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

<table>
<thead>
<tr>
<th>Size</th>
<th>UNCOATED</th>
<th>POLYSACCHARIDE COATED</th>
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</thead>
<tbody>
<tr>
<td>10 nm</td>
<td><img src="Image1.png" alt="Image" /></td>
<td><img src="Image2.png" alt="Image" /></td>
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<tr>
<td></td>
<td>12.78 ± 0.13</td>
<td>9.48 ± 4.286</td>
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<tr>
<td></td>
<td>Dr. Steve Oldenburg, NanoComposix</td>
<td>Dr. Dan Goia, Clarkston University</td>
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<tr>
<td>25 nm</td>
<td><img src="Image3.png" alt="Image" /></td>
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<tr>
<td></td>
<td>27.474 ± 9.062</td>
<td>25.98 ± 8.38</td>
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<tr>
<td></td>
<td>Dr. Karl Martin, Novacentrix</td>
<td>Dr. Dan Goia, Clarkston University</td>
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Hemorrhagic Fever Viruses

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

**Filoviridae**
- 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
Arenavirus Experimental Setup

0 hour

1 h.p.i

Confocal Microscopy

Cell surface expression

4 h.p.i

Confocal Microscopy

Virus internalization

Vero cells

2 h.p.i

12 h.p.i

8 d.p.i

1 h.p.i

TEM

Virus internalization

4 d.p.i

qRT-PCR

Harvest Progeny Virus

TCID<sub>50</sub> determination
TCRV Progeny Virus Production

Tacaribe Virus Neutralization by Silver Nanoparticles

- 10 nm Ag
- 10 nm PS-Ag
- 25 nm Ag
- 25 nm PS-Ag

Log 10 TCID₅₀/ml

Concentration of Ag (µg/ml)

0 1 5 10 25 50 100

* * *** *
Cell Surface TCRV Expression

- Negative Control
- Positive Control

Comparison of TCRV expression:
- 10 nm 50 µg/ml
- 25 nm 50 µg/ml
- 10 nm 10 µg/ml
- 25 nm 10 µg/ml
Dynamic Light Scattering

![Dynamic Light Scattering Graph](image)

- TV
- 10nm Ag
- 10nm PS-Ag
- 25nm Ag
- 25nm PS-Ag

Diameter (nm)

Concentration of Ag (µg/ml)
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

Fold Change Over Control

Concentration of Ag (µg/ml)

- 0
- 10
- 25
- 50

10nm Ag
10nm PS-Ag
25nm Ag
25nm PS-Ag
Mechanism of Ag-NP Inhibition

vRNA

mRNA

viral protein synthesis

packaging

replication
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

eVLP binding to Vero cells +/- Ag-NP

Fold Change over control

Concentration of Ag (μg/ml)
### Cell Surface eVLP Expression

<table>
<thead>
<tr>
<th></th>
<th>10nm uncoated Ag 10µg/ml</th>
<th>10nm uncoated Ag 50µg/ml</th>
<th>10nm PS-coated Ag 10µg/ml</th>
<th>10nm PS-coated Ag 50µg/ml</th>
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<tbody>
<tr>
<td>Vero cells (negative control)</td>
<td><img src="10nm_uncoated_Ag_10ug/ml" alt="Image" /></td>
<td><img src="10nm_uncoated_Ag_50ug/ml" alt="Image" /></td>
<td><img src="10nm_PS-coated_Ag_10ug/ml" alt="Image" /></td>
<td><img src="10nm_PS-coated_Ag_50ug/ml" alt="Image" /></td>
</tr>
<tr>
<td>eVLPs (positive control)</td>
<td><img src="25nm_uncoated_Ag_10ug/ml" alt="Image" /></td>
<td><img src="25nm_uncoated_Ag_50ug/ml" alt="Image" /></td>
<td><img src="25nm_PS-coated_Ag_10ug/ml" alt="Image" /></td>
<td><img src="25nm_PS-coated_Ag_50ug/ml" alt="Image" /></td>
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</table>
eVLP Internalization into Vero Cells

- **Negative Control**
- **Positive Control**
- **10nm 10μg**
- **10nm 50μg**
- **25nm 10μg**
- **25nm 50μg**
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
Cathepsin B Activity in Vero Cells

- 10nm uncoated
- 10nm PS
- 25nm uncoated
- 25nm PS

Cathepsin L Activity in Vero Cells

- 10nm uncoated
- 10nm PS
- 25nm uncoated
- 25nm PS
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
Acknowledgements

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Nanoparticles
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• Dr. Dan Goia (Clarkson University, Center for Advanced Materials Processing, Potsdam, NY)

Ebola virus-like particles
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Comments/Questions