



An Examination of Photonic Nanoparticles' Potential for Causing Nanotoxicity in Cultivated Cells In vitro

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ABSTRACT

Although optical nanoparticles (NPs) have the potential to offer new diagnostic and therapeutic tools for human diseases, it is yet unknown how biocompatible and hazardous they are at the nanoscale. Here, for a test on nanotoxicity, we created a number of common NPs (such as gold nanospheres, gold nanorods, silver nanospheres, silver triangular nanoplates, and quantum dots) with various materials and surface chemical modifications. SRB assay was used to examine cell proliferation in the co-culture of cancer cells with NPs. It was discovered that a number of variables, including material choice, physical size/surface area, shape, and surface chemical property, were strongly connected with the toxicity of NPs. When used as probes in the disciplines of biology and medicine, this work has the potential to deliver consistent and organised information.

Keywords: Nanotoxicity; Gold nanospheres; Gold nanorods; Silver nanospheres

INTRODUCTION

The toxicity of nanoparticles (NPs) is a significant concern in nanotechnology due to the field's fast expanding attention. However, nothing is understood regarding the toxicity and health concerns of these nanomaterials. Additionally, NP side effects are a developing and growing academic and social issue as a result of the expanding production and use of NPs in numerous domains [1]. These preliminary toxicity investigations have led to recommendations for the usage of NPs in a variety of sectors. The toxicity of NPs has been studied in the past based on a number of variables, including form, size, surface chemistry, chemical composition, surface activity, and solubility. For instance, several studies on the nanotoxicology of carbon nanomaterials, ferric oxide NPs, and TiO₂ NPs have been completed [2]. Over the past ten years, there has been a lot of research done on the uses of nanoparticles (NPs) in biomedicine, particularly those that exhibit optical features for drug and bio-labeling, biosensors, cancer treatment, and diagnostic tools. Due to their unique physical-chemical characteristics, NPs with optical features are frequently used in biological domains. Examples include metal plasmonics NPs such as semiconductor quantum dots (QDs), gold nanospheres, silver nanospheres, and gold nanorods. Because biological safety has examined the cytotoxic and genotoxic assessment of glycolipid-reduced/-capped gold and silver spherical NPs, the nanotoxicology of these NPs is particularly noteworthy [3]. They discovered that gold nanoparticles are more cyto-compatible than comparable

silver nanoparticles, proving that silver nanoparticles can damage DNA more than gold nanoparticles. Chan discovered that the poly diallyldimethylammonium chloride-poly 4- styrenesulfonic acid system covering on the gold nanorods' surface had little to no effect on how cells functioned. The effect of surface chemistry on the absorption, toxicity, and gene expression of gold nanorods in mammalian cells results in any meaningful change in the expression of genes. Clift has looked at the effects of oxidative stress and how different surface-coated QDs on macrophage cell signalling in vitro [4].

The QDs' toxicity caused by cellular interaction is dose-dependent. The anionic QDs interact with cells less effectively than the cationic and hydrophobic QDs. More importantly, the most hazardous QDs are the cationic ones; however, the toxicity of these QDs can be decreased by coating them with poly(ethylene glycol, or PEG). Su et al. studied the cytotoxicity of aqueously produced CdTe QDs and the relative contributions from released cadmium ions. They showed that the cytotoxicity of the CdTe QDs will be significantly reduced following surface passivation with CdS and ZnSe shell. Currently, the majority of nanotoxicity investigations are concentrated on a single variable quantity for the control trials, while also examining each result independently [5]. Additionally, the surface of the utilised NPs is frequently adjusted by substituting the original surface group from the batch of conventional synthesis, where the experiments are less applied and followed. However, in order to confidently control the safe use of all forms of NPs with

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optical utilities, a more thorough assessment of nanotoxicology on the same platform is required. There has not yet been a published study on the nanotoxicology of all the widely used optical NPs in a single platform or system. In this study, we made biologically acceptable aqueous gold nanospheres, gold nanorods, silver nanoplates, silver triangular nanoplates, and CdTe/CdS core/shell QDs. We next tested the nanotoxicity of these NPs in human cervical carcinoma Hela cells and human hepatocarcinoma cell line HepG2. These two cell lines were frequently employed to assess the cytotoxicity of anticancer medicines using the Sulforhodamine B (SRB) assay because they exhibit better endocytosis than other cell lines. The toxicity of nanoparticles is closely related to a number of variables, including the choice of material, size and surface area, appearance shape, and surface chemical properties. According to our findings, all nanoparticles' nanotoxicity is dosage-dependent [6].

MATERIALS AND METHODS

Penicillin, streptomycin, DMEM high glucose medium, and foetal bovine serum (FBS) were purchased from the Beyotime Institute of Biotechnology. They were acquired from Sigma-Aldrich along with sulforhodamine B (SRB), trichloroacetic acid (TCA), chloroauric acid trihydrate, and sodium borohydride. L-ascorbic acid (99.5%) and hexadecyltrimethylammonium bromide were bought from Sigma. We bought silver nitrate (AgNO_3 , 99%) from Fluka. The resistivity of the deionized water after purification by a Milli-Q water purification system was 18.2 M cm [7].

Characterization of Gold Nanospheres

GNSs were created using the typical sodium citrate reduction procedure. Usually, a three-neck flask was used to combine 45 mL of deionized water with 5 mL of HAuCl_4 (2.5 mM) aqueous solutions. The flask was then heated to 100 C. 1% sodium citrate solution was then swiftly poured into the flask. The tint yellow colour of the solution quickly turned to black, red, or deep purple [8]. The GNSs were cooled to room temperature after an hour while awaiting the subsequent nanotoxicity assessment. The additional sodium citrate's volume might be adjusted to adjust the size. The size of the as-prepared GNSs was around 15 nm and 30 nm, as demonstrated by the field-emission scanning electron microscope, respectively. Peaks in plasmon absorption were found at 520 nm and 525 nm [9].

Characterization of Gold Nanorods

Traditional seed-mediated growth techniques were used to create gold nanorods, starting with the reduction of Au salt in water at room temperature using a potent reducing agent. Simple reduction of HAuCl_4 (0.25 mM) in CTAB solution with the reduced reagent—ice-cold sodium borohydride—was used to create the Au seed particles [10]. Reduction of additional metal salts afterward in the presence of additives that influence structure, a weak reducing agent causes the controlled production of gold nanorods. In a nutshell, 0.5 mM HAuCl_4 was reduced in a solution containing CTAB, 0.05 mM silver nitrate, and 176 L of ascorbic acid to create a 25 mL growth solution. After adding ascorbic acid, the solution's colour quickly changed from golden to colourless. The growing fluid's colour gradually changed from colourless to deep purple when 36 L of the seed solution was added. After several hours of stirring, the bilayer CTAB-coated gold nanorods were finally produced. According to the FESEM, the prepared nanorods were

primarily 15 nm wide and 44 nm long [11].

Characterization of Silver Triangular Nanoplates

The photo conversion method was used to conduct a typical experiment. First, silver seeds were made by adding a drop wise amount of NaBH_4 solution (8 mM, 1 mL) to an aqueous solution of AgNO_3 (0.1 mM, 99 mL) while vigorously stirring and in the presence of disodium citrate (1 mM). The yellow silver seeds were then exposed under a sodium lamp (the NAV-T 70 model from Osram China Lighting Co., Ltd.) for 100 mL. By measuring the sodium lamp's irradiation power density at the location of the silver solution with a Gentec-EO Solo 2 energy and power metre, it was found to be 80.6 mW/cm^2 . During the irradiation process, the colour of the silver colloids changed from yellow to green and blue [12]. The STNPs were ready and waiting for the subsequent nanotoxicity measurement after two hours. The size of the STNPs as-prepared was around 60 nm, as can be seen in the FESEM of Fig. 1E. Peak absorption of the in-plate dipole plasmon resonance is located at 636 nm.

RESULT

Hepatocellular carcinoma in humans HepG2 cells and human cervical cancer Hela cells were kept alive at 37 degrees Celsius in DMEM high glucose media supplemented with 10% FBS, 100 units/ml penicillin, and 100 g/ml streptomycin. In order to determine cytotoxicity, the SRB assay is frequently employed. This method is based on the measurement of the protein content of live cells. After a 48-hour treatment period, it was carried out in 96-well plates eight times. In a nutshell, cells were plated overnight at a density of 5×10^3 cells per well and exposed to the appropriate NP concentrations during 48 hours. After cooling the culture media in each well for 3 hours at 4 degrees Celsius with 100 L of 20% TCA, the supernatant was removed. Plate cleaned with water five times and let to air dry. Each well received 100 L of SRB solution 0.4% (w/v) in 1% acetic acid, which was then incubated at room temperature for 30 minutes. The plates were air dried after unbounded SRB was brushed off of them. 150 L of 10 mM Tris-HCl were added to each well to solubilize the bound SRB, and the plate was then shaken for 5 minutes. The optical density (OD) at 570 nm wavelength was measured, and the SRB results were used to calculate the ratio of cell viability to the control group.

CONCLUSION

Cellular delivery, subcellular transport, and cellular endocytosis targeting a nanoparticle's ability to cause nanotoxicity. Several factors have an impact. A nanoparticle firstly has a high surface-to-volume ratio, therefore any slight modification in the surface functional group or particle size, shape, or composition may cause a substantial change in how cells interact. Cons thereafter, the subcellular uptake, cytotoxicity, and NP localisation is strongly dependent on particle size, the nature of the form, surface charge, hydrophobicity, and ligands on the skin. Additionally, larger particles and a higher Surface charge would frequently result in high nonspecific rapid absorption of NPs, which made the uptake even more challenging diminishing the specificity of affinity ligands to increase their NPs. Third, NPs frequently land at lysosomes and stop cell-level targeting.

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