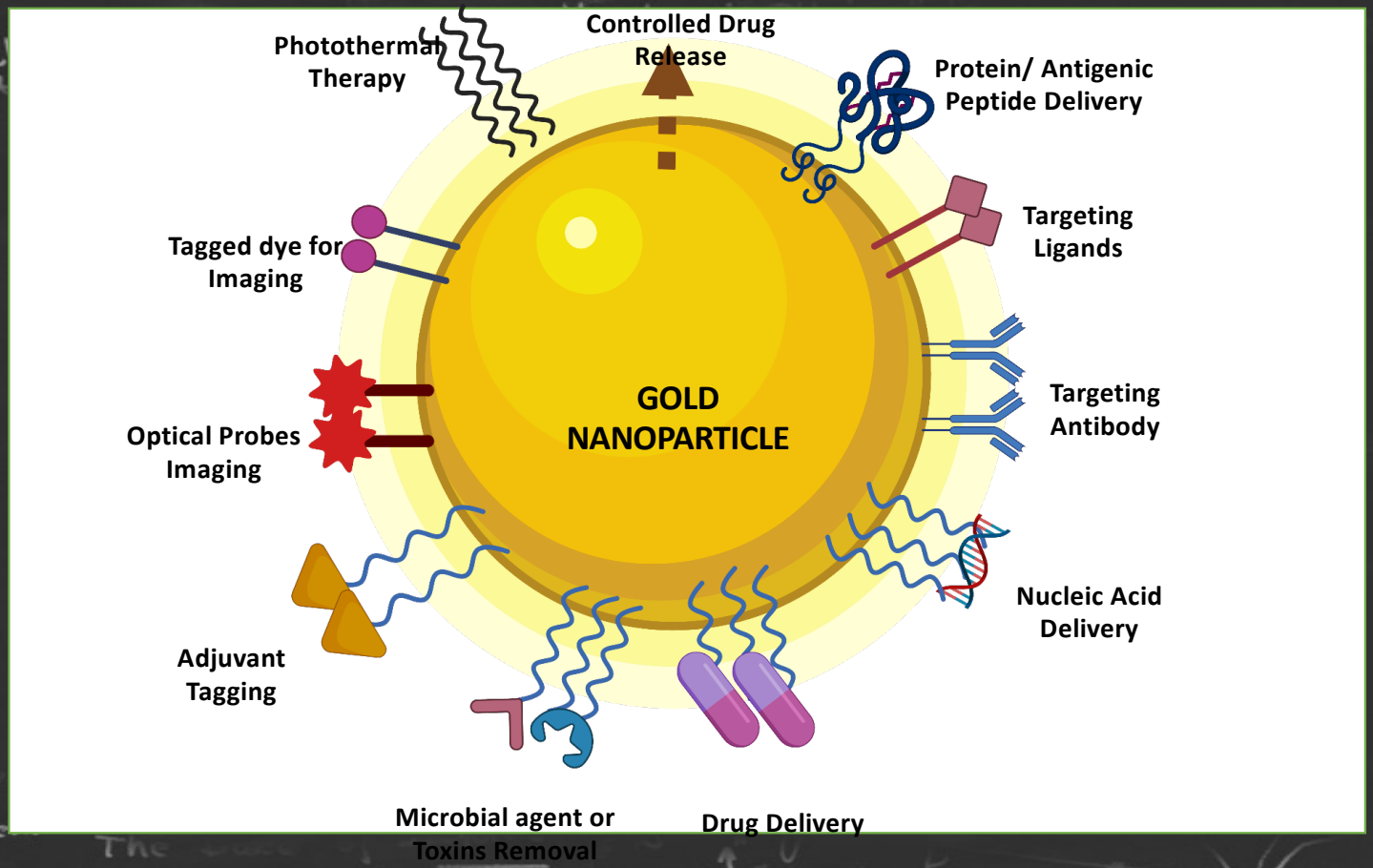
GOLD-NANOPARTICLE-BASED ADJUVANTS FOR VACCINES AGAINST INFECTIOUS DISEASE



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Conference Vaccines
December 9, 2022

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Abstract:
Gold-nanoparticle adjuvants for respiratory tract viral infectious diseases.
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Background
Viral proteins, or recombinant subunit proteins can often be weak immunogens if used alone as vaccine antigens. Enhancement of immune reactivity is therefore often required. This is often adjusted, either by significant increase in antigen amount, or by addition of an adjuvant. Suitable mucosal adjuvants are few and highly desirable, and here we suggest AuGP nanoparticle adjuvants as a stable option. The immune-modifying properties of gold-nanoparticles as adjuvants will be exemplified and presented in an in vitro and pre-clinical setting.

Results:
Results show a significantly elevated humoral immune reactivity against especially the HA and S1 antigens if adjuvants were used both as s.c but especially well if given at a dose of 1ug/antigen/mouse twice intranasally (at days 0 and 21). Serum IgG titers were with highest IgG titers among the nasally immunized (median 12 200+/- 6770), followed by the s.c immunized adjuvants (median 3440+/- 1220) if AuGP was used as adjuvant. Lung-wash anti-viral IgA was seen only in the nasally immunized animals. Cell-mediated immune reactivity was seen against all three included antigens in all vaccinated study groups.
When vaccinated and controls were challenged with influenza A virus, vaccinated animals given low-dose vaccine with AuGP-nanoadjuvant full protection from disease was obtained. Control animals or adjuvant alone immunized animals were not protected from disease.

In conclusion:
AuGP-nano particles are attractive adjuvant options, especially due to their low to modest cell-toxicity, immune-modulating capacity as small but stable vaccine antigen-carriers and their secretion out of systemic compartments at the small size nanoparticle size of 10 to 40 nm.

Acknowledgements:
Yuming Zhang, Siiri Makkonen and Anna Bini for excellent help with biochemical analyses and summary of data.

MATERIALS AND METHODS

Nanoparticle synthesis:

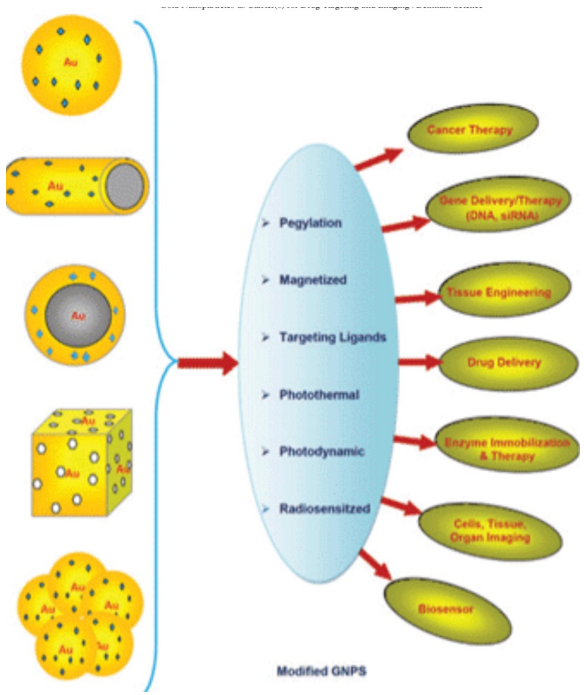
Nanoparticles comes under the range of 1 – 100 nm. They can be synthesized with the help of various methods like chemical vapour deposition, milling, solgel method and self-assembly (bottom-up approach).

Characterization methods:

Synthesized nanomaterials should be characterized in terms of their structure, size and shape. These factors determine their mode of action.

In order to characterize nanoparticles various techniques like scanning electron microscopy (SEM), Transmission electron microscopy (TEM), atomic force microscopy (AFM), X-ray diffraction studies etc. are performed.

These characterization techniques give the assurance of the optimized functions of nanomaterials in the particular application.



vaccines

MDPI

Review

Efficacy and Immune Response Elicited by Gold Nanoparticle-Based Nanovaccines against Infectious Diseases

Anirban Sengupta [1], Mohammad Azharuddin [1], Noha Al-Otaibi [2] and Jorma Hinkula [1,*]

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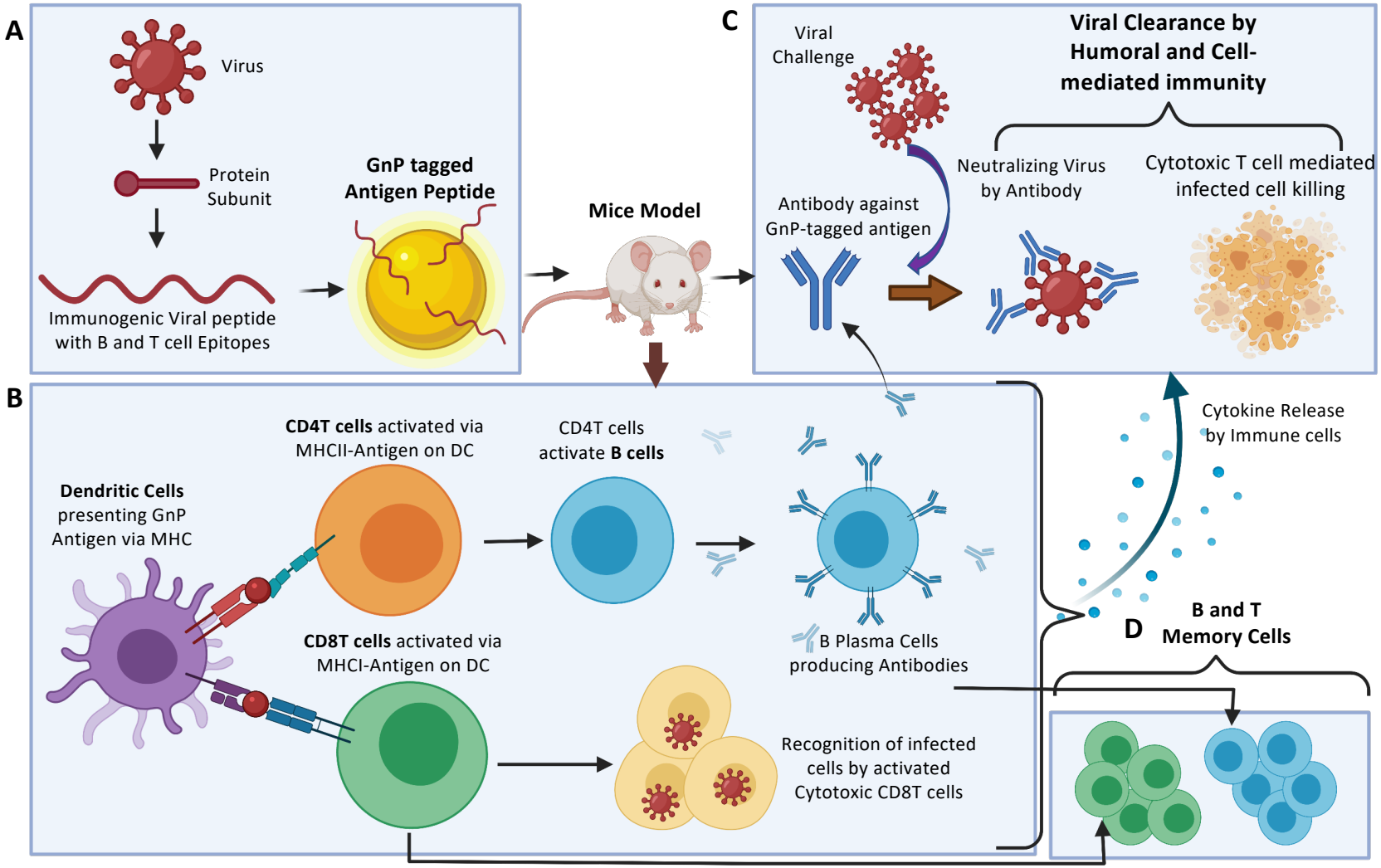
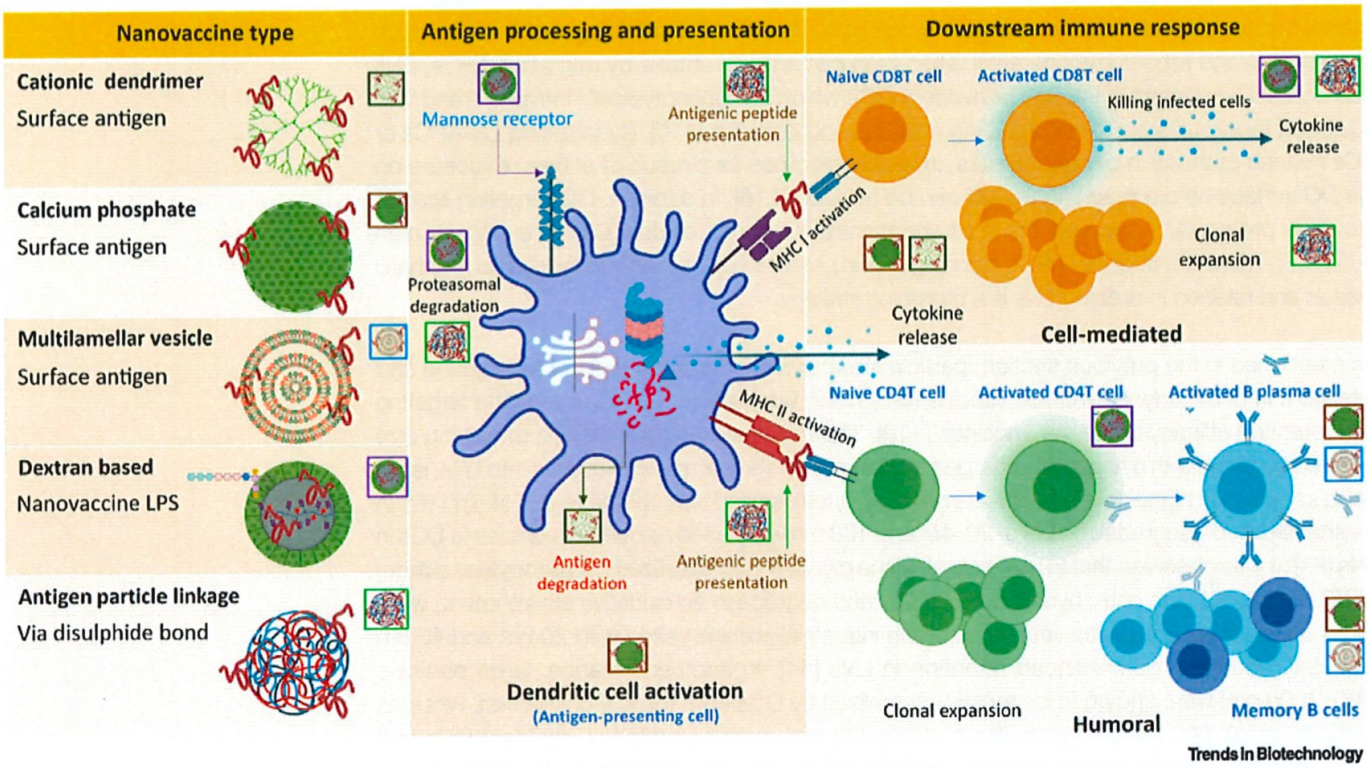

A
Virus
Protein Subunit
Immunogenic Viral peptide with B and T cell Epitopes
GnP tagged Antigen Peptide
Mice Model
C
Viral Challenge
Viral Clearance by Humoral and Cell-mediated immunity
Neutralizing Virus by Antibody
Cytotoxic T cell mediated infected cell killing
Antibody against GnP-tagged antigen
B
Dendritic Cells presenting GnP Antigen via MHC
CD4T cells activated via MHCII-Antigen on DC
CD4T cells activate B cells
CD8T cells activated via MHCI-Antigen on DC
B Plasma Cells producing Antibodies
Recognition of infected cells by activated Cytotoxic CD8T cells
Cytokine Release by Immune cells
D
B and T Memory Cells

Immunological communication/& activation.



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Innate Immune Invisible Ultrasmall Gold Nanoparticles—Framework for Synthesis and Evaluation

Geyunjian Harry Zhu, Mohammad Azharuddin, Rakibul Islam, Hassan Rahmoune, Suryyani Deb, Upasona Kanji, Jyotirmoy Das, Johannes Osterrieth, Parminder Aulakh, Hashi Ibrahim-Hashi, Raghav Manchanda, Per H. Nilsson, Tom Eirik Mollnes, Maitreyee Bhattacharyya, Mohammad M. Islam, Jorma Hinkula, Nigel K. H. Slater, and Hirak K. Patra*

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RESULTS

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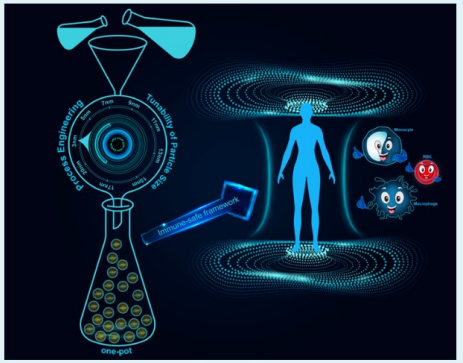
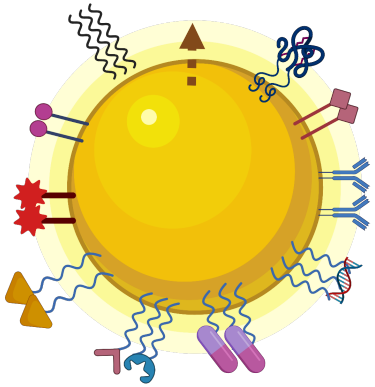
ABSTRACT: Nanomedicine is seen as a potential central player in the delivery of personalized medicine. Biocompatibility issues of nanoparticles have largely been resolved over the past decade. Despite their tremendous progress, less than 1% of applied nanosystems can hit their intended target location, such as a solid tumor, and this remains an obstacle to their full ability and potential with a high translational value. Therefore, achieving immune-tolerable, blood-compatible, and biofriendly nanoparticles remains an unmet need. The translational success of nanoformulations from bench to bedside involves a thorough assessment of their design, compatibility beyond cytotoxicity such as immune toxicity, blood compatibility, and immune-mediated destruction/rejection/clearance profile. Here, we report a one-pot process-engineered synthesis of ultrasmall gold nanoparticles (uGNPs) suitable for better body and renal clearance delivery of their payloads. We have obtained uGNP sizes of as low as 3 nm and have engineered the synthesis to allow them to be accurately sized (almost nanometer by nanometer). The synthesized uGNPs are biocompatible and can easily be functionalized to carry drugs, peptides, antibodies, and other therapeutic molecules. We have performed *in vitro* cell viability assays, immunotoxicity assays, inflammatory cytokine analysis, a complement activation study, and blood coagulation studies with the uGNPs to confirm their safety. These can help to set up a long-term safety-benefit framework of experimentation to reveal whether any designed nanoparticles are immune-tolerable and can be used as payload carriers for next-generation vaccines, chemotherapeutic drugs, and theranostic agents with better body clearance ability and deep tissue penetration.



KEYWORDS: *ultrasmall nanoparticles, process engineering, immunocompatibility, complement-safe, coagulation-safe, pro-inflammatory cytokine, biocompatibility*

Immunization Protocol

- A/H1N1 HA 1,5 μg
- **SARSCoV-2 S1** 1,5 μg
- **M2e peptide** 1,5 μg



- **AuGNP 10nm and 40 nm**
- **N3 cat. lipid emulsion 40 nm**
- Saline/PBS

Biojector administration S.c.

BALB/C & C57BL/6 (M/F 50/50)

FACS and Elispot read out following clinical immunization schedule

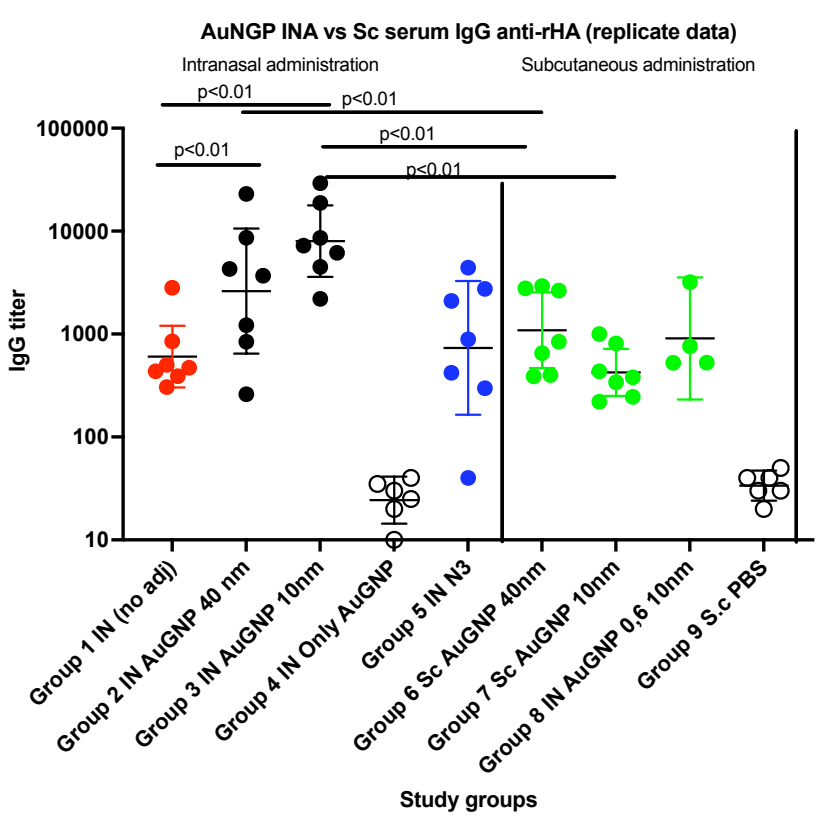
Influenza A/rHA and SARS-CoV-2/S1 mouse safety and immunogenicity study plan

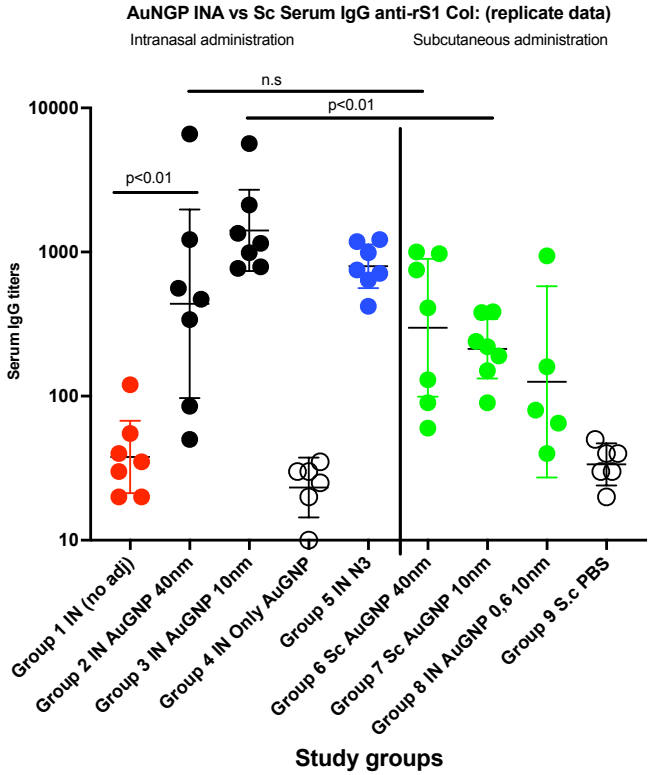
Group	Vaccine (3ug/ml)	Adjuvant	Mouse	N	Virus nasal drop (pfu; 0.05 ml)
1	Influenza H1N1 rHA/SARS-CoV-2 S1 (1,5+1,5ug) INA	-	BALB/C	8 and 6	10^5 i.n.a.
2	Influenza H1N1 rHA/SARS-CoV-2 S1 (1,5+1,5ug) INA	40nm, AuGNP, 4 ug i.n.a. 1*6 ul	BALB/6	8 and 6	10^5 i..n.a.
3	Influenza H1N1 rHA/SARS-CoV-2 S1 (1,5+1,5ug) INA	10 nm, AuGNP 4 ug i.n.a. 1*6 ul	BALB/C	8 and 6	10^5 i.n.a.
4	AuGNP 10nm/40nm (10ug) INA, 2*20 ul	10-40nm, AuGNP, 4 ug i.n.a. 2*6 ul	BALB/C	6 and 4	10^5 i.n.a.
5	Influenza H1N1 rHA/SARS-CoV-2 S1 (1,5+1,5ug) INA, 2*6 ul Naive	Lipid emulsion N3 1,5% (cationic)	BALB/C	8 and 6	-
6	Influenza H1N1 rHA/SARS-CoV-2 S1 (1,5+1,5ug) S.c	40nm, AuGNP, 4 ug S.c. 1*100 ul	Balb/c	8 and 6	10^5 i.n.a.
7	Influenza H1N1 rHA/SARS-CoV-2 S1 (1,5+1,5ug) S.c	10nm, AuGNP, 4 ug S.c. 1*100 ul	Balb/c	8 and 6	10^5 i.n.a.
8	Influenza H1N1 rHA/SARS-CoV-2 S1 (1,5+1,5ug) S.c	10nm, AuGNP, 0,6 ug S.c. 1*100 ul	Balb/c	8 and 6	10^5 i.n.a.
9	Naive (ug) S.c	PBS S.c. 100 ul	Balb/c	6 and 4	10^5 i.n.a.
		-			-

Immunization schedule: Prime day 0; bleed day 21; boost day 28; Harvest spleens and final bleed day 56. Challenge day 60 (of parallell groups with 6 mice/group.

Plasmid components

ELISA Serum IgG



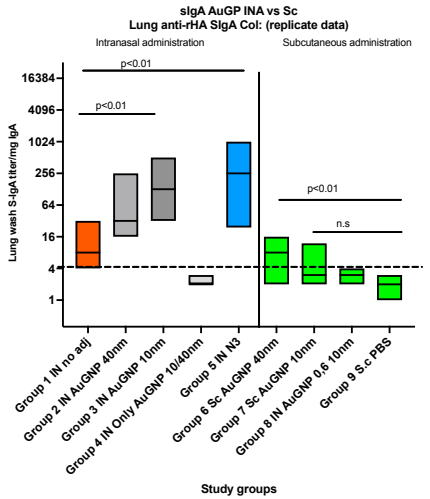


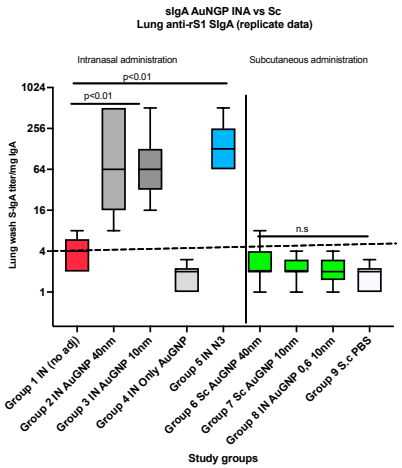
Mucosal respiratory tract IgA

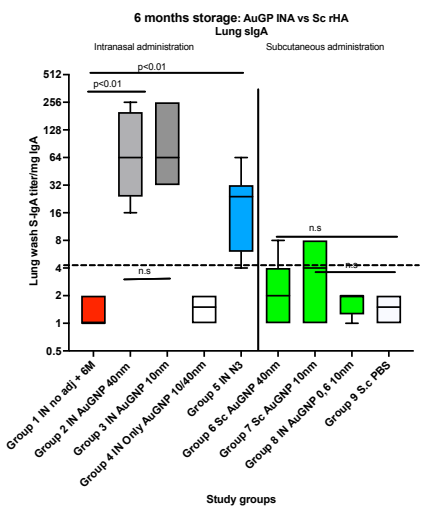
Vaccine and adjuvant stability

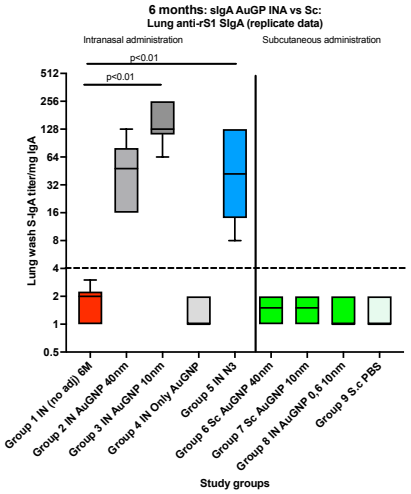
New

Month 6 (at RT)



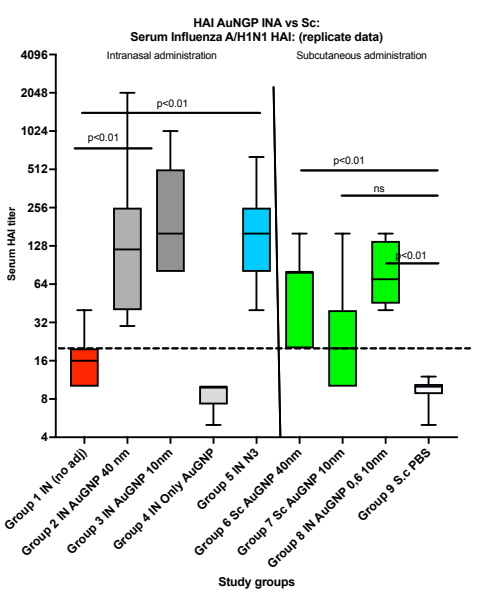


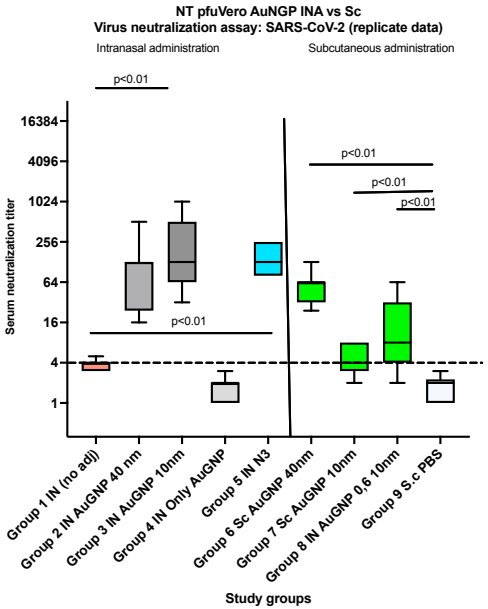




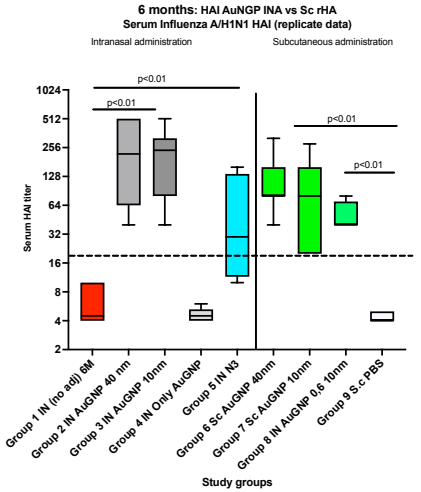
Neutralizing antibodies

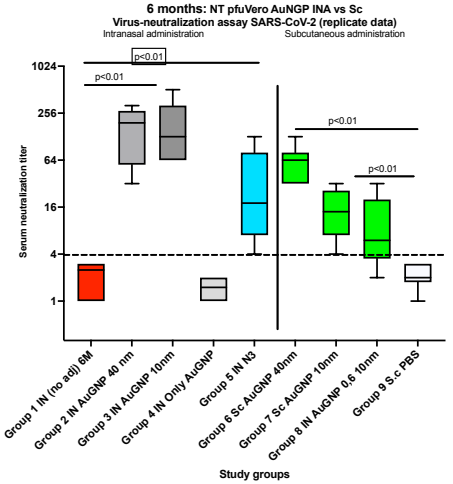
Vaccine & Adjuvant stability:
Serum neutralizing titers
New





Month 6 (at RT)





Conclusions:

°. Intranasal administration of AuGNP at sized 40 and 10 nm induce stronger serum IgG titers than S.c administered AuGNP:s

°. Intranasal and S.c administration of AuGNP at sized 40 and 10 nm induce good virus-neutralizing titers.

°. Intranasal and S.c administration of AuGNP at sized 40 and 10 nm induce full protective immunity against Influenza A virus challenge.

AuGNP influenza rHA/Sars CoV-2 S1-spike vaccine candidates show storage/immunogenicity stability of at least 6 months at room temp.

Ongoing research

Cell-mediated influenza A and SARS-CoV-2 specific immunity analyses ongoing.

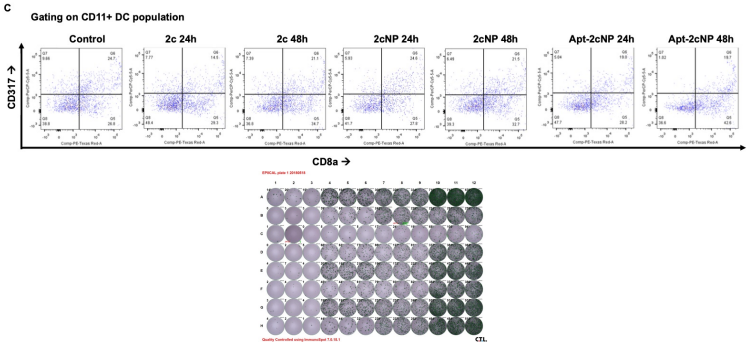
Cross-neutralizing antibody breath, in serum and mucosal tissues?

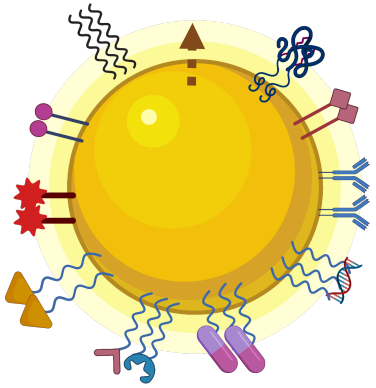
Longevity of induced vaccine-specific immunity at different ages.

Heterologous prime-booster immunization protocols evaluated.

Freeze-drying and powder-lyophilization?

Extensive safety-analyses/Tox studies.





Materials and methods:

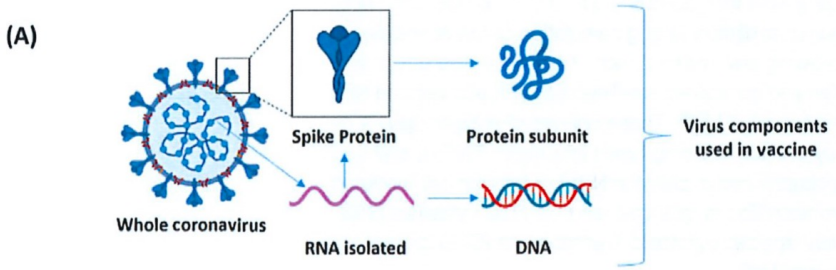
Gold(III) chloride solution (484385-10G), trisodium citrate dihydrate (W302600-1KG-K) were purchased from Sigma Aldrich (MO, USA

GNPs with a diameter of 2-10nm, 20nm, 30nm, 40-55nm were chemically synthesized by Turkevich[39] and Frens[40] method with required modifications[41]. The mechanism behind this method is that gold (III) chloride solution ($HAuCl_4$) is reduced into monovalent gold by reducing agent trisodium citrate dihydrate ($Na_3C_6H_5O_7 \cdot 2H_2O$).

ELISA, binding anti-viral IgG in serum
ELISA, binding anti-viral IgA in respiratory mucosa

Virus-neutralizing, anti-viral IgG in serum
Virus-neutralizing anti-viral IgA in respiratory mucosa

Viral antigens used



rS1 spike protein SARS CoV-2 and rHA/H1N1 Influenza A virus purshased from SinoBiologicals, Germany.

Synthetic influenza A M2-peptides:

M2e peptide number	M2e peptide sequence
Peptide 1 (Human consensus)	SLLTEVETPIRNEWGCRCNDSSD
Peptide 2	SLLTEVETPIRNEWGSRSNDSSDC[20]
Peptide 3	SLLTEVETPIRNEWGSRSNDSSDCG
Peptide 4	SLLTEVETPIRNEWGGRGNDSSDCG

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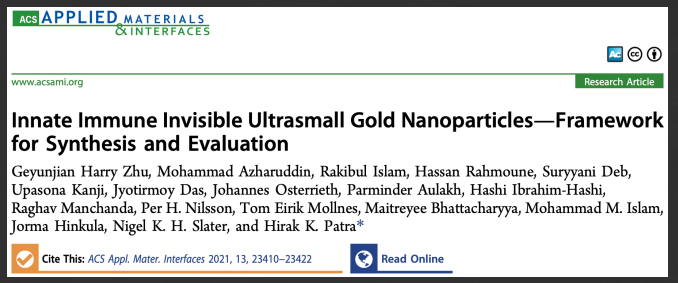

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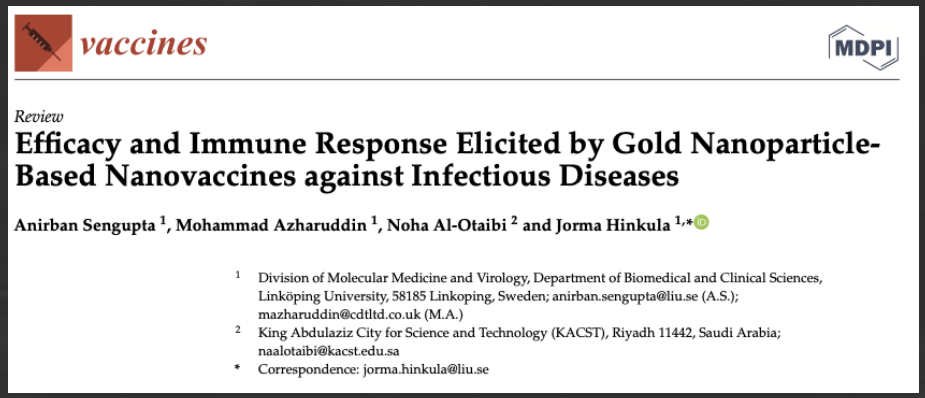

vaccines MDPI

Review

Efficacy and Immune Response Elicited by Gold Nanoparticle-Based Nanovaccines against Infectious Diseases

Anirban Sengupta [1], Mohammad Azharuddin [1], Noha Al-Otaibi [2] and Jorma Hinkula [1,*]

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