

Cairo University
Faculty of Veterinary Medicine
Department of Microbiology

Functionalized nanoparticles and their effect on immune response to co-delivered antigen

A Thesis Presented by: Hossam El-din Mahmoud Abd-Elhady

(B.V.Sc. 2009, M.V.Sc. 2013; Cairo University)
For the Ph.D. Degree in Veterinary Medical sciences.
Microbiology
(Bacteriology- Immunology- Mycology)

Under supervision of:

Prof. Dr. Wagih Armanious Gad El-Said

Professor of Microbiology Faculty of Veterinary Medicine Cairo University

Dr. Mahmoud Dardiri El-Hariri

Assistant Professor of Microbiology Faculty of Veterinary Medicine Cairo University

Prof. Dr. Mona Ibrahim El-Enbaawy

Professor and head of Microbiology Department Faculty of Veterinary Medicine Cairo University

Dr. Taher Ahmed Salah El-din

Chief Researcher of Nanotechnology and Advanced Materials Central Lab. Agriculture Research Center

Supervision sheet

1- Prof. Dr. Wagih Armanious Gad El-Said

Professor of Microbiology Microbiology Department Faculty of Veterinary Medicine, Cairo University

2- Prof. Dr. Mona Ibrahim Hassan El-Enbaawy

Professor and head of Microbiology Department Faculty of Veterinary Medicine, Cairo University

3- Dr. Mahmoud Dardiri Mohamed El-Hariri

Assistant Professor of Microbiology Microbiology Department Faculty of Veterinary Medicine, Cairo University

4- Dr. Taher Ahmed Salah El-din

Chief Researcher Nanotechnology and Advanced Materials Central Lab. Agriculture Research Center

Cairo University
Faculty of Veterinary Medicine
Department of Microbiology

Name: HOSSAM ELDIN MAHMOUD ABD ELHADY

Date of birth: 2 / 11 / 1985
Nationality: Egyptian

Degree: Ph. D.

Specialization: (Bacteriology – Immunology – Mycology)

Supervision: Prof. Dr. Wagih Armanious Gad El-Said; Prof. Dr. Mona Ibrahim Hassan El-Enbaawy; Dr. Mahmoud El-hariri and Dr. Taher Ahmed Salah El-din Title: "Functionalized nanoparticles and their effect on immune response to co-

delivered antigen"

ABSTRACT

The current study investigated the immunological properties of functionalized nanoparticle as antigen carrier and immune stimulant. Formulated nano-vaccine was positioned to build a protection against chronic respiratory disease (CRD) caused by Mycoplasma gallisepticum (MG) which is frequently complicated by avian pathogenic E. coli (APEC) causing complicated chronic respiratory disease (CCRD). Field strains of MG and APEC were isolated and the latter was the source of outer membrane vesicles (OMVs). Poly (D,L lactide-co-glycolic acid) (PLGA) co-polymer was used as nano-capsule in this study. The first and second components of nano-vaccine were MG protein and DNA, they were both entrapped in PLGA nanocapsule through double emulsion process. The third component was OMVs of APEC. 270 SPF experimental chickens were divided into 6 tested groups and 6 control groups. Three groups were administered the nanovaccine subcutaneously (SC) and compared with killed commercial MG vaccine while other three groups were administered via intraocular route (IO) and compared with F strain. Evaluation of immunizing potency of nano-vaccine was measured by monitoring IgG status of MG with ELISA and estimating protection efficacy from CRD and CCRD after challenge test. It was shown that administration of un-capsulated MG protein failed to stimulate immune response while PLGA capsulated did so, thus it could be inferred that PLGA capsule performed adjuvanting effect. SC administration of booster dose from PLGA- MG proteins achieved (80%) protection from CRD and (70%) without booster. IO administration of PLGA-MG proteins achieved (70 %) protection from CRD. The maximum protection from CRD (90%) was attained in case of SC injection of PLGA -MG protein plus PLGA-DNA. OMVs reduced the incidence of CCRD to (0%) through SC injection provided that the booster dose had been administered.

Dedicated to:

My father
My mother
My wife
My daughter
And

Dr. Hamed El-Banna

Acknowledgement

I am greatly indebted in all my work and success to our merciful **Allah**.

I would like to express my heartful thanks and appreciation to **Prof. Dr. Wagih Armanious Gad El-Said**, Professor of Microbiology, Faculty of Veterinary Medicine, Cairo University, who suggested the subject of study, for his kind supervision, interest, valuable advice and giving at most help to accomplish this work.

Word cannot express my deepest thanks and gratitude to **Prof. Dr. Mona Ibrahim Hassan El-Enbaawy**, Professor of Microbiology,
Faculty of Veterinary Medicine, Cairo University, for her supervision,
kindness and faithful till end of the work.

I would like also to express my sincere appreciation and pride with the help and support of **Dr. Mahmoud Dardiri El-Hariri**, Assistant professor of Microbiology, Faculty of Veterinary Medicine, Cairo University.

I would like to express my sincere gratitude to **Dr. Taher Ahmed Salah El-din,** Chief Researcher of Nanotechnology and Advanced Materials Central Lab. Agriculture Research Center for his great support and help.

Also many thanks to all members in Department of Microbiology, Faculty of Vet. Med. Cairo University.

I am also deeply grateful to My Wife and My Family for their continuous help and encouragement.

LIST OF CONTENTS

No.	Topic	Page
1.	Introduction	1
2.	Review of literature	6
2.1.	Polymeric nanoparticles as biomaterials	6
2.2.	Outer membrane vesicles (OMVs)	36
2.3.	Bacterial DNA as potent immune stimulant	45
2.4.	Mycoplasma gallisepticum as significant poultry pathogen	50
2.5.	Avian pathogenic <i>E. coli</i>	57
3.	Material and Methods	59
3.1.	Materials	59
3.1.1.	Flock types	59
3.1.2.	Samples	59
3.1.3.	Isolation and biochemical identification	60
3.1.4.	Media used for preservation and maintenance of culture	61
3.1.5.	Molecular identification (PCR)	62
3.1.6	Precipitation and purification of MG proteins	64
3.1.7.	Extraction of genomic DNA from Bacillus subtilis	64
3.1.8.	Synthesis and characterization of PLGA nano- capsule entrapping MG probe (PLGA-MG)	64
3.1.9.	Synthesis and characterization of PLGA nano- capsule entrapping DNA of <i>B. subtilis</i>	68
3.1.10.	Isolation of nano-sized immune stimulatory outer membrane vesicles from avian pathogenic <i>E. coli</i>	69
3.1.11.	Quality control of prepared nano-vaccine	69
3.1.12.	Commercial vaccines	70
3.1.13.	Equipment and Apparatus	70
3.2.	Methods	72
3.2.1.	Sampling	72
3.2.2.	Isolation and biochemical identification	72
3.2.3.	Molecular identification	77

3.2.4.	Preparation and purification of <i>M. gallisepticum</i> proteins	81
3.2.5.	Extraction of genomic DNA from Bacillus subtilis	82
3.2.6.	Preparation of nano-vaccine	83
3.2.6.1.	Synthesis and characterization of PLGA nano- capsule entrapping <i>M. gallisepticum</i> proteins	83
3.2.6.2.	Synthesis and characterization of PLGA nano- capsule entrapping genomic DNA of <i>B. subtilis</i>	88
3.2.6.3.	Isolation and characterization of immune stimulatory nano-outer membrane vesicle from avian pathogenic <i>E. coli</i>	90
3.2.7.	Quality control of the prepared nano-vaccine	91
4.	Results	109
4.1.	Isolation and biochemical identification	109
4.2.	Molecular identification	111
4.3.	Preparation and purification of <i>M. gallisepticum</i> proteins through ammonium sulfate (salting-out) method	114
4.4.	Extraction of genomic DNA from B. subtilis	114
4.5.	Synthesis of nano-vaccine	114
4.5.1.	Synthesis and Characterization of PLGA nanocapsule entrapping <i>M. gallisepticum</i> proteins (PLGA-MG)	114
4.5.2.	Synthesis and Characterization of PLGA nano- capsule entrapping DNA (PLGA-DNA)	119
4.5.3.	Isolation of immune stimulatory nano-outer membrane vesicles from avian pathogenic <i>E. coli</i>	124
4.6.	Quality control of prepared nano-vaccine	124
5.	Discussion	159
6.	Summary	199
7.	References	203

LIST OF TABLES

Table no.	Title	Page
Table 1	Number of collected samples and its distribution according to governorates and flocks production type	59
Table 2	Protein salting out through different saturation points of ammonium sulfate	81
Table 3	In vitro cultivation and identification of Mycoplasma gallisepticum	110
Table 4	Prevalence of E. coli	111
Table 5	The recovery rate of MG by conventional method and PCR confirmation	111
Table 6	Tissue distribution pattern of MG revealed by PCR confirmation of conventionally isolated strains	112
Table 7	Detection rate of MG through application of PCR on 48-hrs incubation PPLO broth	112
Table 8	Tissue distribution pattern of MG by PCR from 48-hrs incubation PPLO broth	113
Table 9	Detection rate of (MG) through direct tissue PCR	113
Table 10	Results of presence of virulence gene in APEC	114
Table 11	Detection rate of positive virulence genes detection in APEC	114
Table 12	In vitro release of MG proteins from PLGA nano- capsules at room temperature	117
Table 13	<i>In vitro</i> release of MG proteins from PLGA nano capsules at 40 °C	118
Table 14	In vitro release of DNA from PLGA nano capsules at room temperature	122
Table 15	<i>In vitro</i> release of DNA from PLGA capsules nanocapsules at 40 °C	123
Table 16	Geometric mean of anti-MG antibody (IgG) titer and evaluation parameters of challenge test after subcutaneous injection of PLGA nano-capsule entrapping MG proteins (PLGA-MG) with OMVs	126

	•	
Table 17	Geometric mean of anti-MG antibody (IgG) titer and evaluation parameters of challenge test after subcutaneous injection of PLGA nano-capsule entrapping MG proteins (PLGA-MG) without OMVs	128
Table 18	Geometric mean of anti-MG antibody (IgG) titer and evaluation parameters of challenge test after subcutaneous injection of PLGA nano-capsule entrapping MG proteins plus PLGA nano-capsule entrapping DNA	130
Table 19	Geometric mean of anti-MG antibody (IgG) titer and evaluation parameters of challenge test after subcutaneous injection of uncapsulated MG proteins (not entapped in PLGA nano-capsule)	132
Table 20	Geometric mean of anti-MG antibody (IgG) titer and evaluation parameters of challenge test after subcutaneous injection of commercial killed MG vaccine (control positive of subcutaneous route)	134
Table 21	Geometric mean of anti-MG antibody (IgG) titer and evaluation parameters of challenge test for non-vaccinated group (control negative of subcutaneous route)	136
Table 22	Geometric mean of anti-MG antibody (IgG) titer and evaluation parameters of challenge test after intraocular administration of PLGA nano-capsule entrapping MG proteins with OMVs	138
Table 23	Geometric mean of anti-MG antibody (IgG) titer and evaluation parameters of challenge test after intraocular administration of PLGA nano-capsule entrapping MG proteins without OMVs	140
Table 24	Geometric mean of anti-MG antibody (IgG) titer and evaluation parameters of challenge test after intraocular administration of PLGA nano-capsule entrapping MG proteins plus PLGA nano-capsule entrapping bacterial DNA	142
Table 25	Geometric mean of anti-MG antibody (IgG) titer and evaluation parameters of challenge test after intraocular administration of uncapsulated MG antigen (not entrapped in PLGA nano-capsule)	144

Table 26	Geometric mean of anti-MG antibody (IgG) titer and evaluation parameters of challenge test after intraocular administration of commercial F strain (control positive of intraocular route)	146
Table 27	Geometric mean of anti-MG antibody (IgG) titer and evaluation parameters of challenge test after intraocular administration of non-vaccinated group (control negative of intraocular route)	148
Table 28	Comparative results of subcutaneous route	151
Table 29	Comparative results of intraocular route	155