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Cancer cells biomineralize ionic gold into nanoparticles-microplates via secreting defense proteins with specific gold-binding peptides

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ABSTRACT

Cancer cells have the capacity to synthesize nanoparticles (NPs). The detailed mechanism of this process is not very well documented. We report the mechanism of biomineralization of aqueous gold chloride into NPs and microplates in the breast-cancer cell line MCF7. Spherical gold NPs are synthesized in these cells in the presence of serum in the culture media by the reduction of $HAuCl_4$. In the absence of serum, the cells exhibit gold microplate formation through seed-mediate growth albeit slower reduction. The structural characteristics of the two types of NPs under different media conditions were confirmed using scanning electron microscopy (SEM); crystallinity and metallic properties were assessed with transmission electron microscopy (TEM) and x-ray photoelectron spectroscopy (XPS). Gold-reducing proteins, related to cell stress initiate the biomineralization of $HAuCl_4$ in cells (under serum free conditions) as confirmed by infrared (IR) spectroscopy. MCF7 cells undergo irreversible replicative senescence when exposed to a high concentration of ionic gold and conversely remain in a dormant reversible quiescent state when exposed to a low gold concentration. The latter cellular state was achievable in the presence of the rho/ROCK inhibitor Y-27632. Proteomic analysis revealed consistent expression of specific proteins under serum and serum-free conditions. A high-throughput proteomic approach to screen gold-reducing proteins and peptide sequences was utilized and validated by quartz crystal microbalance with dissipation (QCM-D).

Statement of significance

Cancer cells are known to synthesize gold nanoparticles and microstructures, which are promising for bioimaging and other therapeutic applications. However, the detailed mechanism of such biomineralization process is not well understood yet. Herein, we demonstrate that cancer cells exposed to gold ions (grown in serum/serum-free conditions) secrete shock and stress-related proteins with specific gold-binding/reducing polypeptides. Cells undergo reversible senescence and can recover normal physiology when treated with the senescence inhibitor depending on culture condition. The use of mammalian cells as microincubators for synthesis of such particles could have potential influence on their uptake and biocompatibility. This study has important implications for *in-situ* reduction of ionic gold to anisotropic micro-nanostructures that could be used *in-vivo* clinical applications and tumor photothermal therapy.

1. Introduction

Metal and polymeric nanoparticles (NPs) are utilized in a wide range of biomedical applications such as radiotherapy, drug delivery, x-ray imaging due to their size and shape dependent biophysical and optoelectronic properties [1–3]. Metal NPs suitable for biomedical applications are synthesized using a broad range of microbes, such as actinomycetes, algae, bacteria, fungi, viruses, and yeasts [4–6]. It is shown that these particles can also be synthesized by plants [5] and mammalian cells [7]. Using such cells as biological vehicles for the biosynthesis of NPs is highly beneficial, due to their hierarchical structuring, safety, cost-effectiveness, and negligible carbon footprint [8]. Currently, the biological routes for the synthesis of shape- and size-controlled metal NPs are not fully explored. There is documented evidence

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for bacteria [9], diatoms [10] and calcite [11] serving as ideal platforms for the bulk synthesis of NPs [10,12]. However, there is a paucity of their use for biomedical applications due to difficulties in controlling shape and size, which effects long term cell viability.

The biological platforms mentioned above are capable of creating anisotropic NPs in a controlled environment by utilizing polypeptides. These polypeptides bind specifically to inorganic surfaces as functional molecules and/or cellular components such as cell membrane vesicles [13,14]. The formation of gold (Au) NPs are very well documented in human embryonic kidney (HEK293), neuroblastoma (SKNSH) and cervical cancer (HeLa and SiHa) cell lines [7,15]. While the biosynthetic pathway remains elusive, it is postulated that cellular components such as redox enzymes and carbohydrates are a requirement [15]. In the current research paper, we delineate a detailed mechanism of NPs and anisotropic triangular or prismatic microstructure formation in mammalian cells. The process of gold biomineralization is investigated by utilizing the cancer cell line MCF7 as a model, which secretes specific proteins with gold-binding polypeptides (GBP). Additionally, the impact of the biomineralization process on the overall cellular health is investigated. We demonstrate that facet-specific GBP sequences are used as regulating agents for the highly controlled synthesis of nanocrystals with particular shapes.

2. Materials and methods

2.1. Synthesis of NPs and microplates with cell lines

Human breast adenocarcinoma (MCF7) and mouse myoblast (C2C12) cell lines were used as model cells in this investigation. Both cell lines were maintained in T25 culture flasks containing Dulbecco's Modified Eagles Medium (DMEM) supplemented with 10% heat inactivated fetal bovine serum (FBS) and 1% antibiotic (Penicillin/Streptomycin) solution at 37 °C (5% CO_2). Once cells reached a confluent monolayer, they were incubated with filter sterilized Gold (III) chloride hydrate ($HAuCl_4$, Sigma Aldrich) prepared in DMEM (serum containing conditions) or phosphate buffered saline (PBS- serum free conditions) at a final concentration of 1 mM. Four negative controls were used. These consisted of (a) dead cells incubated in PBS only; (b) live cells incubated in PBS and $HAuCl_4$; c) live cells incubated in PBS only and d) PBS with $HAuCl_4$ respectively. Cells were incubated between 36 and 48 h, during which anisotropic prisms formed. Under serum containing conditions, the visual hallmark was a change in the color of the cell culture medium (red to dark pink). No color change was observed in the negative controls. In PBS, gold solutions are stable for months and hence the possibility of autoreduction was ruled out. Human umbilical vein endothelial cells (HUVECs) and human adipose derived stem cells (ADSC) were obtained from Lonza, Germany. Cells were cultured in endothelial growth medium (EGM) and Human Adipose Derived Stem Cell growth medium supplemented with their respective growth factor kits (Lonza, Germany).

2.2. X-ray photoelectron spectroscopy

Samples were prepared by fixing the cells in 4% Paraformaldehyde in PBS for 30 min, dehydrating in graded ethanol (1%–25%–50%–70%–100%) for 10 min each. Samples were then critical point-dried (Leica, EM CPD300, Germany). NPs and microplates suspended in deionized water were drop coated onto silicon wafer chips (5×6 mm) and air-dried overnight. Energy calibration, removal of contaminants, and charge compensation were carried out while recording measurements. The XPS spectra were deconvoluted with the Thermo VG Scientific Advantage software version 5.47 (Thermo Fisher Scientific). XPS analysis was performed with a Thermo VG Thetaprobe 300 (Thermo Fisher Scientific) system using monochromatic incident Al Kα radiation ($h\nu$ = 1486.68 eV; spot size 400 μm; base pressure <10–7 Pa; average detection angle of 53° with respect to the sample surface).

2.3. Fourier transform infrared spectroscopy analysis

Chemical imaging of cells in the presence and absence of $HAuCl_4$ was conducted with a Bruker Tensor II spectrometer over an attenuated total reflectance (ATR) sensor. Cells grown to 100% confluence on flat glass substrate were treated with gold ions for 4 days in a tissue culture incubator. Thereafter, pending media removal cells were washed with PBS and fixed with 4% paraformaldehyde in PBS for 30 min. Prior to IR analysis, the slides were washed with Hank's buffered saline for 3 times. The sample was placed over the ATR-crystal carefully and pressed with a platinum diamond ATR-accessory to avoid uneven in thickness and facilitate close contact of the sample with the ATR crystal [16]. IR Spectra were recorded into absorbance mode with 4 cm^{-1} resolution and 1024 scans. Data analysis was performed with OPUS software (Bruker).

2.4. pH and UV–vis spectroscopy analysis

Changes in the pH or time dependent optical properties were assessed periodically after every 24 h via sampling of aliquots (1 mL) of the aqueous component (LAQUA, HORIBA Scientific, Germany). UV–visible spectra of the solution was recorded using an UV–visible spectrophotometer (Synergy ZEN 5, Biotek Instruments).

2.4. Dynamic light scattering and zeta potential measurement

The hydrodynamic diameter of the anisotropic NPs was determined using a light scattering instrument (MÖBIUζ® analyzer, Wyatt technology, Germany) with an ATLAS pressurization system. The zeta potential was calculated from measurements of electrophoretic mobility performed by the same instrument.

2.5. Scanning electron microscopy

Scanning electron microscopy (SEM) samples were prepared by incubating cells with 1 mM $HAuCl_4$ for 4 days. Thereafter, cells were fixed in 2.5% glutaraldehyde in PBS for 45 min at 4 °C, rinsed with PBS, then water. The samples were dehydrated in an increasing ethanol gradient (30%, 50%, 70% and 90%) for 5 min followed by a 10 min incubation in 100% ethanol. Residual alcohol was removed using an automated critical point dryer (Leica EM, CPD 300). Silicon wafers with cells were air dried and sputter coated with a 5 nm coating of nickel using a Leica coating system (Leica EM, ACE600) and a Zeiss Ultra 55 Gemini scanning electron microscope equipped with a nitrogen cooled EDAX detector system was used for imaging and element dispersive x-ray spectroscopy (EDX) of MCF7 cells containing NPs/microplates. An accelerating voltage of 5 keV and INLENS detector was used for imaging.

2.6. Transmission electron microscopy

MCF7 cells grown in T25 flasks to confluency were harvested using routine trypsinization, resuspended in PBS and centrifuged (2000 rpm, 5 min thrice). Cell pellets were fixed in 3% glutaraldehyde in HEPES buffer (pH 7.4) and dehydrated with graded ethanol (30%, 50%, 70% and 90%) for 5 min followed by a 10 min incubation in 100% ethanol) prior to embedding in epoxy 618. A microtome (EM-UC6) from Leica Co, Austria was used to cut ultrathin sections of samples onto a glass coverslip and stained with uranyl acetate

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