

Peptide-Functionalized Gold Nanoparticles are Potent Inhibitors of Respiratory Syncytial Virus (RSV) Infection

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ABSTRACT

Human respiratory syncytial virus (RSV) is a major cause of severe upper and lower respiratory tract infections in infants, and in high-risk adults. Currently, there is no vaccine and the available therapeutic agents have limited efficacy. Gold Nanoparticles represent good therapeutic agents due their antibacterial and antiviral properties and an important strategy for efficient delivery of therapeutic agents to maximize therapeutic activity. In the present work, we investigated, *in vitro*, the potency enhancement of the antiviral activity by functionalization of gold nanoparticle with different anti-RSV peptides targeting the virus entry. Carboxylated Gold nanoparticles of 15 nm diameter size were conjugated with 1) a dendrimeric Heparan Sulfate-Binding Peptide (SB105-A10); and 2) with a small dendrimer-like peptide (T118). SB105-A10 and T118 are known RSV entry inhibitors by competing for binding to cell surface HSPGs and via interactions with RSV fusion protein, respectively. No significant increase (less than 10%) in cell cytotoxicity was observed with nanoparticle concentration up to 100 µg/mL. NPs were allowed to interact respectively with viral particles before infecting the cells as well as interacted with RSV-infected cells. At 30 µg/mL (~ 1.5 nM) concentration, they showed high inhibitory activity of RSV infection and infectivity (>60% inhibition and ≥3-fold reduction of the virus titer). However, when NPs were added in RSV-infected cells, they exhibited less inhibitory activity of RSV (<50% inhibition). Furthermore, the functionalization of Au-NPs with T118 showed an increase of the antiviral activity as compared to non-functionalized nanoparticles. Our results suggest that the functionalization of gold nanoparticles may be effective strategy for the development of broad-spectrum anti-RSV agents inhibiting RSV infection as well as preventing the spread of the virus among already infected cells

Keywords: Gold nanoparticles, inhibitors, antiviral peptides, nanoparticle conjugation, antiviral agents

1 INTRODUCTION

Human respiratory syncytial virus (RSV) is an enveloped virus with a single-stranded negative-sense RNA of 15.2 kb [1]. RSV is the leading cause of lower

respiratory illness in infants worldwide and a significant cause of morbidity and mortality in adults, particularly in the elderly and in the immunocompromised [1, 2]. To date, no effective therapeutics or vaccines for RSV is available [1] [3]. Therefore, the development of new anti-RSV therapeutics, with improved efficacy and safety is highly needed. Several classes of small molecules inhibiting RSV have been described; of which many of them are entry inhibitors and target the RSV fusion protein or cell surface receptors [1, 4, 5]. These compounds include RSV F protein-derived peptides (T118 and F-481) [4, 6, 7] and dendrimeric Heparan Sulfate-Binding Peptides [5]. Metal-based Nanoparticles have emerged as potent therapeutic agents due to their antibacterial and antiviral properties. They have shown antiviral efficacy against several viruses regardless of the specific structural details of each virus family (eg. HSV, HBV, HIV, Influenza viruses, etc.) [8-10]. In particular, Polyvinylpyrrolidone capped silver nanoparticles (AgNP-PVP) and gold nanoparticles have been shown to be significant inhibitors of RSV [6, 11]. On the other hand, thanks to their functional and physical properties, gold nanoparticles represent an important strategy in the development of drug delivery systems for enhanced uptake and activity [12, 13]. Gold NPs have therefore been used conjugated with several types of molecules able to block different steps of the viral lifecycle and exhibited additive and substantially more potent antiviral activity [14-16]. In the present work, we investigated the additive effect of the functionalization of gold nanoparticle with anti-RSV peptides targeting the virus entry via different mechanisms. Gold nanoparticles of 15 nm diameter size were functionalized with 1) a dendrimeric Heparan Sulfate-Binding Peptide (SB105-A10) targeting RSV entry by competing for binding to cell surface heparan sulfate proteoglycans (HSPGs) [5], and with 2) a small dendrimer-like peptide (T118) derived from the HRB domains in the RSV F protein (aa 488–522) targeting RSV entry via interactions with RSV fusion protein [4, 7]. Our results suggest that the functionalization of gold nanoparticles may be effective strategy for the development of broad-spectrum anti-RSV agents inhibiting RSV infection as well as preventing the spread of the virus among already infected cells.

2 GOLD NANOPARTICLE FUNCTIONALIZATION AND CYTOTOXICITY

Carboxyl-polymer coated spherical gold nanoparticles (AuNPs) 15 nm core size were conjugated with BP-5, SB105-A10 and T118 peptides using EDC chemistry (1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide, EDC/ N-Hydroxysuccinimide, NHS). The conjugation was then assessed and confirmed by UV-Vis spectrophotometry and the stability of the functionalized NPs was evaluated by hydrodynamic size and NP-surface-charge Zeta potential measurements.

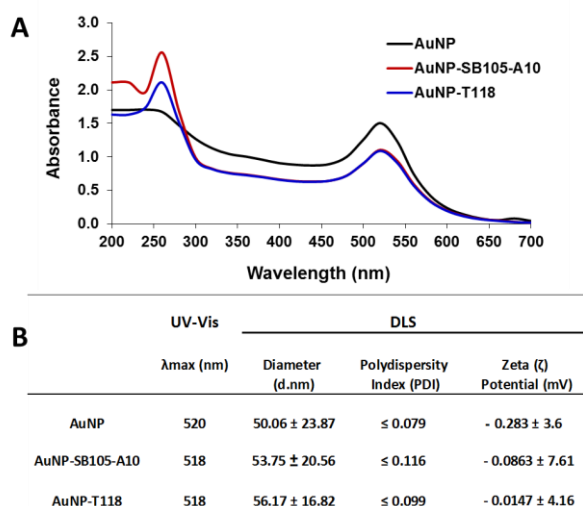


Figure 1. Characterization of AuNPs and peptide-functionalized AuNPs. [A] UV-Vis spectra of AuNPs and SB105-A10 and T118-functionalized AuNPs. [B] DLS characterization of AuNPs before and after functionalization with peptides.

The UV-Vis spectra of the peptide-conjugated AuNP complexes show the change in the nanoparticle absorption indicating the functionalization of AuNPs (Fig. 1A and Fig. 2B). The surface plasmon resonance (SPR) spectrum peak of AuNP shifted from 520 nm to 518 nm (Fig. 1, A-B) after functionalization, thereby confirming the success of the functionalization. Hydrodynamic size and NP-surface-charge Zeta potential measurements also confirmed the functionalization of AuNPs. The zeta potential of AuNPs changed from -0.283 mV to -0.0863 mV and -0.0147 mV; and the hydrodynamic diameter changed from 50.06 nm to 53.75 nm and 56.17 nm after functionalization with SB105-A10 and T118 respectively (Fig. 2B).

The cytotoxicity of the functionalized nanoparticles was determined on HEP-2 cells by colorimetric MTT assay. After a 24-hour exposure, a less than 10% decline in cell viability was observed in HEP-2 cells exposed to up to 100 μ g/mL of AuNPs or peptide-functionalized AuNPs (Fig. 2). Therefore, in this study, AuNPs and peptide-functionalized AuNPs were used at a concentration of 30 μ g/mL (\sim 1.5

nM); this concentration resulted in less than 5% cytotoxicity for all the compounds (Fig. 2).

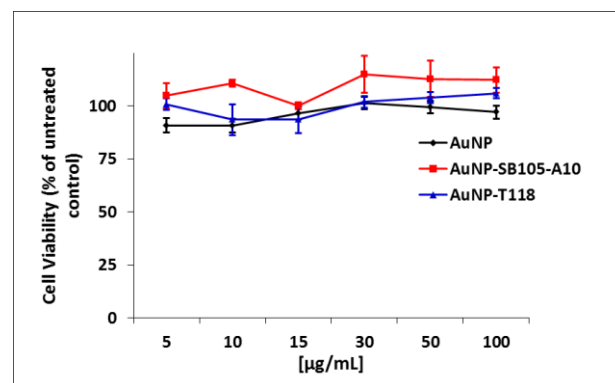


Figure 2. Cytotoxicity analysis of AuNPs and SB105-A10 and T118 peptide-functionalized NPs. After 24-hours incubation, there's less than 15% decline in cell viability with up to 100 μ g/mL of all the compounds.

3 EFFECT OF PEPTIDE-FUNCTIONALIZED GOLD NPS ON RSV INFECTION

The ability of the peptide-functionalized nanoparticles to inhibit RSV infection was investigated by plaque reduction assay and RSV Immunofluorescence imaging. AuNPs and Peptide-functionalized AuNPs were allowed to interact respectively with viral particles before infecting the cells as well as interacted with RSV-infected cells. 1) For RSV infection inhibition assay, RSV stock (100 PFU in 100 μ L) was pre-incubated with compounds (30 μ g/mL) for 30 min at room temperature. HEP-2 cells monolayers were then infected for 1h at 37°C and overlaid with 0.8% methylcellulose medium. After five days, they were analyzed by plaque assay. On the other hand, after 48h, they were stained by incubation with RSV F protein-specific primary antibody and FITC-conjugated secondary antibody immunoglobulin G and then visualized under epifluorescence microscope. 2) For RSV post-infection inhibition assay, HEP-2 cells monolayers were infected with RSV stock (100 PFU in 100 μ L) for 1h at 37°C. Unbound virus was removed and the compounds (30 μ g/mL) added to cells for additional 1h at 37°C, then overlaid with 0.8% methylcellulose medium analysed by plaque assay after five days. 2) For RSV neutralization assay, RSV stock (10^4 in 100 μ L) was pre-treated with 30 μ g/mL of each compound for 2-h at 37°C. The mixture was then titrated by plaque assay on HEP-2 cells.

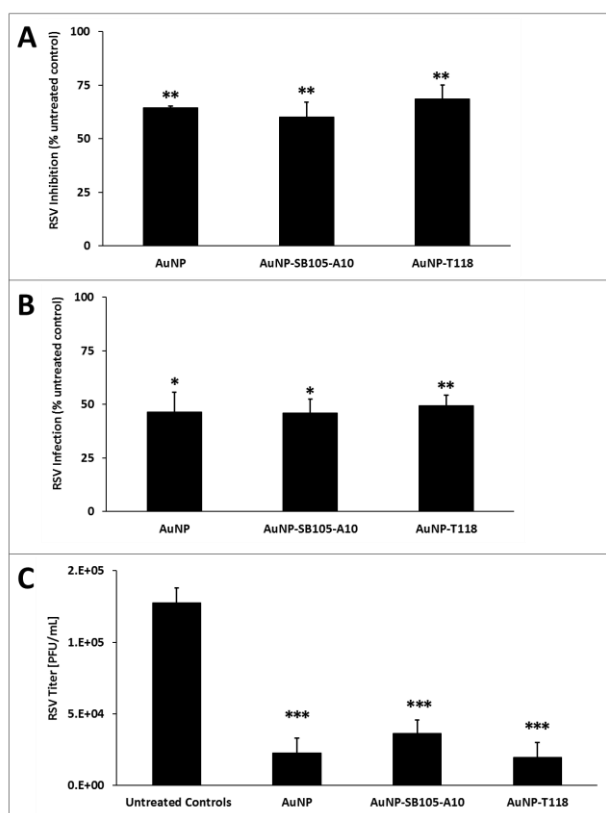


Figure 3. Effect of AuNPs and SB105-A10 and T118-functionalized AuNPs on RSV infection as determined by plaque reduction assay. **[A]** Inhibition of RSV infection: Cells were treated with a mixture of RSV stock (100 PFU in 100 μ L) and compounds (30 μ g/mL) for 1h at 37°C; **[B]** Inhibition of RSV infection in infected cells: Cells were infected with RSV stock (100 PFU in 100 μ L) for 1h at 37°C and then treated with the compounds (30 μ g/mL) for additional 1h at 37°C; **[C]** Inhibition/Neutralization of RSV infectivity: RSV (10^4 in 100 μ L) was pre-treated with 30 μ g/mL of each compound for 2-h at 37°C and then titrated on cells. Five days postinfection, plaques were visualized and counted, and the percent inhibition was determined with respect to untreated control wells. The results are means and standard deviations for three independent experiments performed each in triplicate. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$

AuNPs and SB105 and T118-functionalized Au-NPs, at a concentration of 30 μ g/mL (~ 1.5 nM), showed high inhibitory activity against RSV when added mixed with the viral inoculum ($\geq 60\%$ inhibition, Fig. 3A; and ≥ 3 -fold reduction of virus titer, Fig. 3C). This high inhibitory activity was also confirmed by immunofluorescence imaging of RSV, where a considerable reduction of RSV was observed (Fig. 4). However, as reported in figure 3B, they exhibited less inhibitory activity in infected cells ($< 50\%$ inhibition, Fig. 3). Moreover, the functionalization of Au-NPs with T118 showed an increase of the antiviral

activity as compared to non-functionalized nanoparticles, but however, the differences were not significant ($p > 0.05$).

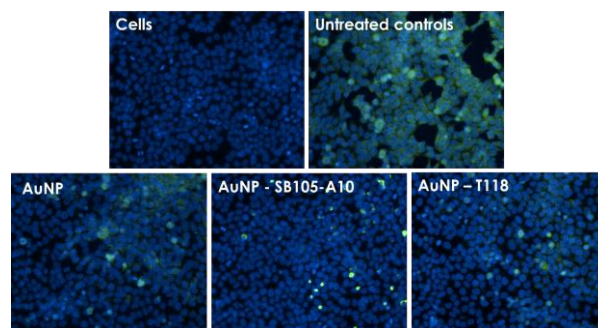


Figure 4. Immunofluorescence microscopic image showing RSV (green, FITC) inhibition by AuNPs and SB105-A10 and T118-functionalized AuNPs in HEp-2 cell line (nucleus: blue, DAPI).

4 CONCLUSION

Several peptides have been reported to exhibit highly potent anti-RSV activity [4]. However, none of these peptides was reported in clinical trials, possibly due, among others, to their relatively low half-life in the circulation. Gold nanoparticles have also been shown to have good antiviral activity against several viruses including RSV [6, 8, 10] and to be suitable platform for development of efficient and selective delivery systems [17, 18]. Therefore, by functionalizing gold nanoparticles with antiviral peptides, one could extend their biological half-life while allowing selective targeting of RSV by multivalent conjugation of AuNPs, which might result in an increase in antiviral activity. This approach has been used against cytomegalovirus and HIV-1 and exhibited additive and substantially more potent antiviral activity [14-16]. Thus, in this study, we functionalized gold nanoparticles with peptides, SB105-A10 [5] and T118 [4], both known RSV entry inhibitors by competing with RSV for binding to cell surface HSPGs and via interactions with RSV fusion protein, respectively; in order to explore the potency enhancement of the antiviral activity. This resulted in a high inhibition of RSV infection and infectivity ($> 60\%$ inhibition and ≥ 3 -fold reduction of the virus titer with 30 μ g/mL). However, when NPs were added in RSV-infected cells, they exhibited less inhibition of RSV ($< 50\%$ inhibition). Although the antiviral activity was higher with peptide-functionalized AuNPs, especially with T118-AuNPs (68.7% inhibition, $p < 0.01$ when added mixed with the viral inoculum; 50% inhibition when added in RSV infected cells, $p < 0.01$; and ≥ 6 -fold reduction of the titer, $p < 0.001$), the differences between non-functionalized AuNPs and peptide-functionalized AuNPs were not significant ($p > 0.05$). This could be due to the low loading efficiency of peptides on AuNPs and/or peptide conformational change upon functionalization with AuNPs. Nevertheless, this increase in antiviral activity, even on a

small-scale, suggest an improvement brought by the functionalization. In conclusion, this approach represents a promising strategy that could be exploited to develop broad spectrum and highly efficient nanoparticle-based antiviral agents.

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