Development and characterization of emulsion-based immunoadjuvant Nano vaccines

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ABSTRACT

Purpose: The main goal of this project is to investigate strategies for development of vaccine nanoparticles co-encapsulating ovalbumin as a model antigen and emulsion based immunoadjuvants. An oil in water emulsion, squalene, and a water in oil emulsion, Montanide 61, will be used in this study to enhance the immunoadjuvant efficiency of the emulsions.

Method: Poly (lactide-co-glycolide) (PLGA) nanoparticles were prepared based on solvent evaporation and ultrasonication techniques. Various formulations of nanoparticles were developed carrying protein and immunoadjuvants, and then characterized with respect to their particle size, zeta potential, and polydispersity index using the ZetaSizer instrument through dynamic light scattering technique. Different formulations parameters such as polymer concentration, volume, ultrasonication speed and time, antigen dose and immunoadjuvant dose were investigated to optimize the nanoparticulate vaccines.

Results: Many experiments and optimization steps were conducted to achieve optimum nano-vaccines loaded with the immunoadjuvants and to evaluate their physicochemical properties. The data obtained for the size of our nanoparticles are shown to be in the range of 200-300 nm and their zeta potential is between -20 mv and -30 mv. Yield of the production technique was also optimized.

Conclusion: These novel nanovaccines are developed in a suitable range for effective immune cell uptake. Our target is to improve the delivery of emulsion-based adjuvants and minimize their dose to avoid their side effects.

INTROUDUCTION

Nanoparticle-based drug delivery systems have remarkable prospects to treat viral infections. The key advantages of nanoparticles utilized as drug carriers are high carrier capacity, suitable stability, feasibility of combination of both hydrophobic and hydrophilic substances, and possibility of variable routes of administration, including inhalation and oral application as well as PLGA as a synthetic polymer that is approved by the U.S. food and drug administration (1). Adjuvants as materials can rise the immune response to a given antigen without being antigenically associated with it. Some compounds for instance, aluminium salts have long been utilized as adjuvants, and in tetanus and diphtheria vaccines the toxoids are combined with aluminium phosphate or hydroxide (2). Here there some other adjuvants such as Montanide 61, Squalene. Montanide ISA 61 VG is a mineral oil-based adjuvant which industrialized by SEPPIC. According to the evidence, Montanide 61 water-in-oil (W/O) emulsion is stable, robust, easy to inject, induces strong immune response and also long-lasting protection which cause it considered suitable for antigens that possess relatively low immunogenicity (3). Squalene adjuvants are oil-in-water (o/w) emulsions developed with fully metabolizable squalene droplets and nontoxic surfactants in the absence or presence of the immunostimulatory α-tocopherol. Squalene exists naturally in the body of human as a precursor to cholesterol, in addition, it is derived from liver of shark (4).

OBJECTIVES

The main objective of the project is to develop polymeric PLGA vaccines loaded with OVA along with immunoadjuvants, which is divided into four short term objectives:

1. To optimize the loading of proteins in nanoparticles with respect to different parameters such as centrifugation time and speed, and polymer concentration.
2. To develop and screen the vaccine nanoparticles co-encapsulating antigens and immune adjuvants to achieve the optimum candidates.
3. To fully characterize the nanoparticles with respect to their physicochemical properties such as size, zeta potential, yield percentage, and protein/adjuvant loading efficiency.
4. To investigate the immunological efficacy and toxicity of various vaccine formulations in dendritic cells in vitro.

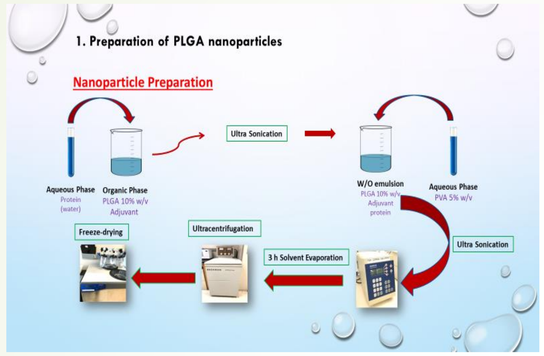
HYPOTHESIS

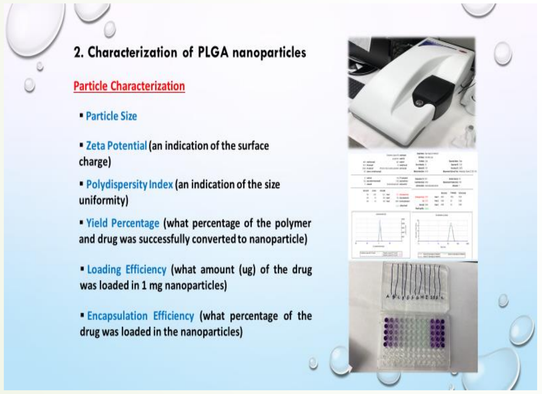
We hypothesize that the PLGA formulation will successfully co-encapsulate OVA and montanide/squalene adjuvants and have the suitable physicochemical properties as a vaccine formulation.

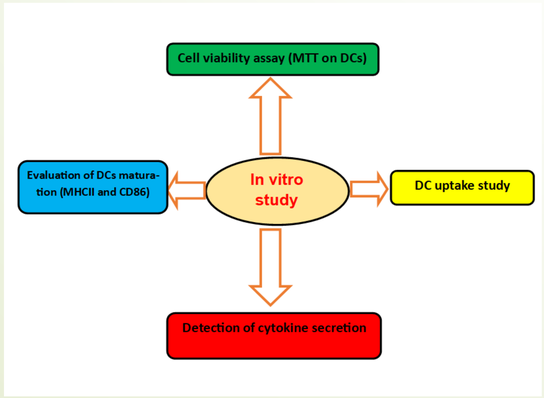
MATERIAL AND METHODS

Proposed methods:

- Preparation of PLGA nanoparticles
- Characterization of PLGA nanoparticles
- In vitro studies







RESULTS

Table1. Particle size and ZP of COOH and ester terminated NPs: We prepared empty nano particles while we tried different Centrifugation time and speed for find the best method.

Formulations	Centrifugation	Size	Zeta potential	Yield
Empty by A method	15 min/15000 and 20 min/18000	212.7± 4.2 nm	-11.6± 1.7	57%
Empty by B method	18 min/15000 and 25 min/18000	197.9± 4.7 nm	-13± 1.4	67%
Empty by C Method	15 min/17000 and 20 min/20000	206.6± 3.2 nm	-15.2± 0.4	63%

Table2. Particle size, ZP, EE of OVA (%) and OVA loading (ug/mg) of COOH and ester terminated NPs: Different tested formulation by vary concentration of OVA and 100 mg PLGA for find optimized method.

Concentration of OVA at 100 mg PLGA	Centrifugation	Size	Zeta potential	Yield	LODING DOSE	EE
2.5mg ova	18 min/15000 and 25 min/18000	247.4± 5.2 nm	-19.1± 0.5	63%	7.375 ± 0.4 ug/mg	14.75%
5 mg ova	18 min/15000 and 25 min/18000	255.9± 1.5 nm	-22.5± 1.1	59%	14.25± 0..7 ug /mg	14.25%
10 mg ova	18 min/15000 and 25 min/18000	240.8± 3.5 nm	-24.2± 1.3	64%	43.075± 1.01 ug /mg	21.53%

Table3. Particle size, ZP, EE of OVA (%) and OVA loading (ug/mg) of COOH and ester terminated NPs: Different tested formulation by vary concentration of OVA and 60 mg PLGA for find optimized method.

Concentration of OVA at 60 mg PLGA	Centrifugation	Size before freeze drying	Zeta potential before freeze drying	Size After freeze drying	Zeta potential	Yield	LODING DOSE	EE
2.5 mg ova	18 min/15000 and 25 min/18000	261.4± 2.2 nm	-23.5± 2,3	281.7± 3.2 nm	-26.6± 2.4	68%	23.375± 0.24 ug/mg	42.075 %
5 mg ova	18 min/15000 and 25 min/18000	231.6± 3.5 nm	-25.2± 1.7	253.8± 2.72 nm	-29± 1.5	59%	25.075± 0.85ug /mg	20.06%
10 mg ova	18 min/15000 and 25 min/18000	248.4± 1.7 nm	-21.4± 2.4	270.1± 3.1 nm	-25.7± 1.9	68%	29.125± 0.68 ug /mg	13.34%

Table4. Particle size, ZP, EE of OVA (%) and OVA loading (ug/mg) of COOH and ester terminated NPs for optimized method.

Concentration of OVA at 60 mg PLGA	Centrifugation	Size before freeze drying	Zeta potential before freeze drying	Size After freeze drying	Zeta potential	Yield	LODING DOSE	EE
2.5 mg ova	18 min/15000 and 25 min/18000	261.4± 4.2 nm	-23.5± 1.4	281.7± 4.2 nm	-26.6± 1.4	68%	23.375± 1.4 ug/mg	42.075%

Figure 1. sample Charts of particle size and ZP results for optimized method

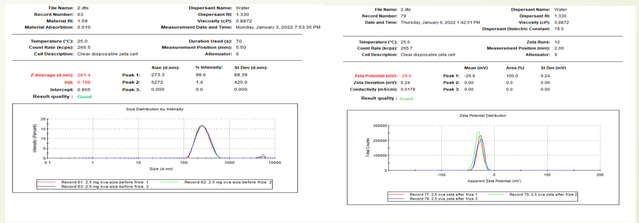


Table5. Summary of various successful formulations of nanoparticles loaded with OVA and montanide 61 (W/O emulsion adjuvant) (various concentrations of montanide)

Formulation	Character	Size	Zeta potential	Yield
2.5mg ova(250PBS) and 60 ul Montanide 61 at 100 mg PLGA	18 min/15000 and 25 min/18000	329.7±2.9	-29.3±1.5	52%
2.5mg ova(250PBS) and 100 ul Montanide 61 at 100 mg PLGA	"	348.3±3.2	-40.2±1.6	54%
2.5mg ova(250PBS) and 150 ul Montanide 61 at 100 mg PLGA	"	545.0±4.7	-35.6±2.2	37%

Table6. Summary of various successful formulations of nanoparticles loaded with OVA and squalene (O/W emulsion adjuvant) (various concentrations of squalene)

formulation	Character	Size	Zeta potential	Yield
2.5mg ova(250PBS) and 100 ul Squalene at 100 mg PLGA	18 min/15000 and 25 min/18000	297.7±2.2	-25.2±1.1	40%
2.5mg ova(250PBS) and 150 ul Squalene at 100 mg PLGA	"	312.5±3.3	-29.5±1.4	42%
2.5mg ova(250PBS) and 250 ul Squalene at 100 mg PLGA	"	287.6±3.1	-29.4±0.8	39%
2.5mg ova(250PBS) and 600 ul Squalene at 100 mg PLGA	"	217.5±1.8	-41.9±1.2	37%

COCLOUSION

W/O and O/W surfactants have immunoadjuvants effects and are regularly used in the vaccines. Their side effects can compromise their application, therefore there is a need for a Nano formulation to enhance the efficacy and reduce the side effects. These novel Nano vaccines were developed and characterized in terms of their physicochemical properties. It was found that the characteristics were in a range suitable for effective immune cell uptake.

FUTURE DIRECTIONS

- ✓ **In vivo studies in pigs by intrauterine administration of vaccine formulations**
- ✓ **In vivo studies in pigs by intradermal vaccination of the formulations**

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REFERENCES

1. Kreuter J. Nanoparticle-based dmg delivery systems. Journal of controlled Release. 1991;16(1-2):169-76.
2. Ellis G, Bone H, Chlebowski R. ADJUVANT THERAPY. J Clin Oncol. 2008;26:4875-82.
3. Khorasani A, Madadgar O, Soleimanjahi H, Keyvanfar H, Mahravani H. Evaluation of the efficacy of a new oil-based adjuvant ISA 61 VG FMD vaccine as a potential vaccine for cattle. Iranian journal of veterinary research. 2016;17(1):8.
4. Nguyen-Contant P, Sangster MY, Topham DJ. Squalene-based influenza vaccine adjuvants and their impact on the hemagglutinin-specific B cell response. Pathogens. 2021;10(3):355.