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Biocompatibility of gold nanoparticles in retinal pigment epithelial cell line

Bedia Begüm Karakoçak [a,b,1], Ramesh Raliya [a,1], Josh T. Davis [b,c], Sanmathi Chavalmane [a], Wei-Ning Wang [a,d], Nathan Ravi [a,b,c,*], Pratim Biswas [a,**]

[a] Aerosol and Air Quality Research Laboratory, Dept. of Energy, Environmental and Chemical Engineering, Washington University in St. Louis, St. Louis, MO, USA
[b] Department of Ophthalmology and Visual Sciences, Washington University in St. Louis, St. Louis, MO, USA
[c] Veterans Affairs Medical Center, St. Louis, MO, USA
[d] Department of Mechanical and Nuclear Engineering, Virginia Commonwealth University, Richmond, VA, USA

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ABSTRACT

Gold nanoparticles (Au NPs) have been tested as targeted delivery agents because of their high chemical stability and surface plasmon properties. Here, we investigated the biocompatibility of Au spheres (5-, 10-, 20-, 30-, 50-, and 100-nm), cubes (50-nm), and rods (10 × 90 nm) on a retinal pigment epithelial (ARPE-19) cell line. The lethal dose for killing 50% of the cells (LD_{50}) was evaluated using an MTT (3-[4, 5 dimethyl-thiazoly-2-yl] 2-5 diphenyl tetrazolium bromide) assay. At and above LD_{50}, based on mass concentrations, the confluent cell layer began to detach, as shown by real-time measurements of electric impedance. We found that the biocompatibility of spheres improved with increasing nanoparticle size. The Au rods were less biocompatible than 10-nm spheres. Confocal microscopy showed that cubic (50-nm) and spherical NPs (50- and 100-nm) neither had cytotoxic effects nor entered cells. Lethal doses for internalized spherical NPs, which were toxic, were recalculated based on surface area ($LD_{50,A}$) concentrations. Indeed, when biocompatibility was expressed as the surface area concentration of NPs, the curve was independent of size. The $LD_{50,A}$ of Au nanospheres was 23 cm^2/ml. Our findings demonstrate that the sole modulation of the surface area would make it possible to use Au NPs for therapeutic purposes.

1. Introduction

Materials at the nanometer scale have a large surface area to mass ratio, which gives them novel properties that differ markedly from those of other material properties with corresponding bulk sizes. Recent advances in the synthesis and biomolecular functionalization of engineered nanoparticles have led to a dramatic expansion of their potential biomedical applications, including their use as nanoprobes (Leduc et al., 2013), nanosensors (Swierczewska et al., 2012) and in bioimaging (Peng et al., 2006; Ruiz-Ederra et al., 2005), photothermal therapy (Ryu et al., 2012), gene therapy (Raju et al., 2011; Shestopalov et al., 2002), targeted drug delivery, and tissue engineering (Austin et al., 2015; Kompella et al., 2013; Naha et al., 2015; Parveen et al., 2012). Specifically, gold nanoparticles (Au NPs) are being used because of their intrinsic characteristics, such as high chemical stability, suitable surface functionalization, and unique surface plasmon properties (Diebold and Calonge, 2010; Parveen et al., 2012).

Researchers have attempted nanoparticle-mediated drug and gene delivery to tissues of the eye, including the retina, to treat major eye diseases such as age-related macular degeneration and diabetes-related retinopathy (Jin Hyoung et al., 2011; Joris et al., 2013; Li et al., 2012a; Ngwa et al., 2012a; Ngwa et al., 2012b). Moreover, intravitreal injection of Au NPs has been investigated for retinal imaging and inhibition of angiogenesis to prevent macular degeneration (Farjo and Ma, 2010; Jeong Hun et al., 2009). Au NPs have been shown to be promising agents for enhanced delivery of anti-VEGF antibody or other antiangiogenic agents to specific sites in the eye (Diebold and Calonge, 2010; Hayashi et al., 2009a; Jeong Hun et al., 2009). Currently, intravitreal and topical routes are most commonly used (Hayashi et al., 2009b).

The use of intravitreal injection of nanoparticles depends on the safety of the particles (Biswas and Wu, 2005; Diebold and Calonge, 2010; Kompella et al., 2013). Therefore, safety concerns necessitate a better understanding of the potential toxicity hazards of novel materials. One method of measuring toxicity is to examine cellular viability (Abe and Saito, 1999) by determining the lethal dose concentration (LD_{50}), the dose required to kill 50% of the cells. Lethal doses of selected

* Correspondence to: N. Ravi, Department of Ophthalmology and Visual Sciences, Washington University in St. Louis, St. Louis, MO, USA.
** Corresponding author.
E-mail addresses: ravi@vision.wustl.edu (N. Ravi), pbiswas@wustl.edu (P. Biswas).
[1] Equal contribution.

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nanoparticles will provide guidance as to safe levels of use for nanomedicine applications.

In this paper, we report on the in-vitro biocompatibility of Au NPs with spontaneously arising retinal pigment epithelial (ARPE-19) cells. This cell line is an acceptable alternative to repeated isolation and standardization of primary RPE cells (Dunn, 1996; Pfeffer and Philp, 2014), provided that its detailed physicochemical characterization is closely similar to that of primary cells for toxicity evaluation. Although important research has been done on Au NPs (Bakri et al., 2008; Pan et al., 2007; Soderstjerna et al., 2014) on various cell lines, no studies have correlated the size, shape, and surface area of the administered Au NPs while reporting biocompatibility with the RPE cells. In the present study, we provided a comprehensive picture of physico-chemical characteristics of NPs and correlated these characteristics to their biocompatibility because it is crucial to predict the response of the targeted retinal cells to the Au NP exposure.

The effect of Au NP shape (spherical, cubic, and rod; size 5–100 nm), and concentration (up to 5 mg/ml) on toxicity was assessed by MTT (3-[4, 5 dimethyl-thiazoly-2-yl] 2–5 diphenyl tetrazolium bromide) assay. In our study, the exposure period was a minimum of 72 h, which is long enough for gold nanoparticles to reach the cells (Bejjani et al., 2005; Cohen et al., 2014) for biocompatibility assessment. To better understand the effect of Au NP on cell toxicity, the electrical impedance of the cells was monitored continuously for 96 h. Furthermore, the presence or absence of NPs inside the cell was verified using plasmonic imaging by confocal microscopy. The correlation was developed for LD_{50} of Au NPs regarding the mass and surface area concentration to toxicity. A mechanistic description of NP toxicity interactions with ARPE-19 cells is presented, along with a simple universal toxicity curve that may apply to all cell types. To the best of our knowledge, this is the first investigation using retinal cells that presents a thorough physico-chemical characterization of NPs along with cellular viability, electrochemical impedance changes, and intracellular location of NPs.

2. Materials and methods

The ARPE-19 cell line (American Type Culture Collection, Manassas, Virginia), Dulbecco's Modified Eagle's medium (DMEM) with F12 supplement (Sigma-Aldrich, St. Louis MO), fetal bovine serum (FBS), and penicillin/streptomycin were purchased from Gibco (Invitrogen, Gaithersburg, MD). 3-Mercaptopropyltrimethoxysilane, dimethyl sulfoxide (DMSO) and sodium hydroxide NaOH were purchased from Sigma-Aldrich (St. Louis, MO).

The methods section is divided into four main subsections: synthesis (seed-mediated method), characterization (size distribution, zeta potential, hydrodynamic diameter, shape, and aggregation status), toxicity assessment in-vitro, and mechanistic evaluation (impedance spectroscopy, confocal spectroscopy). Based on the list of criteria required for a representative biocompatibility study, a methodology and an experimental plan was developed (supplementary information, Table S1). A brief description and objectives are listed for each.

2.1. Synthesis of gold nanoparticles

Au nanoparticles shaped as spheres, cubes, or rods at sizes from 5 to 100 nm were synthesized using a seed-mediated approach (Jana et al., 2001; Wu et al., 2010). Details are provided in the supplementary information.

2.2. Characterization of nanoparticles and exposure to ARPE-19 cell line

Synthesized NPs were characterized by their size, shape, and agglomeration state by transmission electron microscopy (TEM). To confirm the size and the morphology of the Au NPs, micrographs were taken using an FEI Spirit TEM (Hillsboro, OR, USA) operated at 120 kV. The hydrodynamic diameter and zeta potential of NPs in solution before and after their exposure to cell culture medium was measured using dynamic light scattering (DLS). Batch mode measurements were done using a Malvern Zetasizer Nano ZS (Malvern Instruments, Westborough MA). Quantitative determination of gold (Au) concentrations in stock solutions and aliquots before confocal imaging was done with an inductively coupled plasma spectrophotometer, ICP-MS (Elan DRC II, Perkin Elmer, Norwalk, CT).

2.3. Biocompatibility assessment of the ARPE-19 cell line

Since animal cell culture is sensitive to microbial contamination, Au NP solutions were checked for microbial growth before cell exposure. Tests for bacterial and fungal contamination, respectively, were performed on nutrient agar and potato dextrose agar plates both at room temperature and 37 °C.

In a flat-bottom, 96-well plates, 150 μl volumes (1.2×10^4 cells per well) of cells were incubated (37 °C, 5% CO_2) until confluent (supplementary information, Fig. S1), then exposed to different concentrations of NPs. Physically characterized particles were dispersed in cell culture medium containing Dulbecco's Modified Eagle Medium/Ham's F-12 (DMEM/F-12), 10% fetal calf serum (FCS), and antibiotics (C-DMEM/F12) to be used for cell exposure. For each shape and size of Au NPs, concentrations of the exposed solutions were 0.01, 0.05, 0.1, 0.25, 0.5, 0.75, 1, 2.5, and 5 mg/ml. The upper limit of exposure concentration was determined by NP's toxic potential reported in the literature (Choi et al., 2012; Hayashi et al., 2009b; Jin Hyoung et al., 2011; Naha et al., 2015; Pan et al., 2007). Nanoparticle exposure duration was a minimum of 72 h.

The viability of ARPE-19 cells, defined as their ability to maintain its growth, was evaluated using the MTT assay, which is widely used for in-vitro nanotoxicity assessment (Conde et al., 2014; Dumortier et al., 2006; Sayes et al., 2007; Warheit et al., 2007). This technique, while requires the acquisition of optical density at a single wavelength has advantages over other toxicity assays because it requires minimal physical manipulation of ARPE-19 cells and yields quick, reproducible results. Following the exposure of NPs, 100 μl of MTT (1 mg/ml) in growth medium was added to each well, after which the plate was further incubated for 5 h at 37 °C in 5% CO_2. The resulting blue component, produced by the reduction of tetrazolium salt of MTT by mitochondrial dehydrogenase enzyme, was dissolved in 100 μl dimethyl sulfoxide (DMSO). The optical density of the colored product was read photometrically using a spectrophotometer at 540 nm. Possible interactions between the nanoparticles and the formazan crystals, which form as a result of the reduction of MTT dye, can be minimized by selection of the proper dissolution solvent, DMSO, and adjusting the dye concentration as well as the volume added to each well (Kong et al., 2011; Sylvester, 2011). On the other hand, the spectrophotometer plate readings for both positive controls where the ARPE-19 cells were not exposed to Au NPs, and negative controls where the cells were exposed to Au NPs but not treated with MTT were also taken. The results were normalized based on both positive and negative controls. The percentage viability of ARPE-19 cells was calculated using the following formula:

$$\text{Biocompatibility (Cell Viability)} = \left(\frac{\text{Mean optical density of the sample}}{\text{Control optical density}}\right) \times 100 \quad (1)$$

The lethal dose is typically reported as the mass concentration of NPs at which the biocompatibility is 50%. LD_{50} values, based on mass concentration, were calculated by using a four-parameter logistic equation. Data were plotted as sigmoidal dose-response curves using Systat SigmaPlot 11.0 software. $LD_{50,M}$ values were derived from three independent experiments in which eight-well replicate measurements were done. In this work, LD_{50} values are defined based on both mass concentration ($LD_{50,M}$) and surface area concentration ($LD_{50,A}$).