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A silica-gold core-shell structure to mimic the large size of gold particles for promoting cell growth: A comparative study of the silica core size and the nanogold amount in the shell

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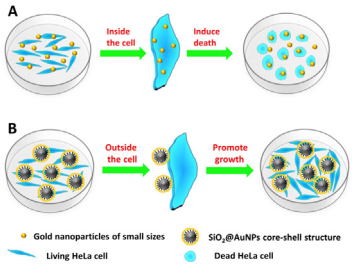
HIGHLIGHTS

- We prepare SiO_2@AuNPs core-shell materials by seed-mediated method.
- SiO_2@AuNPs can promote the growth of HeLa cells, while 4 nm AuNPs lead to cell death.
- The silica substrates hold their attached AuNPs outside the cells.
- The concavo-convex surface of SiO_2@AuNPs is facilitated to cell adhesion, probably resulting in the proliferation of HeLa cells.

GRAPHICAL ABSTRACT



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ABSTRACT

Recently, the biosafety of gold nanomaterials has attracted more attention. Some consider them as an adverse factor for cell growth, whereas others regard them as biocompatible. In this work, we reported a type of silica-gold core-shell structure (SiO_2@AuNPs) with the diameter more than 70 nm that could promote cell growth. The synthesis of SiO_2@AuNPs adopted a step-by-step protocol involving the fabrication of silica substrate followed by their amination, the attachment of fine (about 4 nm) gold seeds, and the formation of a gold shell by reducing $HAuCl_4$ on seeds. The cell viability experiment indicated that AuNPs attached to silica substrate could promote the growth of HeLa cell. With increasing concentration of SiO_2@AuNPs or the content of gold shell, the viability of co-cultured HeLa cells also increased; on the contrary, acute cytotoxicity was observed when pure 4 nm AuNPs were added. Moreover, we found that the proliferative effect could be enhanced by enlarging the size of silica substrate. Due to large SiO_2@AuNPs not entering the cell, their nano-size concavo-convex surface was facilitated to cell adhesion, probably leading to the proliferation of HeLa cells. However, detailed mechanism responsible for cell growth is still unclear and it requires more studies. Although the mechanism remains enigmatic, the findings may have practical significance for cell proliferation, which is in great demand in numerous fields such as tissue engineering. In addition, the core-shell materials were characterized by scanning electron microscope (SEM) and energy-dispersive X-ray spectrum (EDX), and the cell viability was measured by Cell Counting Kit-8 (CCK-8).

1. Introduction

Quite a few literatures hitherto reported the influence of gold nanomaterials upon various cell, indicating that these particular materials were promising candidates for future biomedical

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applications, for instance, biological imaging [1–3], cancer diagnosis and therapy [4–7], gene and drug delivery [8–11], and tissue repair [12,13]. However, there were numerous conflicting reports about the cytotoxicity and proliferation-promoting effect owing to their variable physicochemical properties, which depended on their diameter, shape, and surface modification. Particle size was considered as a key physical parameter that controlled endocytosis effectiveness [14]. As the particles approached nanoscale, they could reach places where large ones could not enter. At the size of less than 50 nm [15], AuNPs easily penetrated into cells and caused cytotoxicity, which resulted from the disruption to DNA [16,17] or the generation of reactive oxygen species (ROS) [18,19] in the blood plasma. Pan et al. [17] showed that small gold clusters had strong potential toxic effect because of the combination with DNA grooves, but the toxicity of large gold nanoparticles sharply decreased. And Li et al. [20] indicated that AuNPs created an oxidative environment and induced cell death. However, if AuNPs could not enter the cell, the findings would be different. Cui et al. [21] observed that when small AuNPs turned into large aggregations on solid plates, they were not toxic to the cell but instead promoted growth. Similar data were recorded by Gu et al. [22], who immobilized 24-nm-gold nanoparticles onto gold film and found that they could enhance hepatocytes proliferation. Likewise, when human mesenchymal stem cells (MSCs) were co-cultured with silk nanofibers (SNFs) decorated with AuNPs (SNF_{Au}), SNF_{Au} induced an increase in the area and the number of cells compared to bare SNFs [23]. These literatures noted that it was fixing gold nanoparticles on one substrate that played an important role in promoting the growth of cells.

To understand fully the effect of large substrates and to develop a new type of platform for investigations, we used silica as a microscopic substrate and prepared SiO_2@AuNPs core-shell materials by seed-mediated growth. We synthesized SiO_2 cores of different sizes from 70 to 500 nm and controlled the amount of gold nanoshell. The influence of the size of SiO_2 core and the amount of gold nanoshell on the viability of co-cultured human cervical carcinoma cells (HeLa cells) was studied. The structure of SiO_2@AuNPs and the content of gold nanoshell were characterized by scanning electron microscopy (SEM) and energy-dispersive X-ray spectrum (EDX), respectively, and the cell viability was characterized by the Cell Counting Kit-8 (CCK-8) assay.

2. Experimental

2.1. Materials

Tetraethoxysilane (TEOS), ammonium hydroxide (NH_4OH, 25% in water), ethanol, and potassium carbonate (K_2CO_3) were obtained from Sinopharm Chemical Reagent Beijing Co. Ltd. (3-Aminopropyl)trimethoxysilane (APTES) was obtained from Aladdin Chemical Co. Ltd. Sodium borohydride ($NaBH_4$) was purchased from Sigma Chemical Co. Poly(*N*-vinylpyrrolidone) (PVP; K-30, $M_w \sim 40{,}000$ g/mol and polymerization $n = 360$) and chloroauric acid ($HAuCl_4 \cdot 4H_2O$) were obtained from Shanghai Reagent Factory. HeLa cell lines were purchased from the American Type Culture Collection (ATCC). Dulbecco's modified Eagle's medium (DMEM), phosphate-buffered saline (PBS buffer; 0.01 M, pH 7.4), newborn calf serum (NC serum), and trypsinase were obtained from Gibco BRL (Grand Island, NY, USA). Cell Counting Kit-8 (CCK-8) was purchased from Dojindo Laboratories, Japan. Polystyrene tissue culture plates (48-well and 96-well) were obtained from Coring Inc. All chemicals and solvents were analytical grade and were used without further purification. Ultrapure water (18 MΩ, prepared from Millipore Q-POD purification system) was used as the solvent unless indicated otherwise.

2.2. Fabrication of SiO_2@AuNPs core-shell materials

SiO_2@AuNPs were designed and fabricated as described in previous studies [24]. Initially, silica nanoparticles with different sizes were grown by Stöber method [25], in which TEOS was reduced by NH_4OH in ethanol in the presence of H_2O. The particle surface was terminated with amine groups by reaction with APTES. Small AuNPs of approximately 4 nm in diameter was synthesized as described [26], and then was attached to the aminated silica surface, which acted as nucleation sites. Gold nanoshells were grown in a solution of $HAuCl_4$ containing potassium carbonate (K_2CO_3) in the presence of the reductant (formaldehyde) and the protectant (PVP). This process reduced additional gold onto the adsorbed gold nanoparticles, causing the small gold nanoparticles to grow and coalesce with neighboring particles, eventually forming a complete metal shell.

2.3. Characterization

SEM images of SiO_2@AuNPs with different sizes were recorded on a scanning electron microscope (Hitachi S4800, Tokyo, Japan) operates at 10 keV. For the SEM observation, samples were obtained by dropping 5 μL of SiO_2@AuNPs solution onto silicon wafer, followed by air-drying. All the images were visualized without staining.

The amount of gold nanoshell was determined using energy-dispersive X-ray spectrum (EDX, Hitachi S4800) operated at 10 keV. For EDX measurement, samples were obtained by dropping 5 μL of SiO_2@AuNPs solution onto aluminum foil and then allowed to dry in ambient air. The data acquisition time was 120 s for one accumulation. To test reproducibility, the measurements were performed at different positions on each sample.

2.4. Cell viability measurement

HeLa cells were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with penicillin/streptomycin (100 units/mL) and 10% (v/v) newborn calf (NC) serum at 37 °C in a humidified atmosphere containing 5% CO_2. Cells were harvested by trypsin-EDTA detachment and centrifuged. The cells were subsequently resuspended for subculture or other uses.

To assess the effect of the SiO_2@AuNPs on the viability of HeLa cells, the Cell Counting Kit-8 (CCK-8) assay was used. HeLa cells in the exponential growth phase were seeded into 96-well plates at a density of 10^4 cells/well and allowed to adhere for at least 12 h at 37 °C in a humidified atmosphere containing 5% CO_2. When HeLa cells achieved 50–60% confluence, SiO_2@AuNPs samples of a series of increasing concentrations were added with 10 μL/well. After incubation for 24 h, CCK-8 was used according to the protocol specification to evaluate the cell viability. The absorbance (OD value) of every well was measured at 450 nm using Multilabel Plate Reader (Perkin Elmer). Cell viability was assessed by calculating the percentage of the OD value with respect to the control experiment without sample (as shown in Eq. (1)). Cells that were cultured under the same volume of DMEM without samples served as the positive control (OD_+), and DMEM without any cells served as the negative control (OD_-). All experiments in this study were performed at least four times.

$$\text{Cell viability} = \frac{OD_{sample} - OD_-}{OD_+ - OD_-} \quad (1)$$