Analysis of the Effect of Gold and Silver Nanoparticles on RSV using AFM

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ABSTRACT

Respiratory Syncytial Virus (RSV) is one of the most common viral causes of upper and lower respiratory tract infections in infants and results in pneumonia. In the present study, we used AFM to observe the effect of Gold and Silver nanoparticles on RSV virus particles. The results indicated that RSV particles were almost round in shape as detected by AFM. Even though the dimensions of the RSV particle exhibited a polymorphous distribution via off-line particle analysis of AFM, most of the RSV particles had a diameter of approximate 135nm. When we mixed RSV with gold nanoparticles, we observed that gold nanoparticles were bound around RSV and RSV started shrinking in size. Further, *in vitro* infection of Hep-2 cells were performed with either RSV alone or RSV mixed with gold nanoparticles. Infected cells were fixed at different time intervals to observe RSV entry into the cells and propagation of the infection.

Keywords: RSV, AFM, gold, silver. Nanoparticles

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. In the present study, we synthesized and used silver and gold nanoparticles to study the inhibition of RSV using Atomic Force Microscopy (AFM). AFM images provide a tool to elucidate the topography of viral and cell surface interactions. Based on the interactions of these structures and their images we can get a better understanding on how the fusion occurs. Since many medically important virus proteins HIV-1 gp41, influenza HA, and coronovirus peplomer, share similar structure and functions, this study may prove valuable to design therapeutic agents and vaccines against these viruses.

2 MATERIALS & METHODS

2.1 Silver nanoparticles

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+%) was mixed in 60ml of DMF (Dimethylformamide) and this reaction mixture was 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 was washed thoroughly with absolute ethanol several times and dried under vacuum for overnight.

2.2 Gold nanoparticles

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

2.3 Cells and virus

HEp-2 (Human body Type-2 epithelial cells) 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 mg/ml Kanamycin and 75 mg/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^{o}C in a humidified atmosphere with 5% CO_2. MEM supplemented with 2% FBS and 2 mM L-Glutamine were added to the flask and infection of HEp-2 cells was observed for 3 days. RSV infected cells were harvested and cell suspensions were 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 as determined by plaque assay revealed a titer of 10^6 p.f.u / ml.

2.4 Preparation of Nanoparticles and RSV mixture.

Nanoparticle samples, silver and gold, were tested to analyse RSV inhibition using Atomic Force microscopy (AFM). The nanoparticle preparations (50 µg/ml) were mixed with 20µl of RSV culture containing 100 PFU of RSV, and incubated for 30 minutes and 1hour at room temperature.

2.5 Infection of HEp-2 cells with nanoparticles + RSV mixture

The nanoparticle+RSV mixtures were added to Hep-2 cells (60-70% confluency) grown on coverslips in 12 well plates to analyze the effect of RSV on the Hep-2 cells. HEp-2 cells infected with RSV without nanoparticles were used as a positive control. Coverslips (20 mm) were put in 12-well tissue culture plates and incubated for 48 h with HEp2 cells. Then, coverslips were washed with 5 ml PBS at various time intervals (15 min, 30 min, 1 h, 2h, 4h, 8h, 16h, 24h, 36h and 48 h) were transferred into small petri dishes. The coverslips were treated with 400 µl of 100% methanol for 30 min to fix the cells. After the fixing process, the coverslips were washed with dH_2O (200 µl/well) followed by washing with 70%, 90%, and 100% ethanol for 5 min each to dehydrate/inactivate cells/virus. Finally, the coverslips were air dried in a BL2 biological safety cabinet for AFM analysis.

2.6 Atomic Force microscopy (AFM) analysis

Approximately 15 µl of RSV and RSV+ nanoparticle solutions were deposited onto a freshly cleaved piece of Mica and air-dried for 30 min under a clean, dry airflow until the surface appeared dry. Previously prepared 20mm coverslips and the dried mica with samples were used for AFM (Nanoscope R2, Pacific Nanotechnology, Santa Clara, CA, USA) studies. Close contact mode and standard silicon cantilevers (Pacific Nanotechnology, Santa Clara, CA, USA) 450 µm in length and 20 µm in width were employed for imaging. The cantilever oscillation frequency was tuned to the resonance frequency of approximately 256 kHz. The set point voltage was adjusted for optimum image quality. Both height and phase information were recorded at a scan rate of 0.5 Hz, in a 512 x 512 pixel format. AFM images containing DCNP in a large scanning area were processed using NanoRule software (Pacific Nanotechnology, Santa Clara, CA, USA).

3 RESULTS&DISCUSION

The advent of AFM since 1986 (Binning et al, 1986) and its applications (Fotiadis et al.2002) have made tremendous impact on studies of microbiological samples, living cells, and a variety of biological specimens. In the present study, AFM was employed to study the interaction of silver and gold nanoparticles with RSV. The results indicated that RSV particles were almost round in shape as detected by AFM. Even though the dimensions of the RSV particle exhibited a polymorphous distribution via off-line particle analysis of AFM, most of the RSV particles had a diameter of approximate 135nm (Figure 1).

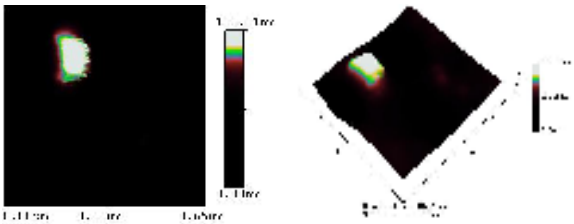

Figure 1: (a) Atomic Force microscopy (AFM) 2D image of RSV (scan size 0.65µm x 0.65 µm) (b)The simulated 3D image of RSV shown in Figure 1a.

When we mixed RSV with gold nanoparticles, we observed that gold nanoparticles bound around RSV and RSV started shrinking in size. In Figure 2, we present AFM images of RSV with gold nanoparticles.

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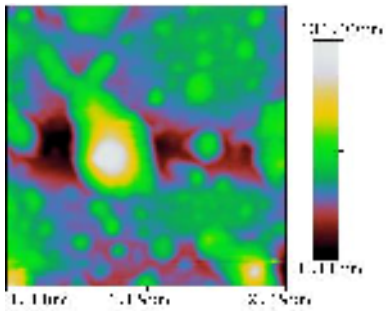
Interestingly, the size of the nanoparticles bound to the virus were exclusively within the range of 10-20 nm (data not shown). We also observed that when we incubated RSV with the gold nanoparticles for 30 min, RSV size was reduced only by 5% but when we increased the incubation time, RSV size was reduced by 25 % after 1 h, and by 71% after 2 h.

A



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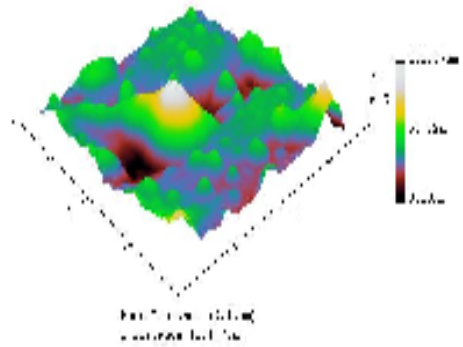

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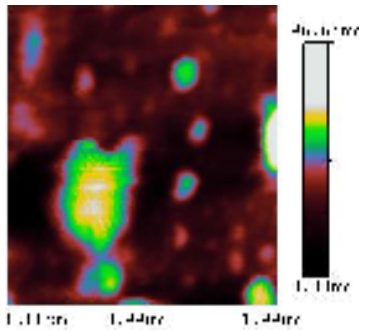

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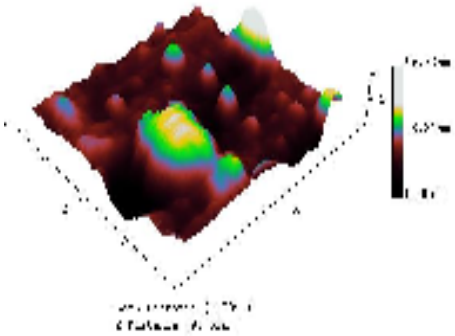

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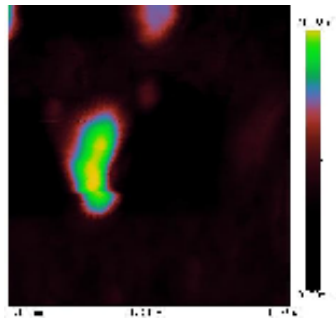

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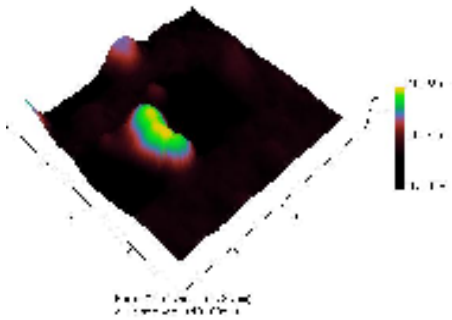

Figure 2: (a) Atomic Force microscopy (AFM) 2D image of RSV with gold nanoparticles after 30 min. incubation (b) The simulated 3D image of RSV+gold nanoparticles shown in Figure 2a. (c) 2D image of RSV with gold nanoparticles after 1h incubation (d) The simulated 3D image of RSV shown in Figure 2c (e) 2D image of RSV with gold nanoparticles after 2h incubation (f) The simulated 3D image of RSV+gold nanoparticles shown in Figure 2e.

Moreover, we studied the effect of RSV on HEp-2 cell surfaces. We observed that the nuclear envelope was deteriorated in RSV infected cells but some nuclear structure was visible when these cells were incubated with the virus for 24 hours. On the other hand, uninfected cells have very well defined nuclear structures and the nuclear membrane is easily distinguishable from the interior organelles. In addition, the membranes appear fuller and more uniform (Figure 3). Additionally, the nuclear envelope has also deteriorated in RSV+gold nanoparticle, which is prepared by incubated only 30min, infected cells for 24h. Since the RSV size reduction was only 5% when virus was incubated with the gold nanoparticles for 30 min, the virus can damage the nuclear envelope. However, when we infected the cells with RSV+Gold nanoparticle mixture incubated for 1h and/or 2 h, the damage on the nuclear envelope was reduced and cells had well defined nuclear structure (data not shown).

A **B**

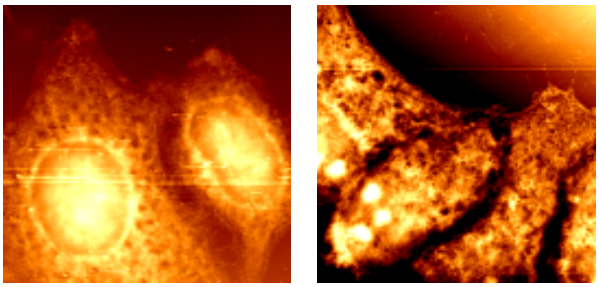

Figure 3: (a) AFM 2D image of uninfected HEp-2 cells (b) AFM 2D image of RSV infected HEp-2 cells after incubation with the virus for 24 hours.

Clearly, our findings demonstrate that Gold nanoparticles have important effects on RSV infection. We are currently analyzing the effect of silver nanoparticles on RSV using AFM. The results obtained from this study will provide more meaningful and clear evidence of the changes in RSV structure in relation to the changes occurring on the cell surface.

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