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Effect of Silver Coating on Barium Titanium Oxide Nanoparticle Toxicity

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Abstract

Nanoparticles are presently being studied for optical and biomedical applications such as medical imaging and drug delivery. Nanoparticles impact the cellular environment due to many variables such as size, shape, and composition. How these factors affect cell viability is not fully understood. The purpose of this study is to test the toxicity effects of silver coating (Ag@) Barium Titanium Oxide (BaTiO₃) nanoparticles on Rhesus Monkey Retinal Endothelial cells (RhREC's) in culture. The addition of silver to the nanoparticles increases their nonlinear optical properties significantly, making the Ag@BaTiO₃ nanoparticles good candidates for nonlinear microscopy contrast agents. We hypothesize that by silver coating nanoparticles, there will be an increase in cell viability at higher concentrations when compared to non-silver coated nanoparticles. RhREC's were treated with BaTiO₃ and Ag@BaTiO₃ at concentrations of 0, 1.0, 10.0, and 100µg/ml for 24 hours at 37°C + 5%CO₂. After 24 hour incubation with respective nanoparticles, cell viability was determined using the trypan blue dye-exclusion method. Treatment with 0, 1.0 and 10.0µg/ml of Ag@BaTiO₃ had minimal effect on cell viability, with 90% viable cells remaining at the end of the 24 hours treatment period. However, cells treated with 100µg/ml of Ag@BaTiO₃ resulted in a decrease to 51% viable cells. Comparatively, cells treated with 0, 1.0 and 10µg/ml of BaTiO₃ had no significant effect on cell viability (90% viable cells after treatment) while the 100µg/ml treatment resulted in a decrease to 29% viable cells. These results show that silver coating of BaTiO₃ nanoparticles has a protective effect on cellular toxicity at high concentrations.

Keywords

Nanoparticles; Rhesus Monkey Retinal Endothelial Cells (RhREC); Cells Toxicity

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Introduction

Nano-technology and materials derived from this technology have become of great interest to the science and medical community alike for use in applications toward biomedical technology, optics, tissue and cell imaging, site-specific drug delivery, and biosensors. While research and bioapplications utilizing nanotechnology has increased over the years, studies characterizing effects of nanoparticle exposure and their potential cytotoxicity are limited [1]. Altering nanoparticle characteristics such as size, surface chemistry, phase, and morphology can tune the cytotoxicity mechanisms, potentially resulting in greatly different cytotoxicity responses for materials of essentially the same composition [2]. The most interesting characteristic of nanoparticles is the quantum size effect due to their minute size [3]. Nanoparticles used in bio-imaging and drug delivery are often bio-conjugated to target specific cells. Because nanoparticles are engineered to interact with cells, it is important to ensure that they do not have any adverse effects [1]. Dye exclusion tests are used to determine the number of viable cells present in a cell suspension. It is based on the principle that live cells possess intact cell membranes that exclude certain dyes, such as trypan blue, eosin, or propidium-iodide, whereas dead cells do not [4]. Knowing the toxicity effects of Barium Titanium Oxide (BaTiO_3) and silver coated (Ag@BaTiO_3) nanoparticles when applied to Rhesus Monkey Endothelial cells (RhRECs) in culture at increasing concentrations will help to determine if these nanoparticles may be used for bio-medical purposes or if these particles are too toxic for possible application to human disease treatment and therapies.

Methods

Culture of RhREC's

Rhesus monkey endothelial cells (RhREC's) were obtained from American Type Tissue Culture (ATCC- CAT # CRL1780) and seeded into T75 flasks per manufacturer's instructions. Cells were grown to confluence (approximately 5 days) at $37^\circ\text{C} + 5\%\text{CO}_2$ in Minimum Essential Medium- alpha (MEM- α ; Invitrogen- CAT #41-061) containing 10% Fetal Bovine Serum (FBS). Confluent cells were trypsinized, harvested, seeded into 24 well plates at 20,000 cells per well and allowed to settle for 24 hours at $37^\circ\text{C} + 5\%\text{CO}_2$ prior to treatments.

Nanoparticles

BaTiO_3 and silver coated (Ag@BaTiO_3) nanoparticles were fabricated per methods described in Yust et al 2012 [5]. In the present study, nanoparticle size was 200nm.

Cell Imaging

RhRECs were seeded at 20,000 cells/well in a 24 well plate for 24 hours at $37^\circ\text{C} + 5\%\text{CO}_2$ with and without respective nanoparticles mentioned previously. After the 24 hour incubation, non-treated, BaTiO_3 treated and Ag@BaTiO_3 treated RhRECs were imaged at $200\times$ with an Olympus Phase Contrast inverted microscope equipped with Star-tech imaging software (www.Startech.com).

Dose-dependent Toxicity study

RhRECs were seeded into 24 well plates at 20,000 cells/well and incubated at 37°C + 5%CO₂ in standard growth media (mentioned above) for 24 hours prior to treatment. After this was done, growth media was removed and respective nanoparticles were introduced in fresh culture media at concentrations of 0, 1.0, 10.0 and 100 µg/ml. Treated cells were incubated for 24 hours at 37°C + 5%CO₂. Cells were then rinsed 3 times with 1× Hanks Buffered Saline Solution (1×HBSS) and harvested using trypsin/EDTA. Cells were then counted using a Neubauer hemacytometer and trypan blue dye-exclusion method for viability (AbCam.com).

Time-dependent Toxicity study

RhRECs were seeded into 24 well plates at 20,000 cells/well as above prior to treatment. Ag@BaTiO₃ nanoparticles were measured and delivered to cells in fresh growth media at a concentration of 100µg/mL. Treated cells were incubated for 0, 12, and 24 hours at 37C + 5%CO₂. At each time point, cells were harvested by trypsin/EDTA and then counted by Neubauer hemacytometer using trypan blue dye-exclusion method for viability.

Results

Cell Imaging

RhREC's treated with 100µg/ml of Ag@BaTiO₃ for 24 hours under normal growth conditions, yielded a dark image with decrease number of cells compared to control (Fig 1A and 1B). RhREC's treated with 100µg/ml of non-silver coated BaTiO₃ nanoparticles yielded an image which showed some darken cell nuclei compared to control (Fig. 1A and 1C).

Dose-dependent Toxicity study

RhREC's incubated with 0, 1, 10, and 100 µg/ml of Ag@BaTiO₃ for 24 hours, under standard culture conditions mentioned previously, showed 90% of viable cells remain after treatment with 1 µg/ml, 95% viability after 10 µg/ml, and a decrease to 51% viable cells after treatment with 100 µg/ml (Fig 2). RhREC's incubated with 0, 1, 10, and 100 µg/ml of non-silver coated BaTiO₃ showed 90% (± 2%) viability after treatment with 1 and 10 µg/ml and decreased to 29% viable cells after treatment with 100 µg/ml after 24 hours treatment (Fig 2).

Time-dependent Toxicity study

RhREC's were treated with 100µg/ml of Ag@BaTiO₃ for 0, 12, and 24 hours. Ag@BaTiO₃ treatment yielded 50% and 51% viable cells at 12 and 24 hours respectively, compared to controls of 100% at 0 hour (Fig 3).

Discussion

At higher concentration of 100µg/ml, silver coating of BaTiO₃ nanoparticles had a protective effect on the viability of RhREC. Results from the present study show that silver coating of BaTiO₃ particles reduced BaTiO₃ particle-induced cell toxicity by 22%. It may, therefore, be preferable to use silver coated BaTiO₃ nanoparticles for bio-medical and bio-

imaging over non-silver coated BaTiO₃ particles. Nevertheless, our results also indicate that at lower concentrations (i.e. 1µg–10µg/ml), BaTiO₃ nanoparticles (with and without silver coating did not significantly reduced cell viability, suggesting that this may be a safe range of effective concentrations for bio-imaging or tissue-specific drug targeting.

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Figure 1.
Photomicrographs of RhRECs with A) no nanoparticles-control B) Ag@BaTiO₃ nanoparticles. c) BaTiO₃ nanoparticles.

Figure 2. Does Dependent Response on Percent Viability of Cells treated with BaTiO₃ and Ag@BaTiO₃ nanoparticles at 0, 1, 10 and 100µg for 24 hours

RhRECs incubated with Ag@BaTiO₃ nanoparticles yield a decrease in viable cells to 90% $\pm$ SE at 1 µg, 95% $\pm$ SE viability at 10µg, and 51% $\pm$ SE viability at 100µg compared to controls (n=3). RhRECs incubated with BaTiO₃ nanoparticles yielded a decrease in viable cells to approximately 90% $\pm$ SE at 1 and 10 µg concentrations, then a decrease to 29% $\pm$ SE viable cells at 100µg compared to controls (n=6). *Significance 5% between data sets.

Figure 3. Toxicity of Ag@BaTiO₃ nanoparticles at 0, 12, and 24 hours at 100 µg concentration
Ag@BaTiO₃ delivery of 100 µg to RhRECs resulted in viability decrease to 50% at 12 hours and approximately 51% ±SE after 24 hours.