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Novel Nanotechnology-Based Antiviral Agents: Silver nanoparticle neutralization of hemorrhagic fever viruses

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Nanomaterials

Unique Properties

- Size (< 100nm)
- Optical (metal & Semiconductors
- Magnetic (metal)
- Surface reactivity
- Catalytic activity (high surface area)
- Bioaffinity
- Surface modification

DOD Applications

• Biosensors

 Antimicrobial Agents

Munitions

- Propellants
- Coatings
- Smart Suits

Challenges

- Toxicity
- Reproducibility
- Stability of coatings/functional groups
- bioaffinity
- Effects on protein activity
- Effects on gene expression



Silver Nanoparticles



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Hemorrhagic Fever Viruses

<u>Arenaviridae</u>

- South american HFV, Lassa Fever, LCMV
- Enveloped, RNA viruses
- No effective therapies
- Candid#1 vaccine
- 5-35% fatality rate



<u>Filoviridae</u>

- Ebola and Marburg
- Enveloped, RNA viruses
- No effective therapies
- Vaccine in Phase I trials
- Up to 90% fatality rate





Tacaribe Virus



- New World (Tacaribe) Complex
 - Junin, Machupo, Guanarito, and Sabia
- Tacaribe virus is a biochemically and serologically close relative of the CDC category A arenaviruses but has a low pathogenic potential for humans
- Experimentally:
 - Cytopathic effect in vero cells
 - lethal meningoencephalitis in mice





TCRV Progeny Virus Production

Tacaribe Virus Neutralization by Silver Nanoparticles





Cell Surface TCRV Expression

Negative Control



PositiveControl





10 nm 10 µg/ml

25 nm 50 µg/ml



25 nm 10 µg/ml









Dynamic Light Scattering





TCRV Internalization into Vero Cells



- Ag-NP-treated TCRV is internalized into infected Vero cells
- Ag-NPs and TCRV interact inside the cell lysosomes



TCRV Internalization into Vero Cells



Ag-NPs facilitate uptake of TCRV into Vero cells



Nucleoprotein RNA Expression

N Protein Gene Expression



Mechanism of Ag-NP Inhibition 000 packaging C ۵ **vRNA** 0 mRNA viral protein synthesis



Filovirus

- qRT-PCR detection of internalized eVLPs using Gp as a marker
- Confocal Microscopy of eVLP cell surface binding
- Confocal Microscopy of eVLP internalization
- Cathepsin B and L activity in Vero cells.



Ebola Virus-Like Particles







Cell Surface eVLP Expression





eVLP Internalization into Vero Cells

Negative Control



Positive Control

10nm 10µg



25nm 10µg



10nm 50µg



25nm 50µg



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Cathepsin Activity

- Bulk and Nano Silver have been shown to inhibit enzyme activity.
- Silver binds readily to thiol groups.
- Cathepsin B has been shown to have an essential role in Ebola virus replication.
- Cathepsin L has an accessory role in Ebola virus replication.



Cathepsin B L. Jayashankar, Acharya Nagarjuna University , Guntur





Conclusions

- Ag-NPs neutralize TCRV infection
 - Decrease in S segment gene expression
 - Decrease in progeny virus production
- Ag-NPs do not prevent the internalization of TCRV
 - Ag-NPs and TCRV interact inside the cell
 - Mechanism of inhibition occurs between endocytosis and vRNA gene production
- Ag-NPs have a similar effect on eVLPs
- Ag-NPs decrease cathepsin activity



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Comments/Questions

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