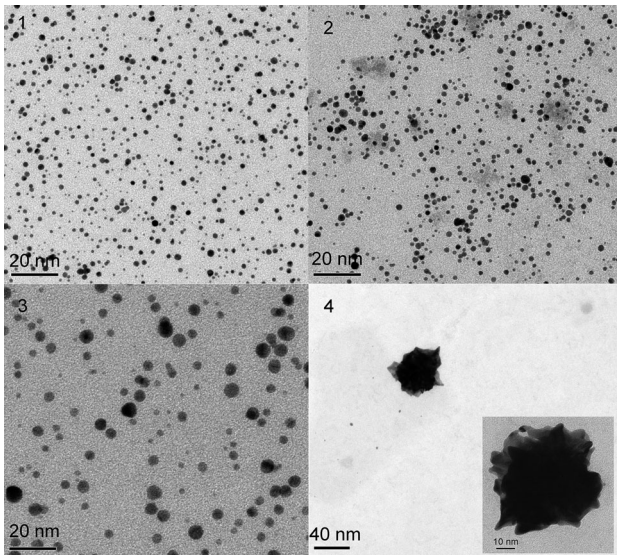

Figure 5. Electron micrographs of synthetic AuNPs (**1**–**4**).

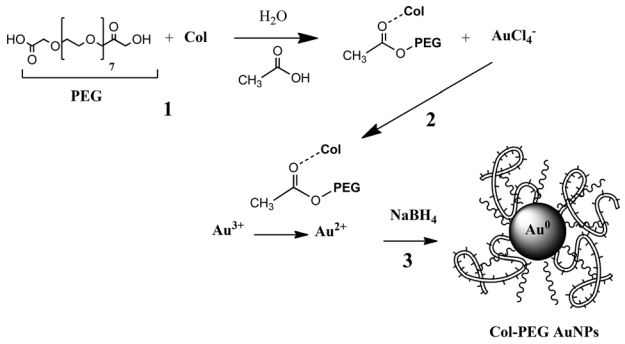

Figure 6. Col-PEG-AuNP reaction during nucleation.

facilitated by Col in acetic aqueous solutions and dicarboxylic acid-terminated PEG to form Au clusters; 3) adsorption of Col-PEG diacid molecules on Au clusters and reduction of metal ions in that vicinity; and 4) growth of AuNPs with some of faceted shape and colloidal stabilization by molecules of PEG polymers (**3**).

Attractive ion–dipole interactions versus repulsive interactions that are due to hydrophobicity between $AuCl_4^-$ ions and a mixture of Col-PEG diacid molecules are important for controlling the competition on the Au seed surface during the NP growth process. TEM micrographs of Col-PEG-AuNPs show a good dispersion of NPs with an average size of 8 ± 0.7 nm (Figure 5-3).

In the synthesis of Col-CTAB-AuNPs (**4**), adding Col changed the color from red to brown and formed snowflake-like NPs of 40 ± 0.7 nm size (Figure 5-4). In a typical AuNP synthesis, Au^{3+} and Au^+ ions form complexes with Br^- of CTAB as follows:

$$AuBr_4^- + 2\,Au^0 + 2\,Br^- \rightarrow 3\,AuBr_2^- \quad (1)$$

The value of the equilibrium constant is 4.92×10^{-6},[5] which indicates that Au^+ ions have a strong tendency to disproportionate in aqueous phase without appropriate ligands. As reported earlier, the extraordinarily strong binding of $AuBr^{2-}$ to the positive CTA^+ head group could stabilize the Au^+ species in aqueous solution. This is due to the cooperative effect of micelles and Br^-, which favors the emergence of soluble Au^+ ions. At the beginning, a small part of Au^+ disproportionate to Au^{3+} and Au^0 species, where the complexation between Au^{3+} and Br^- in the presence of CTAB micelles resulted in the appearance of yellowish-red solution. Collagen and CTAB compete during the synthetic process owing to a higher collision rate of CTAB-Au micelles than that of Col-Au. Consequently, the addiction of Col after $HAuCl_4$ reduction increases the reduction power due to the presence of AA in the solution. This effect is responsible for the change in the final NP shape due to a probable deposition of Col molecules onto Au facet [110].

Collagen can thus be the stabilizer for the AuNP formation under ambient burial conditions of archaeological ivories. It is either arising from the organic fraction of the ivory material or from the fish glue that is rich in collagen (86–93%) obtained from the membrane of the swim bladder of certain species of fish, such as sturgeon, that is used to stick the thin gold foils on the ivory. It is known that fish glue has been used in Mesopotamia and Egypt as early as 3500 years ago and was made by melting over fire and then applying with a brush. Soil constituents slowly dissolve Au foil parts that cause the possible formation of $AuCl_4^-$ ions during the burial time. Because Col is present at the early ivory burial stage, NPs can be formed in a similar above-mentioned process. During diagenesis, Col is degraded, but the NPs can be deposited on the mineral ivory surface, basically HAP, giving rise to the observed purple stains. The mineral phase is preserved in archaeological ivory, in contrast to Col. Even after long-term burial, it can play a supporting role (Figure 7). Today, this finding allows the definition of a new authentication criterion of formerly gilded archaeological ivory sculptures that are very difficult to prove in a non-invasive way.[14]C dating on the ivory requiring sample would be problematic because of the possible re-use of ancient ivory. This new marker could be applied to further precious objects coming from similar contexts, such as from Greece, Mesopotamia, or Egypt.

Experimental Section

$HAuCl_4$, CTAB, $NaBH_4$, $AgNO_3$, AA, PEG-600 diacid, and ethanol (Normapur 99%) were purchased from Sigma Aldrich (Saint-Quentin Fallavier, France), Col as genuine rabbit skin glue plates (no. 63052) from Kremer Pigmente GmbH & Co, KG Aichstetten, Germany, and HAP as bone meal reference material (NIST 1486) were from NIST Gatherburg, MD20899, USA. All chemicals were used as such in Milli Q water. For the description of Col Hyb Au NP syntheses, see the Supporting Information, Section S6.

UV/Vis absorption spectroscopy was performed using a double-beam Varian Cary 500 UV/Vis spectrophotometer for the synthetic samples. PEG-AuNP spectra were recorded between 300–980 nm. The archaeological ivories were measured with a StellarNetInc EPP 2000C apparatus between 400–700 nm. Transmission electron microscopy was performed on a JEOL JEM 1011 microscope operating at 100 KV. The micrographs were taken after separating the surfactant from the metal particles by centrifugation. Typically

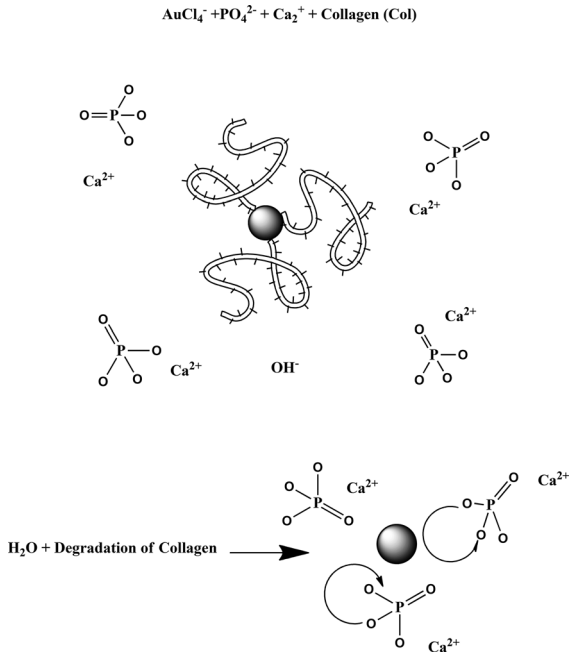

Figure 7. AuNP formation on archaeological ivory: HAP-AuNPs.

1 mL of the sample was centrifuged for 26 min at 15000 r min^{-1}. The upper part of the colorless solution was removed and the solid portion re-dispersed in 1 mL of H_2O. 2 μL of this suspension was placed on a C-coated Cu grid and dried at RT. PM-IRRAS spectra were recorded on a Thermo Nexus spectrometer. The external beam was focused on the sample with a mirror at an incident angle of 80°. A ZnSe grid polarizer and a ZnSe photoelastic modulator, modulating the beam between p- and s-polarizations (HINDS Instruments, PEM 90, modulation frequency = 37 kHz), were placed prior to the sample. The light reflected at the sample was then focused onto aN_2-cooled MCT detector. Spectra were recorded as the sum of 128 scans with 8-cm^{-1} resolution. The external micro-PIXE analyses[26] were performed on a 2 MV tandem accelerator AGLAE (C2RMF, Paris) using a 3 MeV proton beam under He atmosphere using the conditions described in Pichon et al.[27] Quantitative analyses were performed with GUPIXWIN V2.1 by the pivot element method.[28]

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