Use of Nanoparticles as Therapy for Respiratory Syncytial Virus Inhibition

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ABSTRACT

Respiratory Syncytial Virus (RSV) is the leading cause of severe respiratory illnesses including bronchiolitis and pneumonia in young children. In the present study, we synthesized and used silver and gold nanoparticles to study the inhibition of RSV in cell culture. The characteristics of nanoparticles were evaluated by SEM (scanning electron microscopy), TEM (transmission electron microscopy, and EDS (Energy dispersive X-ray) analysis. The nanoparticles were analyzed for cell toxicity. The effectiveness of RSV inhibition was then evaluated by microscopic examination for syncytia formation and by immunofluorescence microscopy. The nanoparticles showed low toxicity to HEp2 cells. We observed RSV inhibition by the nanoparticles used. Our results revealed that PVP-coated silver nanoparticles inhibited RSV infection to a greater extent compared to gold nanoparticles.

Keywords: Respiratory Syncytial Virus, nanoparticles, silver, gold, PVP, TEM, SEM

1. INTRODUCTION

Respiratory Syncytial Virus is the leading cause of severe respiratory illnesses such as bronchiolitis and pneumonia in young children. RSV is a *Paramyxovirus* with negative-sense genomic RNA that encodes for eleven proteins, two of which, F and G are major surface proteins. The G protein is responsible for viral attachment to the host cell, while the F protein facilitates viral entry and spread of the virus from infected to normal cells leading to syncytia formation. Nanoparticles have been gaining extensive usage in medicine and therapy. The structural characteristics of metal nanoparticles and their interactions with surface modifiers are essential to their functions[1], as they should be stable enough to work with at ambient conditions. Silver nanoparticles in particular have strong antimicrobial functions which show potential as antimicrobial agents[2]. Silver nanoparticles have also been shown to attach to gp120 surface glycoprotein of HIV and inhibit infection[3]. As the gp120 surface protein of HIV is similar to the G protein of RSV in structure and function, we hypothesized that the silver and other nanoparticles aided by capping agents would attach to the G protein on the surface of the virus and block the sites responsible for viral attachment to the cell and thus inhibiting RSV infection[4]. In the present study, we synthesized and used silver and gold nanoparticles to study the inhibition of RSV infection in human cell lines.

MATERIALS & METHODS

1.1 Silver nanoparticles

The PVP coated silver nanoparticles were synthesized using a sonochemical method. The experimental procedure for a typical reaction is as follows: 1g of silver(I) acetate (Sigma Aldrich 98+%) and 50 mg of PVP (polyvinylpyrollidone) dissolved in 60ml of DMF (Dimethylformamide) and this reaction mixture is irradiated with a high-intensity ultrasonic horn (Ti-horn, 20 kHz, 100 W/cm2) under argon at room temperature for 3 h. The product obtained is washed thoroughly with absolute ethanol several times and dried in a vacuum for overnight.

2.2 Gold nanoparticles

Gold nanoparticles were synthesized by method of McFarland et al.[5] using gold (III) chloride hydrate (Sigma). The synthesized colloidal gold particles had a concentration of 3μg/ml.

Gold nanoparticle colloidal solution was also bought commercially from Nanoparts™ Inc. with a concentration of 1.51 x 10e + 11 particles/ml.

2.3 Characterization of the synthesized nanoparticles

The synthesized nanoparticles were analyzed using TEM (JEOL-2010 microscope). A small drop (20–30 μL) of each sample was placed on top of a formvar/carbon coated copper grid. The drop of sample was allowed to air dry. Once dry, the grid containing the sample was placed into TEM and viewed. Pictures were taken at 50,000–125,000 magnifications. Samples

were confirmed by EDS (Energy dispersive X-ray) analysis. The XRD measurements were carried out with a Rigaku-D/MAX-2200.

Morphological analysis was carried out using JEOL JSM 5800 SEM. The samples were placed on a double sided carbon tape and coated with gold/palladium to prevent charge buildup in the specimen by electron absorption.

2.4 Cells and virus

HEp-2 cells were purchased from American Type Culture collection (ATCC, Manassas, VA; CCL-23) and were propagated by standard methods using Minimum Essential Medium (MEM) supplemented with 10% Fetal Bovine Serum (FBS), 2 mM L-Glutamine, 75 U/ ml Penicillin, 100 µg/ml Kanamycin and 75 µg/ml Streptomycin.

Human RSV Long strain was purchased from ATCC (VR# 26). Virulent RSV stocks were prepared and propagated in HEp-2 cells. RSV with multiplicity of infection (m.o.i) of 4:1 was added to the flask and virus adsorption was carried out for 1 h at 37°C in a humidified atmosphere with 5% CO_2. MEM supplemented with 2% FBS and 2 mM L-Glutamine was added to the flask and infection of cells was observed for 3 days. RSV infected cells were harvested and cell suspension was subjected to 2 freeze-thaw cycles at -80°C followed by centrifugation at 3,000 x g at 4°C to remove cellular debris. The viral stock was aliquoted and stored at -80°C or liquid nitrogen until further use. Viral titer of the prepared stock was determined by plaque assay and had a titer of 10^6 p.f.u / ml.

2.5 Cell cytotoxicity of nanoparticles

The effect of nanoparticles on cytotoxicity was measured by MTT (3-(4, 5-dimethyl-thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) dye reduction. HEp2 cells were seeded in a 96-well plate at a density of 20,000 cells per well in MEM containing 5% FBS and grown overnight. After 48 h post-incubation of nanoparticles complexes, media were removed and cells were washed twice with sterile PBS. 10 µl of MTT (5 mg/ml) in sterile filtered PBS was added to each well and then incubated for 4 h to allow formation of formazan crystals at 37 °C. After 4 h, 150 µl of DMSO was added to each well to dissolve the MTT formazan crystals and the plate was incubated at 37°C for 30 min. The absorbance of formazan products was measured at 490 nm using a microplate reader.

The effect of nanoparticles on cytotoxicity was also measured by determining cell viability of nanoparticles, calculated as a percentage of the cell viability of untreated cell samples using Trypan Blue Exclusion Assay. HEp-2 cells were plated in 12-well plates and when they reached 60–70% confluency after 24 hours, concentrations of 5, 25, 50 and 100 µg/ml of each nanoparticle sample were added to the cells. After 48 hours, the cells were trypsinized, mixed with Trypan Blue stain, and placed on a hemocytometer for counting under a light microscope. Dead cells stained blue, while live cells did not. A total of 100 cells were counted and the number of unstained cells representing viable cells was used to determine cell viability.

2.6 RSV inhibition experiment

Nanoparticle samples were at various concentrations were tested for RSV inhibition. The nanoparticle preparations were mixed with 10 µl of RSV containing 100 PFU, and incubated for 30 minutes at room temperature. The nanoparticle + RSV mixture was then added to 60–70% confluent HEp-2 cells in 8-chamber slides to determine inhibition of RSV infection. HEp-2 cells infected with RSV without nanoparticles were used as a positive control.

The inhibition of RSV infection by each nanoparticle sample was analyzed both visually using an inverted light microscope and by indirect immunofluroscence using an Olympus IX51 immunofluorescence microscope.

For indirect immunofluorescence HEp-2 cells were grown in 8 chambered slides (BD Biosciences) for 24 h to 60% confluency. The cells were washed with phosphate buffer saline (PBS) and fixed using 10% trichloroacetic acid for 15 min. The cells were then successively washed in 70%, 90%, and 100% ethanol for 5 min. each. After a subsequent wash with PBS, the cells were incubated in blocking buffer (3% dry milk in PBS) for 30 min followed by 3 washes with PBS. The fixed cells were incubated for 1 h at room temperature with monoclonal mouse antibody to RSV F (Biodesign International) in antibody buffer (2% dry milk in PBS). The cells were washed three times for 5 min each in PBS and then incubated for 1 h at room temperature with FITC-conjugated goat anti-mouse IgG (H+L) secondary antibody (Southern Biotechnology) in antibody buffer. Non-specific binding was eliminated by three washes in PBS. Cells were visualized with an fluorescent microscope.

3. RESULTS & DISCUSSION

3.1 TEM and SEM of nanoparticles

TEM was performed in order to analyze to analyze and nanoparticles (Fig. 1). TEM micrographs revealed that the silver nanoparticles capped with PVP are monodispersed have a size of `5-10nm.

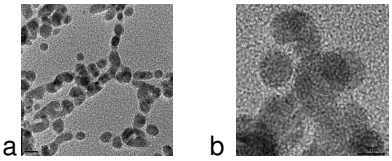


Fig. 1. TEM- micrograph of (a) As prepared Ag nanoparticles (b) Individual Ag nanoparticles with lattice structure.

TEM micrographs of gold procured commercially revealed that they have a size of `30-50nm (Fig. 2). SEM analysis of the nanoparticles was also conducted (Fig. 3).

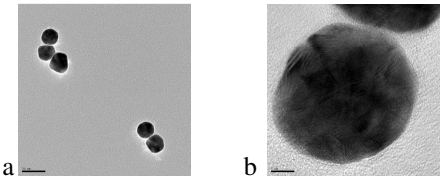


Fig. 2. TEM- micrograph of commercial (a) Au nanoparticles (b) Individual Au nanoparticles with lattice structure.

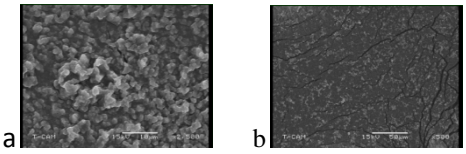


Fig. 3. SEM micrograph of (a) Gold nanoparticles (commercial) (b) Gold synthesized

3.2. Energy Dispersive Spectroscopy (EDS) studies of nanoparticles

Energy Dispersive Spectroscopy (EDS) studies shows that all the particles were either silver or gold and there were no impurities found in the sample (Fig. 4).

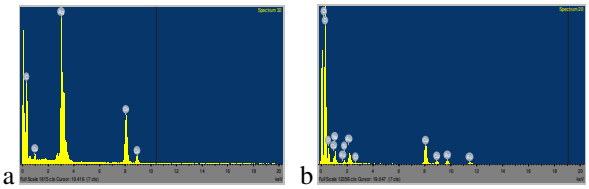


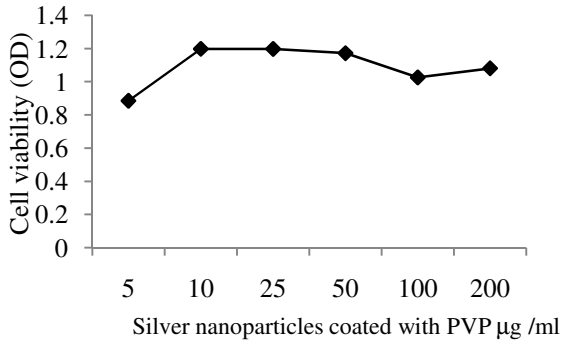
Fig. 4. EDS spectrum of (a) synthesized Ag nanoparticles (b) commercial Au nanoparticles

3.3 Cytotoxicity Analysis of Nanoparticles

After preparing the nanoparticles, it was necessary to test the toxicity of the nanoparticles to the cells. Concentrations ranging from 5 to 200 µg /ml of each nanoparticle sample were tested for toxicity to HEp-2 cells by MTT assay (Fig. 5).

Trypan blue exclusion assay was performed to calculate % viable cells (Fig. 6). The results show 85% cell viability at 5 µg /ml silver nanoparticles coated with PVP which decreased to 70% at 50 µg /ml. Similarly gold nanoparticles also showed 62-77% cell viability even at the highest concentration used showing the nanoparticles were not toxic to the cells.

A.



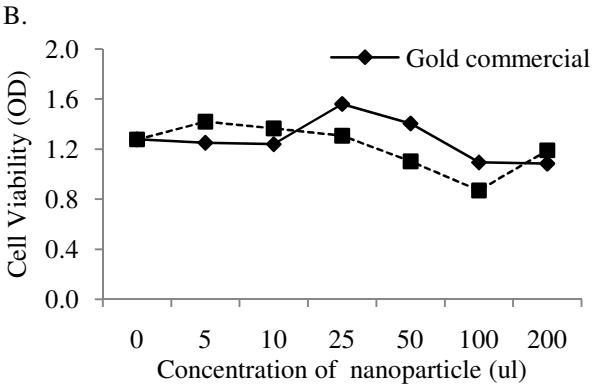


Fig 5. MTT assay for 48 hr cytotoxicity of nanoparticles to HEp2 cells (a) PVP coated silver (b) Gold

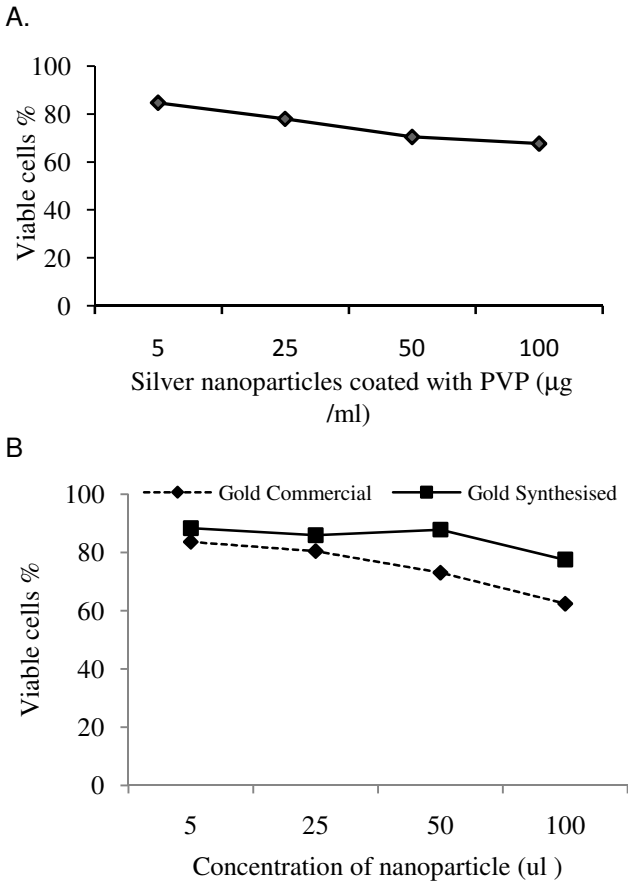


Fig 6 Trypan Blue exclusion assay for cytotoxicity of nanoparticles to HEp2 cells (a) PVP coated silver (b) Gold nanoparticles

3.4 Inhibition of RSV infection

Nanoparticles were mixed with 100 PFU of RSV and added to the cells to determine the inhibition of infection. Reduction in cytopathic effects (syncytia) in HEp-2 cells was observed to determine the extent of RSV infection. The cells infected with RSV showed marked inhibition as seeing by syncytia formation (data not shown) and by immunofluroscence (Fig. 7). Cells infected with RSV mixed with PVP coated silver nanoparticles clearly show significant reduction in RSV infection compared to the cells infected with RSV alone.

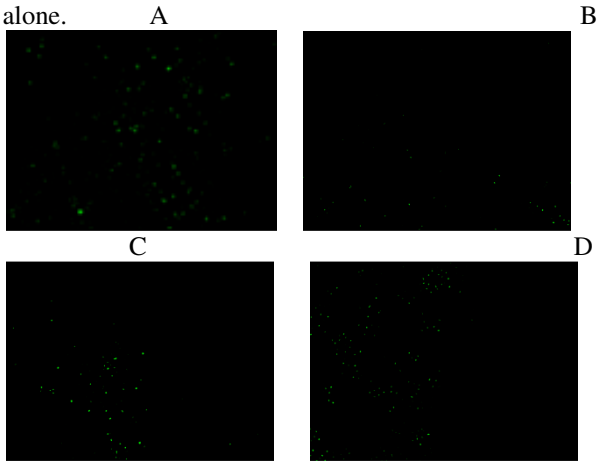


Fig. 7. RSV inhibition by immunofluroscence (a) Cells infected with RSV (b) Cells infected with RSV mixed with PVP coated silver nanoparticles (50 µg/ml) (c) cells infected with RSV mixed with gold nanoparticles (50 ul of prepared stock).

The results of the present study show that PVP-coated silver nanoparticles can inhibit RSV infection can be used in future for therapeutic purposes.

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