PREPARATION AND CHARACTERISATION OF CTAB AND CHITOSAN MODIFIED PLGA NANOPARTICLES CONTAINING PLASMID DNA AND NIGELLA SATIVA OIL FOR ALZHEIMER DISEASE

BY

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ABSTRACT

Background: Alzheimer's disease (AD) is spreading in both developed and developing countries with no comprehensive treatment. Gene therapy provides new treatment strategies where it can interfere the disease on its genetic levels. Viral gene therapy is currently in clinical trial stage but it is facing problems due to safety issues. *Nigella sativa* is an herbal and Prophet medicine having numerous medical effects in AD and other diseases. CTAB and chitosan are frequently used in the preparation of nanoparticle for non-viral gene therapy. **Objective:** To co-encapsulate NSO with pDNA in one gene delivery system based on PLGA nanoparticles for the treatment of AD. **Methodology:** We fabricated PLGA microspheres loaded with pDNA using double-emulsion solvent-evaporation method and investigated the effect of surfactants and PLGA. *N. sativa* oil (NSO) was encapsulated in PLGA microparticle and its cell uptake was evaluated using PC-12 cell line. Two approaches were applied in co-encapsulating pDNA with NSO in one PLGA nanoparticle system i.e. adsorption on pre-prepared cationic nanoparticles and encapsulation within nanoparticles after being complexed with CTAB. CTAB and chitosan-modified PLGA nanoparticles were fabricated and then incorporated with pDNA. NSO was co-encapsulated with pDNA either by adsorption on chitosan-modified nanoparticles or by encapsulation. NSO encapsulation efficiency was evaluated using FTIR. The neuroregenerative effect of the encapsulated NSO was evaluated on N2a cells. **Results and discussion:** Tween blend of HLB-16 revealed the highest supercoil preservation index. Span surfactant and carboxyl terminal low molecular weight PLGA, Tween blend of HLB-16, Triton X-100 revealed exhibited high, moderate and low burst release, respectively. NSO-loaded PLGA microparticles were taken up mainly by mitotic cells in a similar efficiency to Span-modified microparticles but less than Tween 80-modified microparticles. CTAB-modified PLGA nanoparticles were negatively charged while chitosan-PLGA nanoparticles were positively charged. Chitosan-PLGA nanoparticles were taken up by N2a cells higher than other formulations and exhibited good transfection efficiency after being loaded with pDNA. Meanwhile, CTAB-pDNA-PLGA nanoparticles showed the highest transfection efficiency with low burst release. The developed FTIR method exhibited linearity in the range of NSO/PLGA of 5-150%. Both NSO and pDNA were encapsulated in PLGA nanoparticles. NSO encapsulation efficiency was lower in chitosan-modified nanoparticles. The encapsulated pDNA and NSO exhibited the desirable effects in N2a cells. The encapsulated pDNA exhibited the ex-gene expression of green fluorescent protein whereas the encapsulated NSO exhibited neurite outgrowth effect. **Conclusion:** NSO was successfully co-encapsulated with pDNA in PLGA nanoparticles as non-viral gene delivery system. The gene expression from the encapsulated pDNA, the encapsulated NSO was able to exhibit neuroregeneration in N2a cells. CTAB-pDNA-NSO-PLGA nanoparticles revealed the best results with slower pDNA release and higher neurite outgrowth effect.

ملخص البحث

خلفية البحث: تزداد وتيرة حدوث مرض الزهايمر مع عدم وجود علاج فعال. يشكل العلاج الوراثي طريقة جديدة للتفاعل مع المرض على المستوى المورثي. التجارب السريرية للعلاج الوراثي لمرض ألزهايمر تستخدم فيروسات لكنها مرتبطة بمشاكل صحية. حبة السوداء هي نبات طبي ومن الطب النبوي له تأثيرات طبية عديدة في مرض ألزهايمر وغيره. يستخدم الكيتوزان والCTAB في تحضير الجسيمات النانوية للعلاج الوراثي اللافيروسي. **هدف البحث:** تغليف الدنا وزيت حبة السوداء في شكل نانوي واحد لمعالجة مرض ألزهايمر. **طريقة البحث:** في هذه الدراسة قمنا بتصنيع جسيمات مكرونية من الPLGA محملة بالبلاسميد ودرسنا تأثير البوليمير والعديد من العوامل الفاعلة على السطح. أظهرت الدرساة أن خليط التوين ذا الHLB 16 له أكبر قيمة لحفظ البلاسميد. دراسة التحرر الميديد بينت أن مركبات السبان أدت إلى تحرر سريع بينما التريتون أدى إلى تحرر بطيء والتوين بينهما. قمنا بتحضير جسيمات مكرونية من زيت حبة السوداء والبوليمير المذكور أعلاه وتمت دراسة القبض الخلوي لهذه الجسميات من قبل خلايا الPC-12. أظهرت النتائج أن هذه الجسيمات تم قبضها من قبل الخلايا الانقسامية وبشكل أقل من الجسيمات المعدلة بالتوين 80. بعد ذلك هدفت الدراسة لتحضير جسيمات نانوية تضم البلاسميد وزيت حبة السوداء معاً. تم ذلك بطريقتين إما بادمصاص البلاسميد على جسيمات نانوية معدلة بالكيتوزان أو الCTAB أو تغليف البلازميد وحبة السوداء معاً. **النتائج والمناقشة:** الCTAB على عكس الكيتوزان لم يعط الجسميات شحنة موجبة. الجسيمات المعدلة بالكيتوزان تم قبضها بشكل أكبر من قبل الخلايا العصبية (N2a) وأظهرت تعبيراً جينياً جيداً. في المقابل، الجسيمات التي تضمنت البلاسميد داخلها أظهرت أعلى درجة من التعبير الجيني. التحديد الكمي لزيت حبة السوداء أظهر خطية في المجال 5-150%. كلا البلاسميد وزيت حبة السوداء أظهرا التأثيرات الدوائية المطلوبة حيث أدى البلاسميد إلى التعبير الجيني للمورثة الجديدة بينما حرض زيت الحبة السوداء على نمو الامتدادات العصبية للخلايا. **الخلاصة:** من الممكن تغليف زيت حبة السوداء والبلاسميد معاً في جسيمات نانوية واحدة مع المحافظة على فعاليتهما. إضافة الزيت له تأثير داعم في معالجة مرض ألزهايمر. الكبسلة المشتركة معاً تملك الخصائص الأفضل والأكثر ثباتاً.

APPROVAL PAGE

The thesis of Abd Almonem Doolaanea has been approved by the following:

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DECLARATION

I hereby declare that this thesis is the result of my own investigation, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

Abd Almonem Doolaanea

Signature……. Date ….....

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Affirmed by Abd Almonem Doolaanea

……..……..…… ……..

Signature Date

To my grandfather, my parents and my wife…

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LIST OF ABBREVIATIONS

AAV	Adeno-associated virus
AC	Acetone
AD	Alzheimer's diseases
ApoE	Apolipoprotein E
ATCC	American Type Tissue Culture
Aβ	Amyloid-beta
BBB	Blood brain barrier
BCNU	1,3-bis(2-chloroethyl)-1-nitrosourea
BSA	Bovine serum albumin
CID	Compound identification number
CNS	Central nervous system
CTAB	Cetyltrimethylammonium bromide
DCM	Dichloromethane
DD	Degree of deacetylation
DLS	Dynamic light scattering
DMEM	Dulbecco's modified Eagle's medium
DMSO	Dimethyl sulfoxide
DSC	Differential scanning calorimetry
EA	Ethyl acetate
ECB	Encapsulated cell biodelivery
ECL	Effective chain length
EDTA	Ethylenediaminetetraacetic acid
EO	Ethylene oxide
FBS	Foetal bovine serum
FDA	US Food and Drug Administration
FTIR	Fourier transform infrared spectroscopy
GAA	Glacial acetic acid
GDS	Gene delivery system
GFP	Green fluorescent protein
h	hour
HLB	Hydrophile-lipophile balance
ICH	International Conference of Harmonisation
IV	Intrinsic viscosity
kb	kilobases
kDa	Kilo Daltons
LCS	Low molecular weight chitosan
MCS	Medium molecular weight chitosan
MIM	Mendelian Inheritance in Man
min	minute
miRNA	microRNA
2OMR	2`-O-methyl-RNA
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	Molecular weight
N2a	Murine neuroblastoma neuro 2a

NGF	Nerve growth factor
NSO	*Nigella sativa* oil
NMDA	N-methyl-D-aspartate
NP	Nanoparticle
O/W	oil/water
OC	Open circular
OMIM	Online Mendelian Inheritance in Man
OTC	Ornithine transcarbamylase
PB	Phosphate buffer
PBS	Phosphate-buffered saline
PdI	Polydispersity index
pDNA	Plasmid DNA
PEG	Polyethylene glycol
PEI	Polyethylene imine
PGA	Polyglycolic acid
PLA	Polylactic acid
PLGA	Poly (lactic-co-glycolic acid)
PLL	Poly (L-lysine)
PO	Polyoxypropylene
PVA	Poly vinyl alcohol
PVP	Poly(vinyl pyrrolidone)
SC	Supercoiled
SCID	Severe combined immunodeficiency
SDS	Sodium dodecyl sulphate
SEM	Scanning electron microscopy
siRNA	Small interfering RNA
TAE	Tris-Acetate-EDTA
TE	Tris-EDTA
TEM	Transmission electron microscopy
T_g	Glass transition temperature
T_m	Melting temperature
TX	Triton X-100
UK	United Kingdom
US	United States
UV	Ultra violet
W/O	Water/oil
W/O/W	Water/oil/water
Zot	Zonula occludens toxin

CHAPTER ONE

INTRODUCTION

1.1 PROBLEM STATEMENT

Alzheimer's disease (AD) is one of the most common neurodegenerative diseases. The current AD drugs are only symptomatic with no available treatment to stop or slow down the disease. Even though the direct causes that trigger AD are unknown, it is proved that AD has genetic and non-genetic relations. Consequently, it seems rational to be treated with both gene and non-gene therapies. There is a few clinical trials using gene therapy for AD but they employ viral vectors which may have many safety issues. Non-viral gene delivery carriers are under development with noticeable success in the encapsulation plasmid DNA (pDNA). However, combining both pDNA and other therapeutic agents like *Nigella sativa* oil (NSO) in one delivery carrier is challenging. This is due to the difference in the physicochemical properties between the pDNA (very hydrophilic) and the NSO (very hydrophobic).

1.2 SCOPE AND OBJECTIVES

This study aims to develop a nanoparticle carrier to deliver both genetic element (pDNA) and non-genetic element (NSO) to the neuron cells and to evaluate the co-encapsulation of NSO in the treatment of AD. The scope and objectives of this study can be outlined as follows:

1. To fabricate pDNA-loaded PLGA microspheres and study the effects of different surfactants and PLGA molecular weights and hydrophobicities on the microspheres properties.