J Mater Sci (2011) 46:4741–4747
DOI 10.1007/s10853-011-5384-0

Biogenic materialization using pear extract intended for the synthesis and design of ordered gold nanostructures

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Received: 9 September 2010 / Accepted: 9 February 2011 / Published online: 2 March 2011

Abstract We report a facile, green, and biomimetic reduction route coupled with the synthesis and biomolecular-based well-defined self-assembly of gold nanoparticles (AuNPs), without using any organic solvents or any toxic reducing or capping agents. To demonstrate the potential use of pear phytochemicals for the materialization of AuNPs, we propose that the available biomolecules appending on the AuNPs surface and subsequently observed their importance in assembly designs. The spatial array of AuNPs was investigated precisely by using a high-resolution transmission electron microscopy. This method offered biomolecular capping of the AuNPs during and after synthesis and thus provides a promising alternative for nanosurface engineering. The peptide and other biomolecule-based coatings on the AuNPs are applicable for their stability and healthy capping. The hybrid "biomolecular-inorganic" system will be suitable for safe application in medical and diagnostic fields.

Electronic supplementary material The online version of this article (doi:10.1007/s10853-011-5384-0) contains supplementary material, which is available to authorized users.

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Introduction

Interesting physical and chemical properties of nanomaterials mainly depend on the size, shape, and composition of the nanoparticle, and a spatial array of nanomaterial building blocks plays an important role [1–4]. Direct examination of biologically synthesized, nanosized material has become common, whereas the observation of influences on the surface designs of inorganic materials is noteworthy. There are several reasons to utilize biomolecules as nanoparticle building blocks. Biomolecule diversity provides a greater selection of building units, and there are many biological means available to synthesize and modify inorganic materials [5]. DNA, peptides, and proteins bind metals, while semiconductors and ions give us inspiration to design new materials with various sizes and shapes, and various assemblies that have a wide range of applications in nanobiotechnology [6–10]. Bio-conjugated nanomaterials offer exciting opportunities from medicine to electronics at the intersection of nanotechnology and biotechnology [11].

Biological building blocks provide a valuable resource for nanostructure design [12]. The design of environmentally benign systems is also central to exploiting the impact of future technologies. Biological synthesis has focused on synthesizing materials based on molecular interactions between supramolecular organic or bio-organic assemblies and inorganic materials [13]. The functionalization of nanoparticles with biomolecules is expected to lead to biomolecule nanoparticle interactions, and thus to self-assembly [5, 14]. Recently, extensive efforts have aimed to assemble nanoparticles into sophisticated structures [15–19]. Progress in assembling nanoparticles into precise superstructures is a major challenge in the development of more facile and efficient methods; to rationally design and

construct such structures is notably important for the continual development of nanotechnology [20].

Pear fruits are known to contain rich phytochemicals, antioxidants, vitamins, proteins, organic acids, and amino acids. Pear extract contains essential phytochemicals with hydroxy, amino, carboxy, and thio functional groups involved in gold nanoparticle (AuNP) synthesis and healthy capping. Asparagines and serine are the principal amino acids in pear extract, whereas malic acid and citric acid are the principal organic acids. Fructose is the dominant sugar, followed by glucose and sucrose [21, 22]. The peptides, proteins, and saccharides present in pear extract provide rapid, non-enzymatic, and extracellular reduction of gold ions into corresponding AuNPs, subsequently nanosurface engineering via biomolecular capping. We assumed that rapidity in biosynthesis and their capping pattern are sufficient to make this process useful in relation as time function believed to have difference. Bio-inspired AuNPs from time-dependent processes were illustrated for the surface capping of nanoparticles and self-assembled nanostructures partial to the report by [23]. This study will be useful for the rapid preparation of AuNPs via green chemistry principles, and identifies interesting material surface properties based on time-dependent relations. In this article, we propose a green route for rapid production of AuNPs using pear extract as a coupled biomineralization to design the self-assembly of AuNPs.

Experimental details

Pear extract-mediated synthesis and design of AuNPs

Chloroauric acid ($HAuCl_4$) was purchased from Sigma-Aldrich. Fresh, well ripened, and healthy pear fruits were purchased from the local market. Synthesis of AuNPs was started by adding 50% (v/v) pear extract and the pH of reaction mixture was 6.5. The same reaction mixture was further incubated at 50 rpm for 2 h at 30 °C to append biomolecules; in this process, we supposed that rapid synthesis might be insufficient for the capping of available biomolecules. For 2 h we observed the precipitation of incubated particles into superstructures because of intermolecular interactions. Physical properties of the designed AuNPs (including the average core size, morphology, purity, surface capping, crystal structure, and optical properties) were investigated using a transmission electron microscope (TEM) and X-ray diffraction (XRD). Bimolecular and peptide capping were performed by Fourier transform infrared spectroscopy (FTIR) and circular dichroism (CD) spectral analysis to get insights about capping and conformation of peptide chains on AuNPs surfaces intended to design hybrid bio-organic inorganic nanoparticles. Detailed materials and methods are described in supplementary sheet.

Characterization of AuNPs and their self-assembly

The bio-reduction of the $AuCl_4^-$ and its surface plasmon resonance band were studied by sampling aliquots (0.5 mL) and measuring the UV–Visible absorbance between 400 and 700 nm in a 10 mm-path-length quartz cuvette at 1 nm resolution (Shimadzu, UV-1650 PC). Samples for TEM measurements were prepared by dropping casts on carbon-coated lacey films to obtain bright- and dark-field images from self-assembled gold nanostructures. Chemical information of the samples was analyzed using an energy-dispersive X-ray spectroscopy (EDS), which detects the fluorescent X-rays emitted from illuminated regions of the sample.

An aqueous solution of AuNPs was drop-casted onto a glass substrate and dried at 60 °C. XRD data were measured using a X'pert Pro X-ray diffractometer (Phillips). The diffraction data were collected in a θ–2θ mode from 30° to 80°, with a scan speed of 0.02° min^{-1}. A FTIR analysis was performed after the removal of any free residue that was not the capping ligands of the nanoparticles by repeated centrifugation, as discussed above. Subsequently, the purified nanoparticle suspension was drop-casted onto object glass, evaporated at 60 °C, and measured with illuminating ATR-IR equipped with an optical microscope. CD spectral analysis of AuNPs dispersed in water was used to study peptide capping with a 10 mm quartz cuvette. After 5 min at 25 °C, spectra were collected from 250 to 190 nm using a spectrophotometer (Jasco 810). The average of three scans was recorded at a scanning rate of 20 nm min^{-1}, using a response time of 8 s. The peptides on the AuNPs were confirmed by repeated scanning at different concentrations in aqueous suspension.

Results and discussions

The appearance of red color in the reaction mixture was noted immediately after the formation of AuNPs. The growth pattern exhibited a high tendency to initiate, and a complete reduction of 2 mM gold ions was observed within 15–20 min. Analysis of UV–Visible spectrophotometric data from purple solutions confirmed that the SPR band locates at 540 nm for both AuNPs (Fig. 1). Pear extract have ability to reduce Au(III) to Au(0), we believe that the potential reducing power of pear extract is mostly contributed by sugars and unknown peptides and/or proteins. However, this green process presented by pear extract is self-sufficient as electron donor during the bio-reductive precipitation of gold, without using any additional or

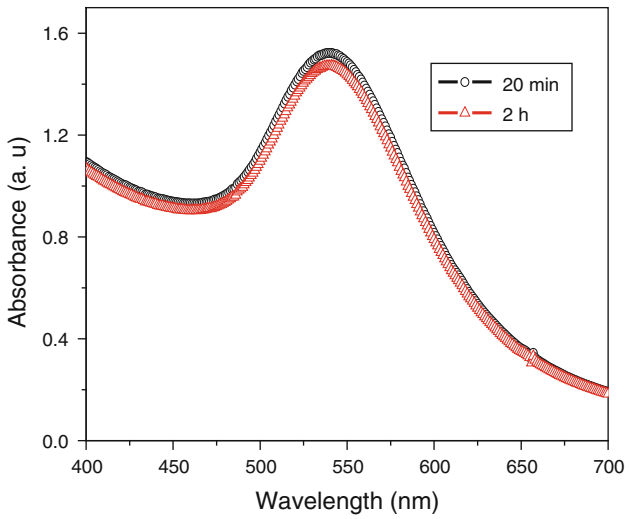

Fig. 1 Absorption spectra of AuNPs in colloidal solution diluted with water (1:1) collected after 20 min and 2 h of incubation with the same reaction mixture of pear fruit extract (Color figure online)

external electron donor. At this stage, it is difficult to conclude any particular electron donors from pear extract, which are involved to reduce Au(III) to Au(0), because the pear extract is the mixture of biomolecules. On the basis of AuNPs surface chemistry, the peptides and/or proteins of pear extract as a key biomolecules engaged in dual function of Au^{III} reduction, subsequently healthy capping of AuNPs. Recently reported by Kumar and Yadav [24], there is need to develop an extracellular green synthesis for the production of metal nanoparticles as an alternative to intracellular microbial methods, because there are possibilities of nanomaterials adsorption to cell wall, intracellular accumulation and thus require the multiple purification steps. However, our result clearly shows that the pear extract-based extracellular biogenic method is as completely safe and rapid route for the synthesis of AuNPs.

The development of high yield, low cost, and safe methods for NPs production is an important challenge. The conversion of $AuCl_4^-$ into nanoparticles was monitored by UV/Vis spectroscopy for SPR (Surface Plasmon Resonance), to know the growth and changes in optical properties of AuNPs. A higher production yield of AuNPs within 20 min of incubation was obtained approximately up to absorbance 1.5 au at 540 nm with 1:1 dilution of original reaction mixture using water as shown in Fig. 1. The conversion of gold ions into AuNPs was rapid and ranges up to 97–98% can be considered a higher production yield within 20 min of incubation at 90 °C. When the solutions were allowed to stand for 2 h after the complete reduction of gold ions, precipitates of AuNPs were formed. However, the UV/Vis spectrum of freshly formed AuNPs within 20 min and the final nanostructures at 2 h demonstrated the same absorbance maxima indicates the establishment of optically stable and identical nature of self-assembled nanostructures (Fig. 1). In addition, the precipitation of incubated particles was the indication of formation of superstructures through intermolecular interactions, thus we successfully developed the green chemistry-based approach for the engineering of surface chemistry and the self-assembled gold nanostructures. In particular, the proposed AuNPs synthesis method was found constructive and extremely reproducible.

The TEM analysis revealed that the nanoparticles are spherical, with an average size of approximately 10 ± 5 nm, and the particles are clustered with sizes from 20 to 50 nm (Fig. 2). Thus, a peripheral interaction of AuNPs is expected to have a tangential driving force when the nanoparticles are aggregated. The aggregation of nanoparticles induced by specific biological interactions attracts interest in the self-assembly process for the construction of complex nanostructures that exhibit new collective properties [25, 26]. As reported earlier, the peptide chains of the AuNPs are responsible for connecting nanoparticles and, thus, forming fibrils via dipole–dipole interactions between the surface peptides [27, 28].

Dark-field S-TEM images of gold nanostructures indicated electron-dense regions (white) and the electron-lucent carbon film background (black) (Fig. 3a). The EDS measurements exhibited strong peaks, corresponding to Au K-, and L-edges, demonstrating that the sample contains AuNPs (Fig. 3b). Copper and weak carbon peaks were also found, which are believed to arise from the carbon-coated copper grid. It was also observed that the nanoparticles form a small nanocluster that constitutes uniform nanochains. Such uniform nanochains are unusual. Structural information of the samples was evaluated by measuring XRD data. Angular positions of the diffracted Bragg peaks (Fig. 4), were compared with standard JCPDS data (card number: 04-0784), and it was confirmed that the nanoparticles have a face-centered-cubic structure with a lattice constant of 4.08 Å.

Spectroscopic studies were performed to better understand the nature of biological capping molecules. Figure 5 shows a FTIR peak at 3301 cm^{-1} that corresponds to the stretching frequency of hydrogen-bonded N–H groups, and a peak at 1657 cm^{-1} that corresponds to the amide-I band [29, 30], which is prominent. However, the amide-I band was reported earlier for the β-sheet [31, 32]. To further explore whether β-sheet formation occurs, we analyzed the assembled species using CD. CD spectral studies were performed to determine the existence and nature of healthy peptide capping in the case of AuNPs synthesized at both the temperatures. The CD spectrum of rapidly synthesized AuNPs demonstrated a negative broad maximum at 217 nm, representing a stable right-handed α-helix structure (Fig. 6). Specifically, we used CD spectroscopy and observed peaks at 222, 217, 213, and 196 nm, which

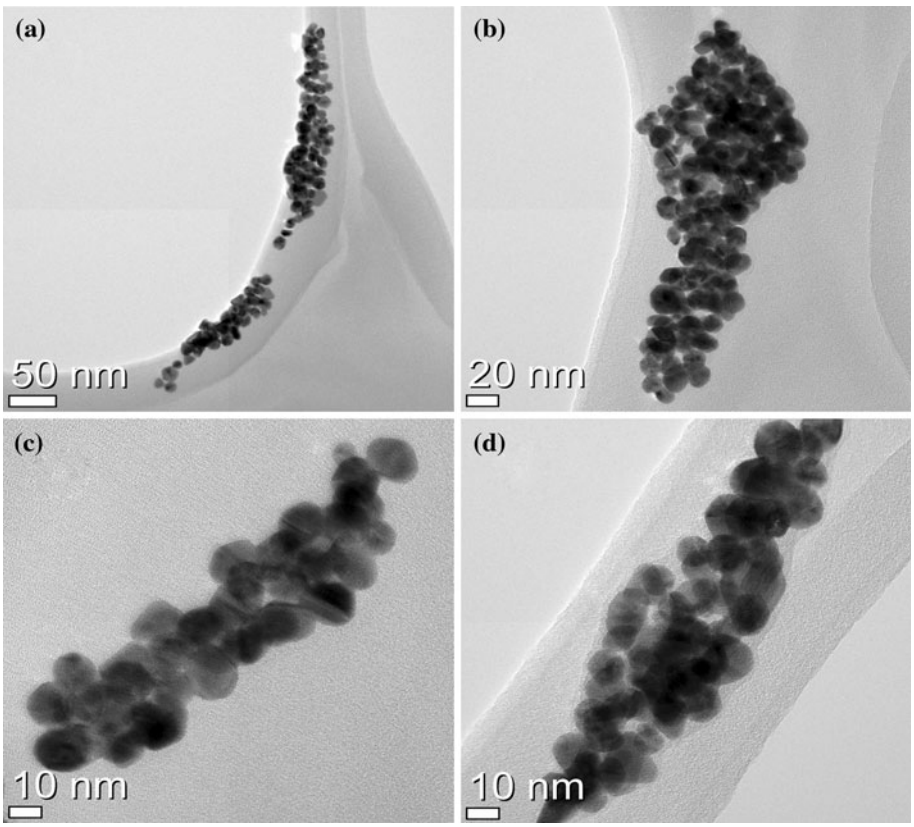

Fig. 2 Representative TEM images are showing a stripe array of self-assembled gold nanostructures obtained after 2 h of incubation (**a**–**d**)

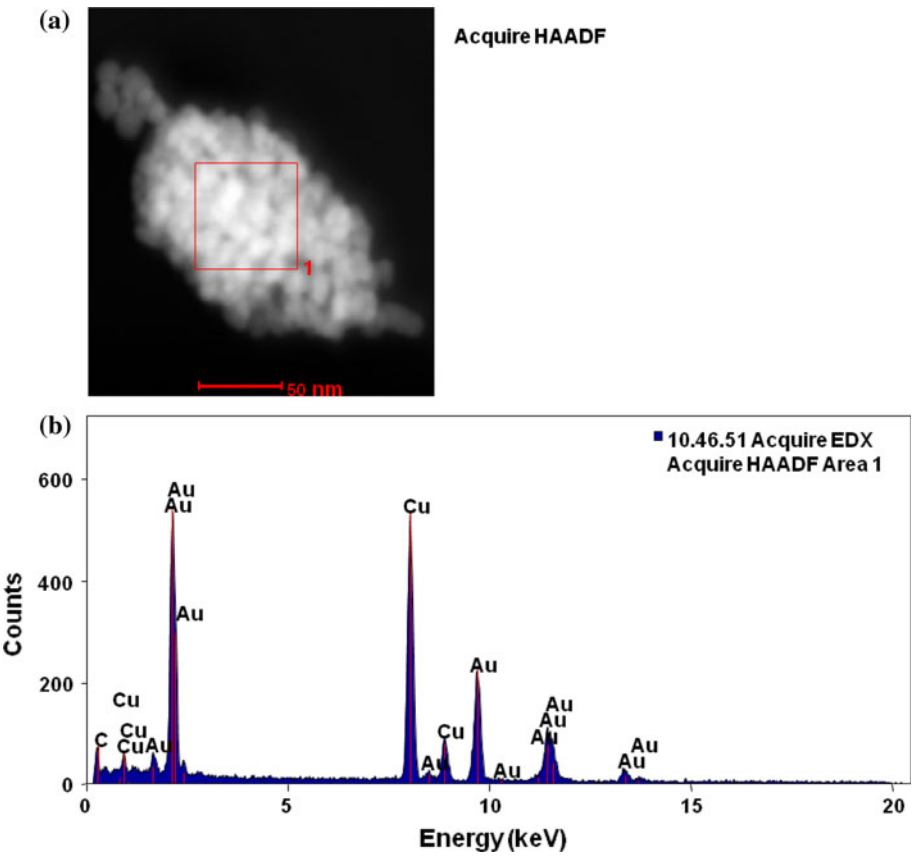

Fig. 3 **a** S-TEM micrographs of the samples obtained after 2 h of incubation and **b** EDS spectra for the selected area (Color figure online)

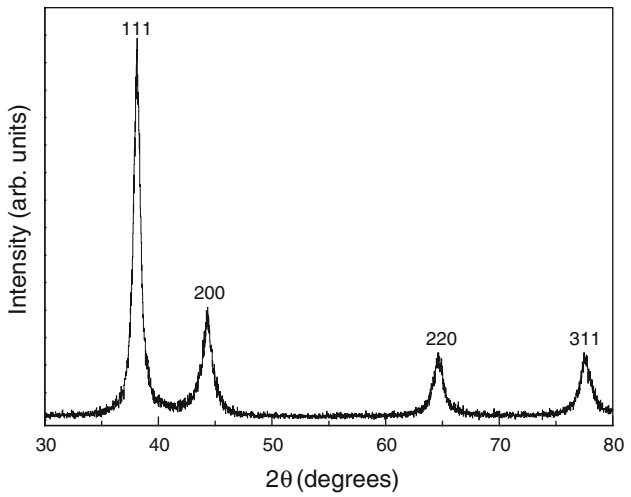

Fig. 4 XRD pattern measured from samples after 2 h of incubation

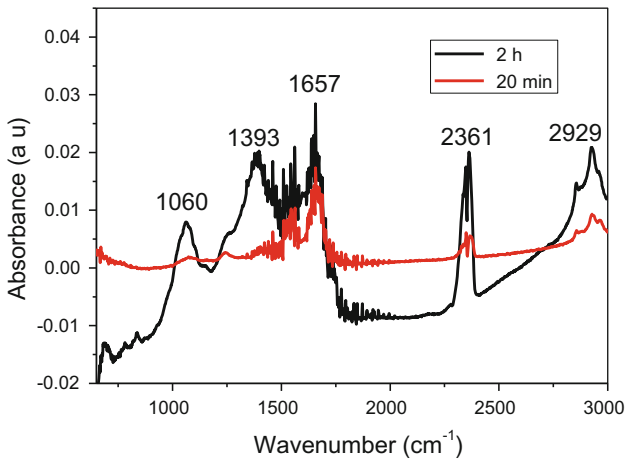

Fig. 5 FTIR spectral analysis of 20 min and AuNPs obtained after 2 h of incubation (Color figure online)

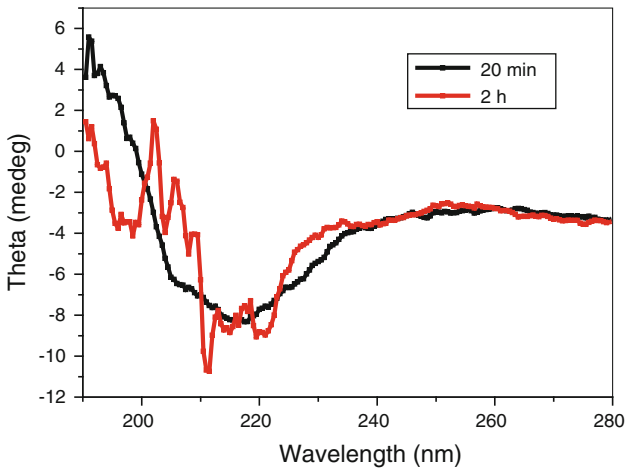

Fig. 6 CD spectral analysis of 20 min and AuNPs obtained after 2 h of incubation (Color figure online)

correspond to the signals produced by a right-handed α-helix with random coil and β-sheet structures. In the past few years, peptides that can form monolayers on nanomaterials have been obtained by a biologically assisted selection process [33]. Proteins and peptides are amino acid polymers that contain a number of reactive side chains. These groups are a part of protein, and serve as "switches" for attaching a wide variety of molecules via different interactions or modifications [34]. Herein, we speculate that peptide-capped AuNPs favor a fluorescent probe attachment, which is mostly controlled by using peptide molecules as a spacer, and is consequently helpful many areas of research. As previously reported, the fluorescent dyes have been conjugated to peptide-coated AuNPs [35]. The unique nanomaterial properties in conjunction with proteins offer particularly exciting opportunities in molecular imaging [36, 37]. Soybean-based biogenic green processes were recently reported to prepare functionalized or hybrid AuNPs to play a crucial role in the overall design and development of AuNP-based nanopharmaceuticals [38].

In addition, we observed a red shift at 222 nm for the nanoparticles obtained after 2 h incubation (Fig. 6), which was initially suggested as efficient for interparticle interactions and supports the formation of meticulous self-assembled nanostructures [39]. Hence, it is clear from these studies that the α-helices in addition to β-sheet and random coil structures play a role in the surface peptide chains. We could also observe self-assembly building higher-order superstructures, which is the result of the repetitive interactions of monomeric building blocks. The folding of immobilized polypeptide on AuNPs and their relation to aggregation was studied by [40]. Importantly, such self-assembled structures are excellent platforms for multivalent ligands that offer high index bonding, instead of a high degree of aggregation. Assuming that peptide secondary structures endorse sticky-ended assembly, we discuss a mechanism at the primary level for the self-assembly of AuNPs. Following nucleation in an axial direction, particles are built-up with ligands from peptides or secondary protein structure, providing proliferation of uniformity and generating self-assembled stripe structures. Building blocks with biomolecular ligands initialize nucleation and follow organization and polymerization via bonding (Fig. 7). The initial organization proliferates and results in self-assembly, mostly through preferred ligands from capped biomolecules and mostly via peptides. Finally, we conclude that the naturally designed biomolecular AuNPs surface templates the self-assembly process, in which they regulate nucleation and orientation (Fig. 7). As reported recently, random coil peptide chains coat the AuNPs core by hydrophobic interaction of the peptide chain and the nanoparticle surface [28]. Thus, nanoparticles that have organic molecules, such

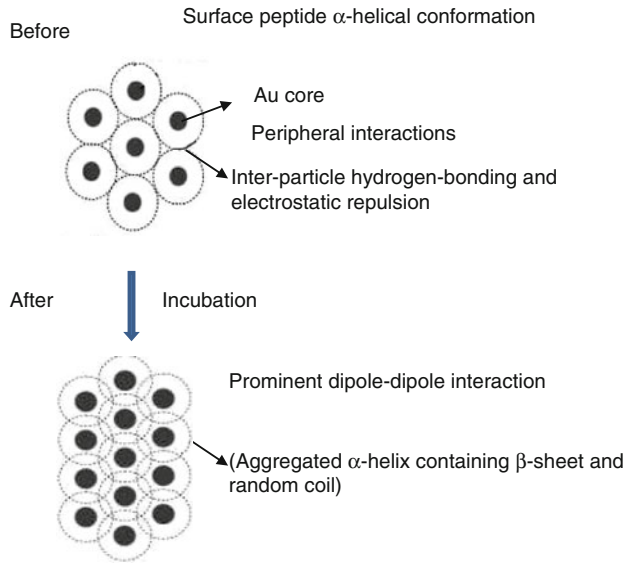

Fig. 7 Schematic representation of AuNP self-assembly before and after incubation (Color figure online)

as peptide building blocks, form ordered and periodic nanoparticle arrangements [41, 42].

Furthermore, we found that this process starts in homogeneous aqueous solution and commences with capped coupling agents for interparticle interactions. The self-assembled nanostructures were studied to integrate the initiators (internal signals) as driving forces for the interparticle interaction. Biomimetic materialization of AuNPs takes advantage of pear extract to rationally design AuNPs surfaces, as demonstrated in self-assembled AuNPs superstructures based on the time function provided for the biomolecular capping. The CD and FTIR, analysis of freshly prepared gold nano crystals suggest that the peptides and/or proteins as a key biomolecules engaged in dual function of Au^{III} reduction subsequently healthy capping of AuNPs (Figs. 5, 6). The friendly organization and/or capping of proteins and peptides with biogenic AuNPs is an example of biomimetic extracellular biomineralization. This would help in understanding the biochemical and molecular mechanism of nanoparticles synthesis and self-assembly. This method is attractive because biocompatible AuNPs can be rapidly produced and stabilized under safe conditions. Previously, we studied that the biocompatibility of obtained AuNPs using pear extract are with human embryonic kidney 293 cells [43]. Accordingly, this green process is suitable for production of nontoxic or biocompatible AuNPs. Natural systems are useful for designing inorganic material and self-assembled structures, because of biomimetic advance. However, such bio-based or biomaterial assembles under constant and ambient physical conditions in response to internal chemical or biological stimuli, which require a signal for initiation, growth, and termination at a precise location and defined time [44], and are still under progress. In this study, we have shown a simple biogenic method to address the assembling of synthesized AuNPs as coupled biomineralization and biomolecular-based, well-defined superstructures in a single step. Consequently, insights of this study will guide understanding of biomimetic surface chemistry, tuning of morphology, and directed-assembly in response to external physical stimuli and their application, are our goals of future studies.

Conclusions

Herein, we reported an efficient, environment-friendly biogenic route as a facile process for the synthesis and hierarchical assembly of nanoparticles through biomolecular nucleation. Pear extract serves dual roles as an effective reducing agent to reduce gold ions and as a stabilizer to provide a healthy coating on AuNPs in a single step. Bio-inspired AuNPs showed time dependence in peptide capping and their self-assembling utility. This initial study establishes the following conclusions: the organized bio-inorganic interface controls the nucleation and organization and thus self-assembly of AuNPs. In the future, this green approach will play an important role in device level applications in opto-electronic and biomedical research fields.

Acknowledgements This study was supported by the National Research Foundation of Korea Grant funded by the Korean Government (MEST) (NRF-C1ABA001-2010-0020501), and was supported in part by grants from the Development of Marine-Bioenergy program and Mass cultivation of marine microalgae and process development of bio-diesel production funded by Ministry of Land, Transport and Maritime Affairs of the Korean Government.

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