- In-vitro Evaluation of Autoinducer-1 Microparticles as potential adjuvant

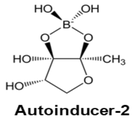
Mohammad N. Uddin[1], Sarthak Shah[1], Christiane Chbib[2] and Martin J. D'Souza[1]

[1]Vaccine Nanotechnology Laboratory, Center for Drug Delivery Research, College of Pharmacy, Mercer University, Atlanta, GA

[2]College of Pharmacy, Larkin University, Miami, FL

PURPOSE

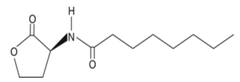
Precursor of bacterial Quorum Sensing (QS) molecule: Autoinducer-2 (AI-2)



Autoinducer-2

Autoinducer-1 molecule:

N-octanol-L-Homoserine lactone



❖ We studied the immunological profile of a bacterial origin adjuvant, autoinducer-1 (N-octanol-L-Homoserine lactone), in a microparticulate form for immunogenicity and its potential for serving as an adjuvant in vaccine formulations.

OBJECTIVE(S)

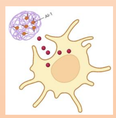
Microparticulate formulation of Autoinducer-1 molecule

In-Vitro immunogenicity evaluation of microparticulate Autoinducer-1

In-Vitro testing of AI-1 MPs with other adjuvants and vaccines.

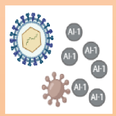
METHOD(S)

Spray dried formulation of AI-1 molecule and vaccine adjuvant microparticles using pre-crosslinked BSA polymer matrix.



Characterization and In-Vitro evaluation of the microparticles for their **immunogenicity**, dose optimization, and cytotoxicity.

Evaluation of immunogenicity of **antigen plus adjuvant**, comparison study with FDA approved adjuvants.



In vitro evaluation of adjuvant effect: AI-1 paired with the **zika MPs, Measles MPs, and Influenza vaccine.**

RESULT(S)

Characterization of microparticles

Recovery yield (%)	74%
Particle size (um)	4.43±0.29
Polydispersity index (PDI)	0.468
Zeta potential	-32.0±0.92 mV

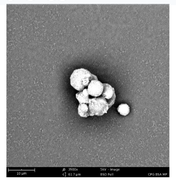
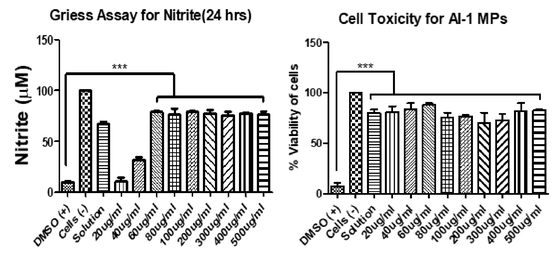


Fig 1: Scanning Electron Microscopy (SEM)of AI-1 MPs

✓ High recovery yield from spray drying.
✓ Small particle size.
✓ Low PDI = Good uniformity distribution of particles.
✓ Zeta potential = particles do not agglomerate.

In-Vitro Immunogenicity and cytotoxicity

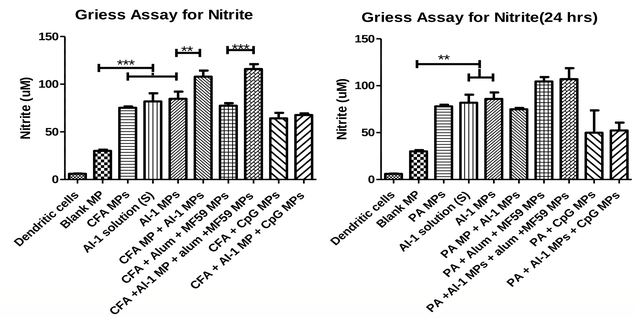


✓ AI-1 MPs showed a max NO release of 60ug/ml when exposed to antigen presenting cells (APCs).

✓ AI-1 MPs were not cytotoxic to the APCs even at higher concentrations.

Fig 2: Nitrite release as obtained by Griess's assay from dendritic cells

Adjuvant potential against bacterial infections

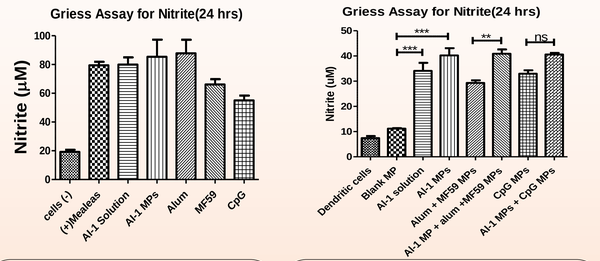


✓ Antigen CFA adjuvanted with AI-1 MPs displayed a comparable nitrite release when combined with FDA approved adjuvants Alum and MF59.

✓ Nitrite release from dendritic cells when paired with antigen PA and adjuvant AI-1 MPs displayed similar immunogenicity as other adjuvants.

Fig 3: Nitrite release as obtained by Griess's assay from dendritic cells.

AI-1 MPs versus FDA approved Adjuvants

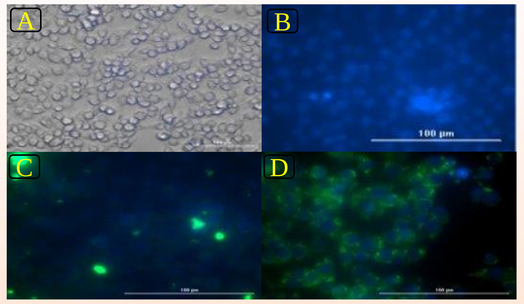


✓ Al-1 MPs are immunogenic as FDA approved adjuvants: Alum, Addavax (MF59), and CpG.

✓ Al-1 MPs are immunogenic as combined FDA approved adjuvants: Alum and MF59.
✓ AI-1 MPs increase immunogenicity of adjuvant CpG MPs.

Fig 4: Nitrite release as obtained by Griess's assay from dendritic cells.

Autophagy in antigen presentation

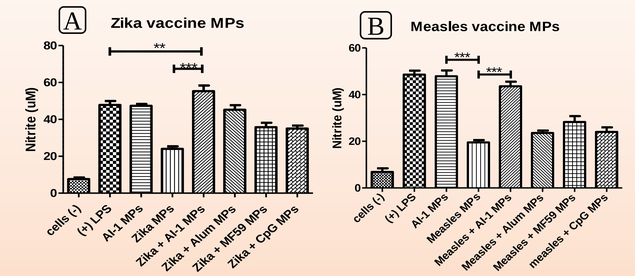


✓ Autophagy is a vital process for antigen presentation to Major Histocompatibility Complexes (MHC) on APCs.

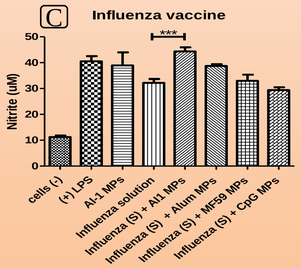
✓ Cells exposed to vaccine MP and AI-1 adjuvanted MPs expressed higher number of autophagosomes.

Fig 5: A: dendritic cells, B: Rapamycin inhibitor, C: PA antigen MPs, D: PA antigen MPs + AI-1 MPs.

Adjuvant potential against viral infections



✓ AI-1 vaccine MPs showed adjuvant potential with the Zika and Measles vaccine MPs.

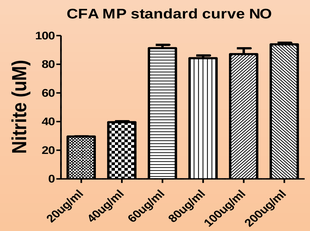


✓ AI-1 vaccine MPs displayed adjuvant potenial with marked influenza vaccine.

✓ AI-1 vaccine MPs displayed adjuvant potenial with three viral antigens: Zika, Measles, and Influenza.

Fig 8: Nitrite release as obtained by Griess's assay from dendritic cells. A: dendritic cells treated with zika MPs. B: cells treated with measles MPs. C: cells treated with influenza marketed vaccine.

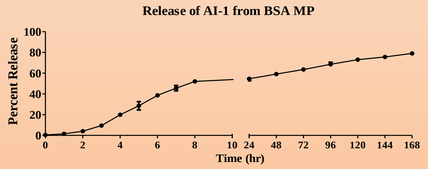
Dose optimization of antigen



✓ Antigens:
❑ Colonization Factor Antigen I (CFA) antigen from E. Coli.
❑ Protective Antigen (PA) antigen from Bacillus anthracis.

Fig 6: Nitrite release as obtained by Griess's assay from dendritic cells. Antigen: Colonization Factor Antigen I (CFA) antigen from E. Coli, and Protective Antigen (PA) antigen from Bacillus anthracis.

Release of AI-1 from BSA MPs



✓ 50% release of AI-1 from BSA matrix in 7 hrs.

Fig 7: Release study of AI-1 adjuvant MPs from the BSA polymer. Analysis done via bicinchoninic acid assay (BCA assay).

CONCLUSION

Spray dried microparticle formulation

AI-1 MP induced innate immune response

All vaccine microparticles were non-cytotoxic

AI-1 MPs were immunogenic with bacterial and viral antigens

AI-1 MPs can be used as vaccine adjuvants