UNIVERSITI PUTRA MALAYSIA

SHIELDING OF VIRUS-LIKE NANOPARTICLES OF HEPATITIS B CORE ANTIGEN BY POLY(2-OXAZOLINE) FOR REDUCED ANTIGENICITY

FAM SEE YEE

FBSB 2021 26

SHIELDING OF VIRUS-LIKE NANOPARTICLES OF HEPATITIS B CORE ANTIGEN BY POLY(2-OXAZOLINE) FOR REDUCED ANTIGENICITY

By

FAM SEE YEE

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science

January 2021

COPYRIGHT

DEDICATION

Wholeheartedly dedicated to my beloved family,

who always support me,

and encourage me to go on every adventure,

especially this one

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

SHIELDING OF VIRUS-LIKE NANOPARTICLES OF HEPATITIS B CORE ANTIGEN BY POLY(2-OXAZOLINE) FOR REDUCED ANTIGENICITY

By

FAM SEE YEE

January 2021

Chair : Tan Wen Siang, PhD
Faculty : Biotechnology and Biomolecular Sciences

The rapid advances of nanotechnology over the past decades have led to increasing development of nano-sized drug delivery systems for various biomedical applications, including cancer therapies. Virus-like nanoparticles (VLNPs) have received considerable interest as nanocarriers for targeted drug delivery to cancer cells. This is owing to their numerous advantages over synthetic nanomaterials, including their biocompatible and biodegradable properties as well as their distinct interfaces for functionalization. Despite the remarkable features, VLNPs have intrinsic drawbacks, in particular, potential antigenicity and immunogenicity, which hamper their clinical applications in nanomedicine. Thus, they can be eliminated easily and rapidly by the host immune systems upon administration, rendering these nanoparticles ineffective for drug delivery to the target site. Recombinant hepatitis B core antigen (HBcAg) VLNPs have been widely employed as a smart drug delivery system as their large surface area exposes numerous amino acid residues for bioconjugation and cross-linking of therapeutic agents. However, HBcAg VLNPs are highly antigenic and immunogenic, compromising their drug delivery efficacy for cancer treatments. The aim of this study was to reduce the antigenicity of HBcAg VLNPs by shielding them with a hydrophilic polymer, poly(2-ethyl-2-oxazoline) (PEtOx). In the present study, an amine-functionalized PEtOx (PEtOx-NH_2) was synthesized using the living cationic ring-opening polymerization (CROP) technique and characterized by nuclear magnetic resonance (NMR) and mass spectrometry (MS). The synthesized PEtOx-NH_2 was then covalently conjugated to HBcAg VLNPs via carboxyl groups. The PEtOx-conjugated HBcAg (PEtOx-

HBcAg) VLNPs were characterized with dynamic light scattering and UV-visible spectroscopy. The colloidal stability study indicated that both HBcAg and PEtOx-HBcAg VLNPs maintained their particle size in Tris-buffered saline (TBS) at human body temperature (37°C) for at least five days. Enzyme-linked immunosorbent assay (ELISA) also demonstrated that the antigenicity of PEtOx-HBcAg VLNPs reduced significantly as compared with unconjugated HBcAg VLNPs, indicating that the external surface of HBcAg VLNPs shielded by PEtOx exhibits a stealth behavior that restrains the binding of antibody to the nanoparticles. This novel surface functionalization with PEtOx provides a general platform for resolving the antigenicity of VLNPs, enabling them to be developed into a variety of powerful drug deliveries in nanotechnology with the ability to evade the immune surveillance. These PEtOx-HBcAg VLNPs could serve as a promising candidate for targeted drug delivery in animal and clinical studies.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PERLINDUNGAN NANOPARTIKEL MENYERUPAI VIRUS ANTIGEN TERAS HEPATITIS B DENGAN POLI(2-OXAZOLINE) UNTUK MENGURANGKAN ANTIGENIK

Oleh

FAM SEE YEE

Januari 2021

Pengerusi : Tan Wen Siang, PhD
Fakulti : Bioteknologi dan Sains Biomolekul

Kemajuan nanoteknologi selama beberapa dekad telah meningkatkan pembangunan sistem penghantaran ubat bersaiz nano untuk pelbagai aplikasi bioperubatan, termasuk terapi kanser. Nanopartikel menyerupai virus (VLNPs) telah menarik minat sebagai pembawa-nano untuk penghantaran ubat ke sel-sel kanser. Ini adalah kerana VLNPs mempunyai banyak kelebihan berbanding dengan nanopartikel sintetik, termasuk ciri-ciri biokompatibel dan biodegradasi serta antara muka yang berbeza untuk fungsian. Walaupun VLNPs memiliki ciri-ciri yang luar biasa, mereka mempunyai kelemahan intrinsik, khususnya potensi antigenik dan imunogenik yang menghalangi aplikasi klinikal mereka dalam perubatan nano. Oleh itu, mereka boleh dihapuskan dengan mudah dan cepat oleh sistem imun, menjadikan nanopartikel ini tidak berkesan untuk penghantaran ubat ke destinasi yang disasarkan. Rekombinan antigen teras hepatitis B (HBcAg) VLNPs digunakan sebagai sistem penghantaran ubat kerana permukaan besar mereka mendedahkan residu asid amino untuk biokonjugasi dan silang agen terapeutik. Walau bagaimanapun, HBcAg VLNPs adalah sangat antigenik dan imunogenik. Ini akan menjejaskan keberkesanan penghantaran ubat untuk rawatan kanser. Tujuan kajian ini adalah untuk mengurangkan antigenik HBcAg VLNPs dengan melindungi mereka dengan polimer hidrofilik, poli(2-etil-2-oxazoline) (PEtOx). Dalam kajian ini, amina berfungsi PEtOx (PEtOx-NH_2) telah disintesis dengan teknik pempolimeran pembukaan cincin kationik hidup (CROP) dan dicirikan dengan resonans magnetik nuklear (NMR) dan spektrometri jisim (MS). Polimer PEtOx-NH_2 itu

seterusnya dikonjugasi dengan HBcAg VLNPs melalui kumpulan karboksil. HBcAg VLNPs yang dikonjugasi dengan PEtOx (PEtOx-HBcAg) telah disifatkan dengan penyerakan cahaya dinamik dan UV spektroskopi. Kajian kestabilan koloid menunjukkan bahawa kedua-dua HBcAg dan PEtOx-HBcAg VLNPs mengekalkan saiz zarah mereka di dalam Tris penimbal air masin (TBS) pada suhu badan manusia (37°C) selama sekurang-kurangnya lima hari. Assay imunosorben berkaitan enzim (ELISA) juga menunjukkan bahawa antigenik PEtOx-HBcAg VLNPs berkurangan dengan ketara berbanding dengan HBcAg VLNPs yang tidak dikonjugasi. Ini menunjukkan bahawa permukaan luaran HBcAg VLNPs yang dilindungi oleh PEtOx menghalangi pengikatan antibodi terhadap nanopartikel. Pemfungsian permukaan novel dengan PEtOx merupakan platform umum untuk menyelesaikan potensi antigenik VLNPs. Ini akan membangunkan VLNPs sebagai sistem penghantaran ubat dalam nanoteknologi dengan keupayaan untuk mengelakkan pengawasan imun. PEtOx-HBcAg VLNPs ini juga merupakan calon yang berpotensi untuk penghantaran ubat secara khusus dalam kajian haiwan dan klinikal.

ACKNOWLEDGEMENTS

I wish to express my deepest gratitude to my supervisor, Prof. Tan Wen Siang for his professional competence and valuable guidance throughout the entire research period.

The door to his office was always open whenever I ran into a trouble spot or had a question about my research or writing. I would not have been able to complete my research without his constant support and encouragement.

I would also like to extend my gratitude to my co-supervisors, Dr. Ho Kok Lian and Dr. Mariatulqabtiah Abdul Razak for their passionate participation and helpful advice throughout my research study.

Besides, I am thankful to my collaborators, Dr. Chee Chin Fei from Universiti Malaya and Dr. Lau Han Yih from MARDI for providing me the technical support and resources.

I would also like to thank the staff members from Faculty of Biotechnology and Biomolecular Sciences for their help and assistance, and Universiti Putra Malaysia for the Graduate Research Fellowship (GRF) financial support.

Many thanks to seniors and labmates, especially Dr. Yong, Bee Koon, Chuan Loo, Sandy, Made, and Bethilda, for their continuous support and guidance. I enjoyed every moment with them in the lab, not only for the helpful discussion we had, but also their funny jokes and laugh. I have indeed gained a lot from them throughout my research study and I am very grateful to them.

Finally, I must express my very profound gratitude to my beloved mum, my dearest sister and brothers, for their sacrifices, supports, encouragements and unconditional love. I would not have made it through my Master's study without their selfless support and generous care. I know that my family have always been there for me and they are forever my pillar of strength.

Besides, I owe thanks to my partner, Lee Wai, who always support and encourage me all this time. Indeed, his passion in research and academia had helped me a lot during my pursuit of Master's degree and this accomplishment would not have been possible without him.

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Tan Wen Siang, PhD
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Mariatulqabtiah Abdul Razak, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

Ho Kok Lian, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

_____
ZALILAH MOHD SHARIFF, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 08 April 2021

Declaration by graduate student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other
- degree at any other institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and Innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature: _____ Date: _____

Name and Matric No.: Fam See Yee (GS50851)

Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature: _____
Name of Chairman of Supervisory Committee: Professor Dr. Tan Wen Siang

Signature: _____
Name of Member of Supervisory Committee: Associate Professor Dr. Mariatulqabtiah Abdul Razak

Signature: _____
Name of Member of Supervisory Committee: Associate Professor Dr. Ho Kok Lian

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF ABBREVIATIONS

Å	Angstrom
ABC	accelerated blood clearance
ABPs	albumin-binding peptides
APCs	antigen-presenting cells
APS	ammonium persulphate
BCR	B cell membrane receptors
BSA	bovine serum albumin
CBB	Coomassie Brilliant Blue
CCMV	cowpea chlorotic mottle virus
$CDCl_3$	deuterated chloroform
CIP	cell-internalizing peptide
CNTs	carbon nanotubes
CPMV	cowpea mosaic virus
CROP	cationic ring-opening polymerization
cryoEM	cryo-electron microscopy
CT	C-terminal
Da	Dalton
dH_2O	distilled water
DLS	dynamic light scattering
EDC	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride
ELISA	enzyme-linked immunosorbent assay
EtOx	2-ethyl-2-oxazoline
FDA	Food and Drug Administration
g	gravity
g	gram

^{1}H	proton or hydrogen
h	hours
HBcAg	hepatitis B core antigen
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus
HCl	hydrochloric acid
HSP	heat shock protein
IPTG	isopropyl-β-thiogalactoside
kDa	kiloDalton
kPa	kiloPascal
kV	kilovolt
L	liter
LB	Luria Bertani
M	molar
mA	milliampere
MAC	membrane attack complex
MASP	MBL-associated serine proteases
MBL	mannan-binding lectin
MeOTs	methyl p-toluenesulfonate
mg	milligram
$MgCl_2$	magnesium chloride
MHz	megahertz
min	minutes
MIR	major immunodominant region
mL	milliliter
mM	millimolar
mm	millimeter
mmol	millimole

mPEG-NH_2	methoxypolyethylene glycol amine
MPS	mononuclear phagocyte system
MS	mass spectrometry
Mw	molecular weight
Na_2HPO_4	disodium hydrogen phosphate
NaCl	sodium chloride
NaH_2PO_4	sodium dihydrogen phosphate
nm	nanometer
NMR	nuclear magnetic resonance
NP	nanoparticle
NPs	nanoparticles
NSA	N-terminal self-assembly
OD	optical density
PAGE	polyacrylamide gel electrophoresis
PEG	poly(ethylene glycol)
PEtOx	poly(2-ethyl-2-oxazoline)
PEtOx-HBcAg	PEtOx-conjugated HBcAg
PEtOx-NH_2	amine-functionalized PEtOx
PLA	poly(lactic acid)
PLGA	poly(lactic acid-*co*-glycolic acid)
PMeOx	poly(2-methyl-2-oxazoline)
p-NPP	p-Nitrophenyl Phosphate
POx	poly(2-oxazoline)
ppm	parts per million
RBC	red blood cell
RBCs	red blood cells
RES	reticuloendothelial system
R_h	hydrodynamic radii

rpm	revolutions per minute
RT	room temperature
s	seconds
SDS	sodium dodecyl sulfate
SIRPα	signal regulatory protein alpha
SM	skim milk
SP	self peptide
sulfo-NHS	*N*-hydroxysulfosuccinimide
T	triangulation number
TBS	Tris-buffered saline
TBST	mixture of TBS and Tween 20
TEMED	tetramethylethylenediamine
tHBcAg	truncated HBcAg
UV	ultraviolet
UV-VIS	ultraviolet–visible
VLNP	virus-like nanoparticle
VLNPs	virus-like nanoparticles
v/v	volume over volume
w/v	weight over volume
α	alpha
β	beta
δ	delta
ε	epsilon
λ	lamda
μg	microgram
μL	microliter
μm	micrometer

Amino acids

Ala, A	alanine
Arg, R	arginine
Asn, N	asparagine
Asp, D	aspartic acid
Cys, C	cysteine
Gln, Q	glutamine
Glu, E	glutamic acid
Gly, G	glycine
His, H	histidine
Ile, I	isoleucine
Leu, L	leucine
Lys, K	lysine
Met, M	methionine
Phe, F	phenylalanine
Pro, P	proline
Ser, S	serine
Thr, T	threonine
Trp, W	tryptophan
Tyr, Y	tyrosine
Val, V	valine

CHAPTER 1

INTRODUCTION

Nanotechnology has contributed tremendously to the rapid development of nano-sized materials and nanoparticles (NPs) as therapeutics and diagnostics agents for cancer treatments (Patra et al., 2018). The use of today's therapeutic drugs is often hindered by their intrinsic drawbacks including poor solubility as well as adverse pharmacokinetics and biodistribution (Ordikhani et al., 2020). Nonetheless, NPs that serve as drug reservoirs can potentially improve the drug solubility and prolong the blood circulation half-life, releasing the drug in a controlled and sustained manner to minimize the systemic side effects and further improve the pharmacokinetics (Zhang et al., 2008).

Several classes of nanomaterials including synthetic materials such as liposomes, micelles, dendrimers, hydrogels, and natural biomaterials such as virus-like nanoparticles (VLNPs) are widely employed as smart drug delivery systems for cancer therapies (Chen et al., 2016; Hao et al., 2016; Kesharwani et al., 2014; Li & Mooney, 2016; Yildiz et al., 2011). The encapsulation and conjugation of therapeutic payloads such as chemotherapeutic drugs, peptides, nucleic acids, and ligands can enhance the efficacy of targeted delivery to specific tumor cells with fewer adverse effects (Sanna et al., 2014).

VLNPs that serve as nanocarriers provide an ideal platform for the development of targeted delivery systems owing to their distinguishing features over synthetic nanomaterials, including their biocompatible and biodegradable properties, highly symmetrical structure and unrivaled monodispersity (Rohovie et al., 2017). The term "VLNPs" refers to the non-infectious protein shells or capsids that consist of virus-derived structural proteins. The structures of self-assembled recombinant VLNPs are highly tunable as the empty interior cavity is amenable to the loading of drug molecules and imaging agents, whereas the exterior surface can be presented with targeting ligands for cell-specific delivery (Yildiz et al., 2011).

Recombinant hepatitis B core antigen (HBcAg) produced in *Escherichia coli* self-assembles into small and large icosahedral VLNPs with a high degree of geometric symmetry and polyvalency (Crowther et al., 1994). A variety of cargos including DNA, RNA, peptides, green fluorescent protein, and

chemotherapeutic drugs have been loaded into HBcAg VLNPs for biomedical applications (Biabanikhankahdani et al., 2018; Dhason et al., 2012; Lee et al., 2012; Lee & Tan, 2008; Strods et al., 2015). The large surface area of HBcAg VLNPs exposes a series of amino acid residues with specific functional groups (eg. Asp, Glu, Lys, and Cys) readily available for both genetic and chemical modifications. Hence, HBcAg VLNPs can be functionalized with diverse targeting moieties and drugs to attain targeted drug delivery for cancer treatments (Biabanikhankahdani et al., 2016, 2017, 2018; Gan et al., 2018).

Despite the remarkable applications, HBcAg VLNPs are highly antigenic, and they were reported to behave as T-cell-independent and T-cell-dependent antigens (Milich & McLachlan, 1986). The configuration of the HBcAg capsid spikes protruding from the surface of the capsid may serve as a recognition site for B cell membrane receptors (BCR) (Milich et al., 1997), owing to the presence of dominant B cell epitopes at the tip of the spikes (Belnap et al., 2003). Furthermore, it has been reported that B cells rather than non-B cell antigen-presenting cells (APCs) such as macrophages and dendritic cells act as the primary APCs for HBcAg, which explains its enhanced immunogenicity in terms of antibody production (Milich et al., 1997).

Regardless of the therapeutic purpose of NPs, a prolonged circulation is a requisite for effective drug delivery and therapeutic efficacy. However, the intrinsic antigenicity and immunogenicity may lead to the rapid clearance of NPs due to their interactions with the mononuclear phagocyte system (MPS) and/or complement system (Ilinskaya & Dobrovolskaia, 2016). This resulting premature NP elimination from blood circulation will cause the release of therapeutic agent at off-target site, compromising drug delivery efficacy to tumor cells.

Studies over the past decades have proved that grafting a stealth coating layer onto the surfaces of NPs can potentially improve their blood circulation half-life by restricting the interactions between NPs and opsonin proteins that mediate the phagocytic clearance (Aggarwal et al., 2009; Dobrovolskaia & McNeil, 2007; Salmaso & Caliceti, 2013). This stealth delivery system commonly recruits polymers with high hydrophilicity and high flexibility to impart MPS-avoidance properties to NPs (Suk et al., 2016).

Poly(2-oxazoline) (POx) is a hydrophilic polymer that has been known for half a century (Hoogenboom, 2017). It is highly tunable through living cationic ring-

opening polymerization (CROP) synthesis (Sedlacek et al., 2012). In recent years, the increasing concern regarding the use of poly(ethylene glycol) (PEG) and its potential immunogenicity has led to a renewed interest towards POx as a promising alternative polymer (Abu Lila et al., 2013; Verhoef et al., 2014). POx is a non-toxic polymer with similar stealth properties as PEG, and it offers numerous advantages such as excellent biocompatibility, non-immunogenic, and high stability with a range of end-group and side-chain functionalization (de la Rosa, 2014; Luxenhofer et al., 2012).

POxylation, the coating of POx chains on the surface of NPs, can improve the circulation time of the particles in the bloodstream due to their hydrophilic nature (Bludau et al., 2017; Moreadith et al., 2017; Viegas et al., 2011). Although the applications of POx in biomedical field arose only recently, two POx polymers, namely poly(2-methyl-2-oxazoline) (PMeOx) and poly(2-ethyl-2-oxazoline) (PEtOx), are widely studied for polymeric therapeutics (de la Rosa, 2014).

Taken all together, with the aim of reducing the antigenicity of HBcAg VLNPs, it is hypothesized that shielding the HBcAg VLNPs with PEtOx will provide a stealth coating for the capsid against the antibody recognition and binding. The specific objectives of this study include:

1. To purify and characterize recombinant HBcAg VLNPs,
2. To synthesize and characterize amine-functionalized PEtOx (PEtOx-NH_2),
3. To conjugate PEtOx-NH_2 to HBcAg VLNPs,
4. To characterize the PEtOx-conjugated HBcAg (PEtOx-HBcAg) VLNPs,
5. To determine the antigenicity of PEtOx-HBcAg VLNPs.

REFERENCES

Abu Lila, A. S., Kiwada, H., & Ishida, T. (2013). The accelerated blood clearance (ABC) phenomenon: Clinical challenge and approaches to manage. *Journal of Controlled Release*, *172*(1), 38–47.

Aggarwal, P., Hall, J. B., McLeland, C. B., Dobrovolskaia, M. A., & McNeil, S. E. (2009). Nanoparticle interaction with plasma proteins as it relates to particle biodistribution, biocompatibility and therapeutic efficacy. *Advanced Drug Delivery Reviews*, *61*(6), 428–437.

Aljabali, A. A. A., Shukla, S., Lomonossoff, G. P., Steinmetz, N. F., & Evans, D. J. (2013). CPMV-DOX delivers. *Molecular Pharmaceutics*, *10*(1), 3–10.

Amoozgar, Z., & Yeo, Y. (2012). Recent advances in stealth coating of nanoparticle drug delivery systems. *Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology*, *4*(2), 219–233.

Babij, N. R., McCusker, E. O., Whiteker, G. T., Canturk, B., Choy, N., Creemer, L. C., Amicis, C. V. De, Hewlett, N. M., Johnson, P. L., Knobelsdorf, J. A., Li, F., Lorsbach, B. A., Nugent, B. M., Ryan, S. J., Smith, M. R., & Yang, Q. (2016). NMR chemical shifts of trace impurities: Industrially preferred solvents used in process and green chemistry. *Organic Process Research & Development*, *20*(3), 661–667.

Bachmann, B. J. (1972). Pedigrees of some mutant strains of *Escherichia coli* K-12. *Bacteriological Reviews*, *36*(4), 525–557.

Barbero, F., Russo, L., Vitali, M., Piella, J., Salvo, I., Borrajo, M. L., Busquets-Fité, M., Grandori, R., Bastús, N. G., Casals, E., & Puntes, V. (2017). Formation of the protein corona: The interface between nanoparticles and the immune system. *Seminars in Immunology*, *34*, 52–60.

Barenholz, Y. (Chezy). (2012). Doxil® — The first FDA-approved nano-drug: Lessons learned. *Journal of Controlled Release*, *160*(2), 117–134.

Barwal, I., Kumar, R., Kateriya, S., Dinda, A. K., & Yadav, S. C. (2016). Targeted delivery system for cancer cells consist of multiple ligands conjugated genetically modified CCMV capsid on doxorubicin GNPs complex. *Scientific Reports*, *6*(1), 37096.

Beatty, P. H., & Lewis, J. D. (2019). Cowpea mosaic virus nanoparticles for cancer imaging and therapy. *Advanced Drug Delivery Reviews*, *145*, 130–144.

Belnap, D. M., Watts, N. R., Conway, J. F., Cheng, N., Stahl, S. J., Wingfield, P. T., & Steven, A. C. (2003). Diversity of core antigen epitopes of hepatitis B virus. *Proceedings of the National Academy of Sciences*, *100*(19), 10884–10889.

Bhatt, R., de Vries, P., Tulinsky, J., Bellamy, G., Baker, B., Singer, J. W., & Klein, P. (2003). Synthesis and in vivo antitumor activity of poly(L-glutamic acid) conjugates of 20(*S*)-camptothecin. *Journal of Medicinal Chemistry*, *46*(1), 190–193.

Bhattacharya, S. (2020). Fabrication of poly(sarcosine), poly (ethylene glycol), and poly (lactic-co-glycolic acid) polymeric nanoparticles for cancer drug delivery. *Journal of Drug Delivery Science and Technology*, 102194.

Biabanikhankahdani, R., Alitheen, N. B. M., Ho, K. L., & Tan, W. S. (2016). pH-responsive virus-like nanoparticles with enhanced tumour-targeting ligands for cancer drug delivery. *Scientific Reports*, *6*(1), 37891.

Biabanikhankahdani, R., Bayat, S., Ho, K. L., Alitheen, N. B. M., & Tan, W. S. (2017). A simple add-and-display method for immobilisation of cancer drug on His-tagged virus-like nanoparticles for controlled drug delivery. *Scientific Reports*, *7*(1), 5303.

Biabanikhankahdani, R., Ho, K. L., Alitheen, N. B. M., & Tan, W. S. (2018). A dual bioconjugated virus-like nanoparticle as a drug delivery system and comparison with a pH-responsive delivery system. *Nanomaterials*, *8*(4), 236.

Bilim, V. (2003). Technology evaluation: PK1, Pfizer/Cancer Research UK. *Current Opinion in Molecular Therapeutics*, *5*(3), 326–330.

Bludau, H., Czapar, A. E., Pitek, A. S., Shukla, S., Jordan, R., & Steinmetz, N. F. (2017). POxylation as an alternative stealth coating for biomedical applications. *European Polymer Journal*, *88*, 679–688.

Borisova, G., Bundule, M., Grinstein, E., Dreilina, D., Dreimane, A., Kalis, J., Kozlovskaya, T., Loseva, V., Ose, V., & Pumpen, P. (1987). Recombinant capsid structures for exposure of protein antigenic epitopes. *Mol. Genet.*, *6*, 169–174.

Böttcher, B., Wynne, S. A., & Crowther, R. A. (1997). Determination of the fold of the core protein of hepatitis B virus by electron cryomicroscopy. *Nature*, *386*(6620), 88–91.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*(1–2), 248–254.

Buzea, C., Pacheco, I. I., & Robbie, K. (2007). Nanomaterials and nanoparticles: Sources and toxicity. *Biointerphases*, *2*(4), MR17–MR71.

Cameron, C. M., Barrett, J. W., Mann, M., Lucas, A., & McFadden, G. (2005). Myxoma virus M128L is expressed as a cell surface CD47-like virulence factor that contributes to the downregulation of macrophage activation in vivo. *Virology*, *337*(1), 55–67.

Caracciolo, G. (2015). Liposome–protein corona in a physiological environment: challenges and opportunities for targeted delivery of nanomedicines. *Nanomedicine: Nanotechnology, Biology and Medicine*, *11*(3), 543–557.

Chapman, R. G., Ostuni, E., Takayama, S., Holmlin, R. E., Yan, L., & Whitesides, G. M. (2000). Surveying for surfaces that resist the adsorption of proteins. *Journal of the American Chemical Society*, *122*(34), 8303–8304.

Chen, Q., Ding, H., Zhou, J., Zhao, X., Zhang, J., Yang, C., Li, K., Qiao, M., Hu, H., Ding, P., & Zhao, X. (2016). Novel glycyrrhetinic acid conjugated pH-sensitive liposomes for the delivery of doxorubicin and its antitumor activities. *RSC Advances*, *6*(22), 17782–17791.

Chen, X., Liu, B., Tong, R., Zhan, L., Yin, X., Luo, X., Huang, Y., Zhang, J., He, W., & Wang, Y. (2021). Orchestration of biomimetic membrane coating and nanotherapeutics in personalized anticancer therapy. *Biomaterials Science*.

Chenthamara, D., Subramaniam, S., Ramakrishnan, S. G., Krishnaswamy, S., Essa, M. M., Lin, F.-H., & Qoronfleh, M. W. (2019). Therapeutic efficacy of nanoparticles and routes of administration. *Biomaterials Research*, *23*(1), 20.

Cho, K., Wang, X., Nie, S., Chen, Z. (Georgia), & Shin, D. M. (2008). Therapeutic nanoparticles for drug delivery in cancer. *Clinical Cancer Research*, *14*(5), 1310–1316.

Choi, S. H., Kwon, I. C., Hwang, K. Y., Kim, I.-S., & Ahn, H. J. (2011). Small heat shock protein as a multifunctional scaffold: Integrated tumor targeting and caspase imaging within a single cage. *Biomacromolecules*, *12*(8), 3099–3106.

Chung, Y. H., Cai, H., & Steinmetz, N. F. (2020). Viral nanoparticles for drug delivery, imaging, immunotherapy, and theranostic applications. *Advanced Drug Delivery Reviews*, *156*, 214–235.

Clarke, B. E., Newton, S. E., Carroll, A. R., Francis, M. J., Appleyard, G., Syred, A. D., Highfield, P. E., Rowlands, D. J., & Brown, F. (1987). Improved immunogenicity of a peptide epitope after fusion to hepatitis B core protein. *Nature*, *330*(6146), 381–384.

Cooper, A., Tal, G., Lider, O., & Shaul, Y. (2005). Cytokine induction by the hepatitis B virus capsid in macrophages is facilitated by membrane heparan sulfate and involves TLR2. *The Journal of Immunology*, *175*(5), 3165–3176.

Crowther, R. A., Kiselev, N. A., Böttcher, B., Berriman, J. A., Borisova, G. P., Ose, V., & Pumpens, P. (1994). Three-dimensional structure of hepatitis B virus core particles determined by electron cryomicroscopy. *Cell*, *77*(6), 943–950.

Dai, Y., Xing, H., Song, F., Yang, Y., Qiu, Z., Lu, X., Liu, Q., Ren, S., Chen, X., & Li, N. (2016). Biotin-conjugated multilayer poly (D,L-lactide-co-glycolide)-lecithin-polyethylene glycol nanoparticles for targeted delivery of doxorubicin. *Journal of Pharmaceutical Sciences*, *105*(9), 2949–2958.

Dams, E. T. M., Laverman, P., Oyen, W. J. G., Storm, G., Scherphof, G. L., van der Meer, J. W. M., Corstens, F. H. M., & Boerman, O. C. (2000). Accelerated blood clearance and altered biodistribution of repeated injections of sterically stabilized liposomes. *Journal of Pharmacology and Experimental Therapeutics*, *292*(3), 1071–1079.

Dawidczyk, C. M., Kim, C., Park, J. H., Russell, L. M., Lee, K. H., Pomper, M. G., & Searson, P. C. (2014). State-of-the-art in design rules for drug delivery platforms: Lessons learned from FDA-approved nanomedicines. *Journal of Controlled Release*, *187*, 133–144.

de la Rosa, V. R. (2014). Poly(2-oxazoline)s as materials for biomedical applications. *Journal of Materials Science: Materials in Medicine*, *25*(5), 1211–1225.

Deng, Y., Zhang, X., Shen, H., He, Q., Wu, Z., Liao, W., & Yuan, M. (2020). Application of the nano-drug delivery system in treatment of cardiovascular diseases. *Frontiers in Bioengineering and Biotechnology*, *7*, 489.

Desai, N. (2016). Nanoparticle albumin-bound paclitaxel (Abraxane®). In M. Otagiri & V. T. G. Chuang (Eds.), *Albumin in Medicine* (pp. 101–119). Springer Singapore.

Dhason, M. S., Wang, J. C. Y., Hagan, M. F., & Zlotnick, A. (2012). Differential assembly of hepatitis B virus core protein on single- and double-stranded nucleic acid suggest the dsDNA-filled core is spring-loaded. *Virology*, *430*(1), 20–29.

Di, J., Gao, X., Du, Y., Zhang, H., Gao, J., & Zheng, A. (2020). Size, shape, charge and "stealthy" surface: Carrier properties affect the drug circulation time *in vivo*. *Asian Journal of Pharmaceutical Sciences*.

Diao, Y. Y., Han, M., Ding, P. T., Chen, D. W., & Gao, J. Q. (2010). DOX-loaded PEG-PLGA and Pluronic copolymer composite micelles enhances cytotoxicity and the intracellular accumulation of drug in DOX-resistant tumor cells. *Die Pharmazie*, *65*(5), 356–358.

Dobrovolskaia, M. A., & McNeil, S. E. (2007). Immunological properties of engineered nanomaterials. *Nature Nanotechnology*, *2*(8), 469–478.

Drexler, K. E. (1986). *Engines of Creation. The Coming Era of Nanotechnology*. Anchor Books.

Dyson, M. R., & Murray, K. (1995). Selection of peptide inhibitors of interactions involved in complex protein assemblies: Association of the core and surface antigens of hepatitis B virus. *Proceedings of the National Academy of Sciences*, *92*(6), 2194–2198.

Fakruddin, M., Hossain, Z., & Afroz, H. (2012). Prospects and applications of nanobiotechnology: A medical perspective. *Journal of Nanobiotechnology*, *10*(1), 31.

Fang, R. H., Kroll, A. V, Gao, W., & Zhang, L. (2018). Cell membrane coating nanotechnology. *Advanced Materials*, *30*(23), 1706759.

Ferrari, C., Bertoletti, A., Penna, A., Cavalli, A., Valli, A., Missale, G., Pilli, M., Fowler, P., Giuberti, T., & Chisari, F. V. (1991). Identification of immunodominant T cell epitopes of the hepatitis B virus nucleocapsid antigen. *Journal of Clinical Investigation*, *88*(1), 214–222.

Feynman, R. P. (1960). There's plenty of room at the bottom. *Handbook of nanoscience, engineering, and technology* (pp. 22–36). CRC Press.

Flenniken, M. L., Willits, D. A., Harmsen, A. L., Liepold, L. O., Harmsen, A. G., Young, M. J., & Douglas, T. (2006). Melanoma and lymphocyte cell-specific targeting incorporated into a heat shock protein cage architecture. *Chemistry & Biology*, *13*(2), 161–170.

Frank, M. M., & Fries, L. F. (1991). The role of complement in inflammation and phagocytosis. *Immunology Today*, *12*(9), 322–326.

Freund, S. M. V., Johnson, C. M., Jaulent, A. M., & Ferguson, N. (2008). Moving towards high-resolution descriptions of the molecular interactions and structural rearrangements of the human hepatitis B core protein. *Journal of Molecular Biology*, *384*(5), 1301–1313.

Gabizon, A. A. (2001). Pegylated liposomal doxorubicin: metamorphosis of an old drug into a new form of chemotherapy. *Cancer Investigation*, *19*(4), 424–436.

Gan, B. K., Yong, C. Y., Ho, K. L., Omar, A. R., Alitheen, N. B., & Tan, W. S. (2018). Targeted delivery of cell penetrating peptide virus-like nanoparticles to skin cancer cells. *Scientific Reports*, *8*(1), 8499.

Ganem, D. (1991). Assembly of hepadnaviral virions and subviral particles. In W. S. Mason & C. Seeger (Eds.), *Hepadnaviruses* (pp. 61–83). Springer Berlin Heidelberg.

Gao, W., Hu, C. M. J., Fang, R. H., Luk, B. T., Su, J., & Zhang, L. (2013). Surface functionalization of gold nanoparticles with red blood cell membranes. *Advanced Materials*, *25*(26), 3549–3553.

Gazit, E., & Mitraki, A. (2013). *Plenty of room for biology at the bottom: An introduction to bionanotechnology*. Imperial College Press.

Gustafson, H. H., Holt-Casper, D., Grainger, D. W., & Ghandehari, H. (2015). Nanoparticle uptake: The phagocyte problem. *Nano Today*, *10*(4), 487–510.

Hadjesfandiari, N., & Parambath, A. (2018). Stealth coatings for nanoparticles: Polyethylene glycol alternatives. In A. Parambath (Ed.), *Engineering of Biomaterials for Drug Delivery Systems: Beyond Polyethylene Glycol* (pp. 345–361). Woodhead Publishing.

Hao, W., Liu, D., Shang, Y., Zhang, J., Xu, S., & Liu, H. (2016). pH-triggered copolymer micelles as drug nanocarriers for intracellular delivery. *RSC Advances*, *6*(35), 29149–29158.

Harris, J. M., & Chess, R. B. (2003). Effect of pegylation on pharmaceuticals. *Nature Reviews Drug Discovery*, *2*(3), 214–221.

Harris, L., Batist, G., Belt, R., Rovira, D., Navari, R., Azarnia, N., Welles, L., & Winer, E. (2002). Liposome-encapsulated doxorubicin compared with conventional doxorubicin in a randomized multicenter trial as first-line therapy of metastatic breast carcinoma. *Cancer*, *94*(1), 25–36.

Hashimoto, Y., Shimizu, T., Mima, Y., Abu Lila, A. S., Ishida, T., & Kiwada, H. (2014). Generation, characterization and in vivo biological activity of two distinct monoclonal anti-PEG IgMs. *Toxicology and Applied Pharmacology*, *277*(1), 30–38.

Hermanson, G. T. (2008). Zero-length crosslinkers. *Bioconjugate Techniques* (pp. 213–233). Elsevier.

Ho, C. W., Tan, W. S., Kamaruddin, S., Ling, T. C., & Tey, B. T. (2008). The direct recovery of recombinant hepatitis B core antigen from disruptate derived from continuous-flow bead milling. *Biotechnology and Applied Biochemistry*, *50*(1), 49.

Hoare, D. G., & Koshland, D. E. J. (1967). A method for the quantitative modification and estimation of carboxylic acid groups in proteins. *The Journal of Biological Chemistry*, *242*(10), 2447–2453.

Hoogenboom, R. (2017). 50 years of poly(2-oxazoline)s. *European Polymer Journal*, *88*, 448–450.

Hu, C. M. J., Fang, R. H., Luk, B. T., Chen, K. N. H., Carpenter, C., Gao, W., Zhang, K., & Zhang, L. (2013). 'Marker-of-self' functionalization of nanoscale particles through a top-down cellular membrane coating approach. *Nanoscale*, *5*(7), 2664.

Hu, C.-M. J., Zhang, L., Aryal, S., Cheung, C., Fang, R. H., & Zhang, L. (2011). Erythrocyte membrane-camouflaged polymeric nanoparticles as a biomimetic delivery platform. *Proceedings of the National Academy of Sciences*, *108*(27), 10980–10985.

Ilinskaya, A. N., & Dobrovolskaia, M. A. (2016). Understanding the immunogenicity and antigenicity of nanomaterials: Past, present and future. *Toxicology and Applied Pharmacology*, *299*, 70–77.

Ishida, T., Maeda, R., Ichihara, M., Mukai, Y., Motoki, Y., Manabe, Y., Irimura, K., & Kiwada, H. (2002). The accelerated clearance on repeated injection of pegylated liposomes in rats: Laboratory and histopathological study. *Cellular & Molecular Biology Letters*, *7*(2), 286.

Jain, K. K. (2020). Role of nanobiotechnology in drug delivery. In K. K. Jain (Ed.), *Methods in Molecular Biology* (pp. 55–73). Springer New York.

Jaiswal, S., Jamieson, C. H. M., Pang, W. W., Park, C. Y., Chao, M. P., Majeti, R., Traver, D., van Rooijen, N., & Weissman, I. L. (2009). CD47 is upregulated on circulating hematopoietic stem cells and leukemia cells to avoid phagocytosis. *Cell*, *138*(2), 271–285.

Jeon, S., Lee, J., Andrade, J., & De Gennes, P. (1991). Protein-surface interactions in the presence of polyethylene oxide. *Journal of Colloid and Interface Science*, *142*(1), 149–158.

Jiang, Z., Tian, Y., Shan, D., Wang, Y., Gerhard, E., Xia, J., Huang, R., He, Y., Li, A., Tang, J., Ruan, H., Li, Y., Li, J., Yang, J., & Wu, A. (2018). pH protective Y1 receptor ligand functionalized antiphagocytosis BPLP-WPU micelles for enhanced tumor imaging and therapy with prolonged survival time. *Biomaterials*, *170*, 70–81.

Karadag, K., Yamada, S., & Endo, T. (2018). Synthesis of poly(2-ethyl-2-oxazoline)-block-polypeptide copolymers by combination of ring-opening polymerization of oxazoline and polycondensation of activated urethane derivatives of α-amino acids. *Polymer Bulletin*, *75*(11), 5075–5088.

Kesharwani, P., Jain, K., & Jain, N. K. (2014). Dendrimer as nanocarrier for drug delivery. *Progress in Polymer Science*, *39*(2), 268–307.

Kim, T. Y., Kim, D. W., Chung, J. Y., Shin, S. G., Kim, S. C., Heo, D. S., Kim, N. K., & Bang, Y. J. (2004). Phase I and pharmacokinetic study of Genexol-PM, a cremophor-free, polymeric micelle-formulated paclitaxel, in patients with advanced malignancies. *Clinical Cancer Research*, *10*(11), 3708–3716.

Kinoshita, T. (1991). Biology of complement: The overture. *Immunology Today*, *12*(9), 291–295.

Kroll, A. V, Fang, R. H., & Zhang, L. (2017). Biointerfacing and applications of cell membrane-coated nanoparticles. *Bioconjugate Chemistry*, *28*(1), 23–32.

Kukowska-Latallo, J. F., Candido, K. A., Cao, Z., Nigavekar, S. S., Majoros, I. J., Thomas, T. P., Balogh, L. P., Khan, M. K., & Baker, J. R. (2005). Nanoparticle targeting of anticancer drug improves therapeutic response in animal model of human epithelial cancer. *Cancer Research*, *65*(12), 5317–5324.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, *227*(5259), 680–685.

Laverman, P., Carstens, M. G., Boerman, O. C., Th. M. Dams, E., Oyen, W. J. G., van Rooijen, N., Corstens, F. H. M., & Storm, G. (2001). Factors affecting the accelerated blood clearance of polyethylene glycol-liposomes upon repeated injection. *Journal of Pharmacology and Experimental Therapeutics*, *298*(2), 607–612.

Lazdina, U., Alheim, M., Nyström, J., Hultgren, C., Borisova, G., Sominskaya, I., Pumpens, P., Peterson, D., Milich, D., & Sällberg, M. (2003). Priming of cytotoxic T cell responses to exogenous hepatitis B virus core antigen is B cell dependent. *Journal of General Virology*, *84*(1), 139–146.

Lazdina, U., Cao, T., Steinbergs, J., Alheim, M., Pumpens, P., Peterson, D. L., Milich, D. R., Leroux-Roels, G., & Sallberg, M. (2001). Molecular basis for the interaction of the hepatitis B virus core antigen with the surface immunoglobulin receptor on naive B cells. *Journal of Virology*, *75*(14), 6367–6374.

Lee, B. R., Jo, E., Yoon, H. Y., Yoon, C. J., Lee, H. J., Kwon, K. C., Kim, T. W., & Lee, J. (2018). Nonimmunogenetic viral capsid carrier with cancer targeting activity. *Advanced Science*, *5*(8), 1800494.

Lee, B. O., Tucker, A., Frelin, L., Sallberg, M., Jones, J., Peters, C., Hughes, J., Whitacre, D., Darsow, B., Peterson, D. L., & Milich, D. R. (2009). Interaction of the hepatitis B core antigen and the innate immune system. *The Journal of Immunology*, *182*(11), 6670–6681.

Lee, K. W., & Tan, W. S. (2008). Recombinant hepatitis B virus core particles: Association, dissociation and encapsidation of green fluorescent protein. *Journal of Virological Methods*, *151*(2), 172–180.

Lee, K. W., Tey, B. T., Ho, K. L., Tejo, B. A., & Tan, W. S. (2012). Nanoglue: An alternative way to display cell-internalizing peptide at the spikes of hepatitis B virus core nanoparticles for cell-targeting delivery. *Molecular Pharmaceutics*, *9*(9), 2415–2423.

Lee, L. A., & Wang, Q. (2006). Adaptations of nanoscale viruses and other protein cages for medical applications. *Nanomedicine: Nanotechnology, Biology and Medicine*, *2*(3), 137–149.

Li, J., & Mooney, D. J. (2016). Designing hydrogels for controlled drug delivery. *Nature Reviews Materials*, *1*(12), 16071.

Liang, T. J. (2009). Hepatitis B: the virus and disease. *Hepatology*, *49*(S5), S13–S21.

Lipka, J., Semmler-Behnke, M., Sperling, R. A., Wenk, A., Takenaka, S., Schleh, C., Kissel, T., Parak, W. J., & Kreyling, W. G. (2010). Biodistribution of PEG-modified gold nanoparticles following intratracheal instillation and intravenous injection. *Biomaterials*, *31*(25), 6574–6581.

Ludgate, L., Liu, K., Luckenbaugh, L., Streck, N., Eng, S., Voitenleitner, C., Delaney, W. E., & Hu, J. (2016). Cell-free hepatitis B virus capsid assembly dependent on the core protein C-terminal domain and regulated by phosphorylation. *Journal of Virology*, *90*(12), 5830–5844.

Luxenhofer, R., Han, Y., Schulz, A., Tong, J., He, Z., Kabanov, A. V., & Jordan, R. (2012). Poly(2-oxazoline)s as polymer therapeutics. *Macromolecular Rapid Communications*, *33*(19), 1613–1631.

Luxenhofer, R., López-García, M., Frank, A., Kessler, H., & Jordan, R. (2006). First poly (2-oxazoline)-peptide conjugate for targeted radionuclide cancer therapy. *PMSE Prepr*, *95*, 283–284.

Ma, Y., Yang, Q., Wang, L., Zhou, X., Zhao, Y., & Deng, Y. (2012). Repeated injections of PEGylated liposomal topotecan induces accelerated blood clearance phenomenon in rats. *European Journal of Pharmaceutical Sciences*, *45*(5), 539–545.

Majumder, J., Taratula, O., & Minko, T. (2019). Nanocarrier-based systems for targeted and site specific therapeutic delivery. *Advanced Drug Delivery Reviews*, *144*, 57–77.

Malik, N., Evagorou, E. G., & Duncan, R. (1999). Dendrimer-platinate: A novel approach to cancer chemotherapy. *Anti-Cancer Drugs*, *10*(8), 767–776.

Markman, M. (2006). Pegylated liposomal doxorubicin in the treatment of cancers of the breast and ovary. *Expert Opinion on Pharmacotherapy*, *7*(11), 1469–1474.

Matsumura, Y., Hamaguchi, T., Ura, T., Muro, K., Yamada, Y., Shimada, Y., Shirao, K., Okusaka, T., Ueno, H., Ikeda, M., & Watanabe, N. (2004). Phase I clinical trial and pharmacokinetic evaluation of NK911, a micelle-encapsulated doxorubicin. *British Journal of Cancer*, *91*(10), 1775–1781.

Mero, A., Fang, Z., Pasut, G., Veronese, F. M., & Viegas, T. X. (2012). Selective conjugation of poly(2-ethyl 2-oxazoline) to granulocyte colony stimulating factor. *Journal of Controlled Release*, *159*(3), 353–361.

Milich, D. R., Chen, M., Schodel, F., Peterson, D. L., Jones, J. E., & Hughes, J. L. (1997). Role of B cells in antigen presentation of the hepatitis B core. *Proceedings of the National Academy of Sciences*, *94*(26), 14648–14653.

Milich, D. R., & McLachlan, A. (1986). The nucleocapsid of hepatitis B virus is both a T-cell-independent and a T-cell-dependent antigen. *Science*, *234*(4782), 1398–1401.

Misra, R., Acharya, S., & Sahoo, S. K. (2010). Cancer nanotechnology: Application of nanotechnology in cancer therapy. *Drug Discovery Today*, *15*(19–20), 842–850.

Mitchell, R. N. (2013). Innate and adaptive immunity. *Biomaterials Science* (pp. 512–533). Elsevier.

Moreadith, R. W., Viegas, T. X., Standaert, D. G., Bentley, M. D., Fang, Z., Dizman, B., Yoon, K., Weimer, R., Harris, J. M., & Ravenscroft, P. (2012). SER-214, a novel polymer-conjugated rotigotine formulation affords greatly extended duration of anti-parkinsonian effect and enhanced plasma exposure following a single administration in rodents and primates. *Proceedings of the 16th International Conference of Parkinson's Disease and Movement Disorders, Movement Disorder Society*, 17–21.

Moreadith, R. W., Viegas, T. X., Bentley, M. D., Harris, J. M., Fang, Z., Yoon, K., Dizman, B., Weimer, R., Rae, B. P., Li, X., Rader, C., Standaert, D., & Olanow, W. (2017). Clinical development of a poly(2-oxazoline) (POZ) polymer therapeutic for the treatment of Parkinson's disease – proof of concept of POZ as a versatile polymer platform for drug development in multiple therapeutic indications. *European Polymer Journal*, *88*, 524–552.

Mudshinge, S. R., Deore, A. B., Patil, S., & Bhalgat, C. M. (2011). Nanoparticles: Emerging carriers for drug delivery. *Saudi Pharmaceutical Journal*, *19*(3), 129–141.

Murata, Y., Kotani, T., Ohnishi, H., & Matozaki, T. (2014). The CD47-SIRP signalling system: Its physiological roles and therapeutic application. *Journal of Biochemistry*, *155*(6), 335–344.

Murray, K., Bruce, S. A., Hinnen, A., Wingfield, P., van Erd, P. M., de Reus, A., & Schellekens, H. (1984). Hepatitis B virus antigens made in microbial cells immunise against viral infection. *The EMBO Journal*, *3*(3), 645–650.

Nagao, A., Abu Lila, A. S., Ishida, T., & Kiwada, H. (2013). Abrogation of the accelerated blood clearance phenomenon by SOXL regimen: Promise for clinical application. *International Journal of Pharmaceutics*, *441*(1–2), 395–401.

Nasrollahzadeh, M., Sajadi, S. M., Sajjadi, M., & Issaabadi, Z. (2019). An introduction to nanotechnology. In M. Nasrollahzadeh, S. M. Sajadi, M. Sajjadi, Z. Issaabadi, & M. B. T. I. S. and T. Atarod (Eds.), *An Introduction to Green Nanotechnology* (pp. 1–27). Elsevier.

Nassal, M. (1992). The arginine-rich domain of the hepatitis B virus core protein is required for pregenome encapsidation and productive viral positive-strand DNA synthesis but not for virus assembly. *Journal of Virology*, *66*(7), 4107–4116.

Nassal, M., & Schaller, H. (1993). Hepatitis B virus replication. *Trends in Microbiology*, *1*(6), 221–228.

Newton, S. E., Clarke, B. E., Appleyard, G., Francis, M. J., Carroll, A. R., Rowlands, D. J., Skehel, J., & Brown, F. (1987). New approaches to FMDV antigen presentation using vaccinia virus. In R. M. Chanock, R. A. Lerner, F. Brown, & H. Ginsberg (Eds.), *Vaccines 87: Modern Approaches to New Vaccines. Prevention of AIDS and Other Viral, Bacterial and Parasitic Diseases.* (pp. 12–21). Cold Spring Harbor Lab. Press.

Ngui, S. L., Hallet, R., & Teo, C. G. (1999). Natural and iatrogenic variation in hepatitis B virus. *Reviews in Medical Virology*, *9*(3), 183–209.

Oldenborg, P. A., Zheleznyak, A., Fang, Y. F., Lagenaur, C. F., Gresham, H. D., & Lindberg, F. P. (2000). Role of CD47 as a marker of self on red blood cells. *Science*, *288*(5473), 2051–2054.

Ordikhani, F., Zandi, N., Mazaheri, M., Luther, G. A., Ghovvati, M., Akbarzadeh, A., & Annabi, N. (2020). Targeted nanomedicines for the treatment of bone disease and regeneration. *Medicinal Research Reviews*, med.21759.

Owens, D. E., & Peppas, N. A. (2006). Opsonization, biodistribution, and pharmacokinetics of polymeric nanoparticles. *International Journal of Pharmaceutics*, *307*(1), 93–102.

Papini, E., Tavano, R., & Mancin, F. (2020). Opsonins and dysopsonins of nanoparticles: Facts, concepts, and methodological guidelines. *Frontiers in Immunology*, *11*, 567365.

Parodi, A., Quattrocchi, N., van de Ven, A. L., Chiappini, C., Evangelopoulos, M., Martinez, J. O., Brown, B. S., Khaled, S. Z., Yazdi, I. K., Enzo, M. V., Isenhart, L., Ferrari, M., & Tasciotti, E. (2013). Synthetic nanoparticles functionalized with biomimetic leukocyte membranes possess cell-like functions. *Nature Nanotechnology*, *8*(1), 61–68.

Pasut, G. (2019). Grand challenges in nano-based drug delivery. *Frontiers in Medical Technology*, *1*, 1.

Patra, J. K., Das, G., Fraceto, L. F., Campos, E. V. R., Rodriguez-Torres, M. del P., Acosta-Torres, L. S., Diaz-Torres, L. A., Grillo, R., Swamy, M. K., Sharma, S., Habtemariam, S., & Shin, H. S. (2018). Nano based drug delivery systems: Recent developments and future prospects. *Journal of Nanobiotechnology*, *16*(1), 71.

Pelaz, B., del Pino, P., Maffre, P., Hartmann, R., Gallego, M., Rivera-Fernández, S., de la Fuente, J. M., Nienhaus, G. U., & Parak, W. J. (2015). Surface functionalization of nanoparticles with polyethylene glycol: Effects on protein adsorption and cellular uptake. *ACS Nano*, *9*(7), 6996–7008.

Prajapati, V. K., Awasthi, K., Gautam, S., Yadav, T. P., Rai, M., Srivastava, O. N., & Sundar, S. (2011). Targeted killing of *Leishmania donovani in vivo* and *in vitro* with amphotericin B attached to functionalized carbon nanotubes. *Journal of Antimicrobial Chemotherapy*, *66*(4), 874–879.

Pumpens, P., Borisova, G. P., Crowther, R. A., & Grens, E. (1995). Hepatitis B virus core particles as epitope carriers. *Intervirology*, *38*(1–2), 63–74.

Pumpens, P., Sasnauskas, K., Kazaks, A., & Grens, E. (2008). Construction of novel vaccines on the basis of the virus-like particles: Hepatitis B virus proteins as vaccine carriers. In Y. Khudyakov (Ed.), *Medicinal Protein Engineering* (pp. 205–248). CRC.

Pumpens, P., & Grens, E. (1999). Hepatitis B core particles as a universal display model: A structure-function basis for development. *FEBS Letters*, *442*(1), 1–6.

Pumpens, P., & Grens, E. (2001). HBV core particles as a carrier for B cell/T cell epitopes. *Intervirology*, *44*(2–3), 98–114.

Qamar, S. A., Asgher, M., Khalid, N., & Sadaf, M. (2019). Nanobiotechnology in health sciences: Current applications and future perspectives. *Biocatalysis and Agricultural Biotechnology*, *22*, 101388.

Razieh, M. (2011). *Detection of hepatitis B core antigen by phage display immuno-TaqMan real-time polymerase chain reaction*. Universiti Putra Malaysia, Malaysia.

Rodriguez, P. L., Harada, T., Christian, D. A., Pantano, D. A., Tsai, R. K., & Discher, D. E. (2013). Minimal "self" peptides that inhibit phagocytic clearance and enhance delivery of nanoparticles. *Science*, *339*(6122), 971–975.

Rohovie, M. J., Nagasawa, M., & Swartz, J. R. (2017). Virus-like particles: Next-generation nanoparticles for targeted therapeutic delivery. *Bioengineering & Translational Medicine*, *2*(1), 43–57.

Roldo, M. (2016). Carbon nanotubes in drug delivery: Just a carrier? *Therapeutic Delivery*, *7*(2), 55–57.

Roose, K., Baets, S. De, Schepens, B., & Saelens, X. (2013). Hepatitis B core–based virus–like particles to present heterologous epitopes. *Expert Review of Vaccines*, *12*(2), 183–198.

Rosenthal, E., Poizot-Martin, I., Saint-Marc, T., Spano, J.-P., Cacoub, P., & Study Group, D. N. X. (2002). Phase IV study of liposomal daunorubicin (DaunoXome) in AIDS-related Kaposi sarcoma. *American Journal of Clinical Oncology*, *25*(1).

Saeednia, L., Yao, L., Cluff, K., & Asmatulu, R. (2019). Sustained releasing of methotrexate from injectable and thermosensitive chitosan–carbon nanotube hybrid hydrogels effectively controls tumor cell growth. *ACS Omega*, *4*(2), 4040–4048.

Sainsbury, F. (2017). Virus-like nanoparticles: Emerging tools for targeted cancer diagnostics and therapeutics. *Therapeutic Delivery*, *8*(12), 1019–1021.

Salfeld, J., Pfaff, E., Noah, M., & Schaller, H. (1989). Antigenic determinants and functional domains in core antigen and e antigen from hepatitis B virus. *Journal of Virology*, *63*(2), 798–808.

Sällberg, M., Pushko, P., Berzinsh, I., Bichko, V., Sillekens, P., Noah, M., Pumpens, P., Grens, E., Wahren, B., & Magnius, L. O. (1993). Immunochemical structure of the carboxy-terminal part of hepatitis B e antigen: Identification of internal and surface-exposed sequences. *Journal of General Virology*, *74*(7), 1335–1340.

Sällberg, M., Rudén, U., Magnius, L. O., Harthus, H. P., Noah, M., & Wahren, B. (1991). Characterisation of a linear binding site for a monoclonal antibody to hepatitis B core antigen. *Journal of Medical Virology*, *33*(4), 248–252.

Sällberg, M., Rudén, U., Wahren, B., Noah, M., & Magnius, L. O. (1991). Human and murine B-cells recognize the HBeAg/beta (or HBe2) epitope as a linear determinant. *Molecular Immunology*, *28*(7), 719–726.

Salmaso, S., & Caliceti, P. (2013). Stealth properties to improve therapeutic efficacy of drug nanocarriers. *Journal of Drug Delivery*, *2013*, 1–19.

Sanna, V., Pala, N., & Sechi, M. (2014). Targeted therapy using nanotechnology: Focus on cancer. *International Journal of Nanomedicine*, *9*, 467.

Sathyamoorthy, N., & Dhanaraju, M. D. (2016). Shielding therapeutic drug carriers from the mononuclear phagocyte system: A review. *Critical Reviews in Therapeutic Drug Carrier Systems*, *33*(6), 489–567.

Schmidt, M., Harmuth, S., Barth, E. R., Wurm, E., Fobbe, R., Sickmann, A., Krumm, C., & Tiller, J. C. (2015). Conjugation of ciprofloxacin with poly(2-oxazoline)s and polyethylene glycol via end groups. *Bioconjugate Chemistry*, *26*(9), 1950–1962.

Sedlacek, O., Monnery, B. D., Filippov, S. K., Hoogenboom, R., & Hruby, M. (2012). Poly(2-oxazoline)s - are they more advantageous for biomedical applications than other polymers? *Macromolecular Rapid Communications*, *33*(19), 1648–1662.

Sheehan, J., Cruickshank, P., & Boshart, G. (1961). A convenient synthesis of water-soluble carbodiimides. *The Journal of Organic Chemistry*, *26*(7), 2525–2528.

Sheehan, J., Preston, J., & Cruickshank, P. (1965). A rapid synthesis of oligopeptide derivatives without isolation of intermediates. *Journal of the American Chemical Society*, *87*(11), 2492–2493.

Shim, G., Miao, W., Ko, S., Park, G. T., Kim, J. Y., Kim, M. G., Kim, Y. B., & Oh, Y. K. (2017). Immune-camouflaged graphene oxide nanosheets for negative regulation of phagocytosis by macrophages. *Journal of Materials Chemistry B*, *5*(32), 6666–6675.

Shimizu, T., Ichihara, M., Yoshioka, Y., Ishida, T., Nakagawa, S., & Kiwada, H. (2012). Intravenous administration of polyethylene glycol-coated (PEGylated) proteins and PEGylated adenovirus elicits an anti-PEG immunoglobulin M response. *Biological and Pharmaceutical Bulletin*, *35*(8), 1336–1342.

Shin, H., Jo, S., & Mikos, A. G. (2003). Biomimetic materials for tissue engineering. *Biomaterials*, *24*(24), 4353–4364.

Silva, G. A. (2004). Introduction to nanotechnology and its applications to medicine. *Surgical Neurology*, *61*(3), 216–220.

Sominskaya, I., Skrastina, D., Petrovskis, I., Dishlers, A., Berza, I., Mihailova, M., Jansons, J., Akopjana, I., Stahovska, I., Dreilina, D., Ose, V., & Pumpens, P. (2013). A VLP library of C-terminally truncated hepatitis B core proteins: Correlation of RNA encapsidation with a Th1/Th2 switch in the immune responses of mice. *PLOS ONE*, *8*(9), e75938.

Steinmetz, N. F. (2010). Viral nanoparticles as platforms for next-generation therapeutics and imaging devices. *Nanomedicine: Nanotechnology, Biology and Medicine*, *6*(5), 634–641.

Stephan, M. T., & Irvine, D. J. (2011). Enhancing cell therapies from the outside in: Cell surface engineering using synthetic nanomaterials. *Nano Today*, *6*(3), 309–325.

Stewart, F. J. (1993). *Mutations that affect the structure and interactions of the core antigen of hepatitis B virus*. University of Edinburgh, U. K.

Strods, A., Ose, V., Bogans, J., Cielens, I., Kalnins, G., Radovica, I., Kazaks, A., Pumpens, P., & Renhofa, R. (2015). Preparation by alkaline treatment and detailed characterisation of empty hepatitis B virus core particles for vaccine and gene therapy applications. *Scientific Reports*, *5*(1), 11639.

Suk, J. S., Xu, Q., Kim, N., Hanes, J., & Ensign, L. M. (2016). PEGylation as a strategy for improving nanoparticle-based drug and gene delivery. *Advanced Drug Delivery Reviews*, *99*, 28–51.

Sun, H., Su, J., Meng, Q., Yin, Q., Chen, L., Gu, W., Zhang, Z., Yu, H., Zhang, P., Wang, S., & Li, Y. (2017). Cancer cell membrane-coated gold nanocages with hyperthermia-triggered drug release and homotypic target inhibit growth and metastasis of breast cancer. *Advanced Functional Materials*, *27*(3), 1604300.

Suzuki, T., Ichihara, M., Hyodo, K., Yamamoto, E., Ishida, T., Kiwada, H., Ishihara, H., & Kikuchi, H. (2012). Accelerated blood clearance of PEGylated liposomes containing doxorubicin upon repeated administration to dogs. *International Journal of Pharmaceutics*, *436*(1–2), 636–643.

Svenson, S., & Tomallia, D. (2005). Dendrimers in biomedical applications—reflections on the field. *Advanced Drug Delivery Reviews*, *57*(15), 2106–2129.

Tan, W. S., Dyson, M. R., & Murray, K. (2003). Hepatitis B virus core antigen: Enhancement of its production in *Escherichia coli*, and interaction of the core particles with the viral surface antigen. *Biological Chemistry*, *384*(3), 363–371.

Tan, W. S. (1997). *Interactions between the surface and core antigens of hepatitis B virus*. University of Edinburgh, U.K.

Tan, W. S., McNae, I. W., Ho, K. L., & Walkinshaw, M. D. (2007). Crystallization and X-ray analysis of the T = 4 particle of hepatitis B capsid protein with an N-terminal extension. *Acta Crystallographica Section F Structural Biology and Crystallization Communications*, *63*(8), 642–647.

Taniguchi, N. (1974). On the basic concept of nanotechnology. *Proceedings of the International Conference on Production Engineering*, 18–23.

Tong, J., Yi, X., Luxenhofer, R., Banks, W. A., Jordan, R., Zimmerman, M. C., & Kabanov, A. V. (2013). Conjugates of superoxide dismutase 1 with amphiphilic poly(2-oxazoline) block copolymers for enhanced brain delivery: Synthesis, characterization and evaluation in vitro and in vivo. *Molecular Pharmaceutics*, *10*(1), 360–377.

Ulrich, R., Nassal, M., Meisel, H., & Krüger, D. H. (1998). Core particles of hepatitis B virus as carrier for foreign epitopes. *Advances in Virus Research, 50*, 141–182.

Vanlandschoot, P., Houtte, F. Van, Serruys, B., & Leroux-Roels, G. (2005). The arginine-rich carboxy-terminal domain of the hepatitis B virus core protein mediates attachment of nucleocapsids to cell-surface-expressed heparan sulfate. *Journal of General Virology*, *86*(1), 75–84.

Verhoef, J. J. F., Carpenter, J. F., Anchordoquy, T. J., & Schellekens, H. (2014). Potential induction of anti-PEG antibodies and complement activation toward PEGylated therapeutics. *Drug Discovery Today*, *19*(12), 1945–1952.

Viegas, T. X., Bentley, M. D., Harris, J. M., Fang, Z., Yoon, K., Dizman, B., Weimer, R., Mero, A., Pasut, G., & Veronese, F. M. (2011). Polyoxazoline: Chemistry, properties, and applications in drug delivery. *Bioconjugate Chemistry*, *22*(5), 976–986.

Vijayan, V., Uthaman, S., & Park, I. K. (2018). Cell membrane-camouflaged nanoparticles: A promising biomimetic strategy for cancer theragnostics. *Polymers*, *10*(9), 983.

Vonarbourg, A., Passirani, C., Saulnier, P., & Benoit, J. P. (2006). Parameters influencing the stealthiness of colloidal drug delivery systems. *Biomaterials*, *27*(24), 4356–4373.

Walkey, C. D., & Chan, W. C. (2012). Understanding and controlling the interaction of nanomaterials with proteins in a physiological environment. *Chemical Society Reviews*, *41*(7), 2780–2799.

Werner, M. E., Cummings, N. D., Sethi, M., Wang, E. C., Sukumar, R., Moore, D. T., & Wang, A. Z. (2013). Preclinical evaluation of Genexol-PM, a nanoparticle formulation of paclitaxel, as a novel radiosensitizer for the treatment of non-small cell lung cancer. *International Journal of Radiation Oncology, Biology, Physics*, *86*(3), 463–468.

Whitacre, D. C., Lee, B. O., & Milich, D. R. (2009). Use of hepadnavirus core proteins as vaccine platforms. *Expert Review of Vaccines*, *8*(11), 1565–1573.

Wynne, S. A., Crowther, R. A., & Leslie, A. G. W. (1999). The crystal structure of the human hepatitis B virus capsid. *Molecular Cell*, *3*(6), 771–780.

Xuan, M., Shao, J., & Li, J. (2019). Cell membrane-covered nanoparticles as biomaterials. *National Science Review*, *6*(3), 551–561.

Xuan, M., Shao, J., Dai, L., He, Q., & Li, J. (2015). Macrophage cell membrane camouflaged mesoporous silica nanocapsules for in vivo cancer therapy. *Advanced Healthcare Materials*, *4*(11), 1645–1652.

Yadav, L. D. S. (2005a). Proton nuclear magnetic resonance (PMR or ^{1}H NMR) spectroscopy. *Organic Spectroscopy* (pp. 133–194). Springer Netherlands.

Yadav, L. D. S. (2005b). Ultraviolet (UV) and visible spectroscopy. *Organic Spectroscopy* (pp. 15–17). Springer Netherlands.

Yildiz, I., Shukla, S., & Steinmetz, N. F. (2011). Applications of viral nanoparticles in medicine. *Current Opinion in Biotechnology*, *22*(6), 901–908.

Yu, X., Jin, L., Jih, J., Shih, C., & Zhou, Z. H. (2013). 3.5Å cryoEM structure of hepatitis B virus core assembled from full-length core protein. *PLOS ONE*, *8*(9), e69729.

Zhang, L., Gu, F., Chan, J., Wang, A., Langer, R., & Farokhzad, O. (2008). Nanoparticles in medicine: therapeutic applications and developments. *Clinical Pharmacology & Therapeutics*, *83*(5), 761–769.

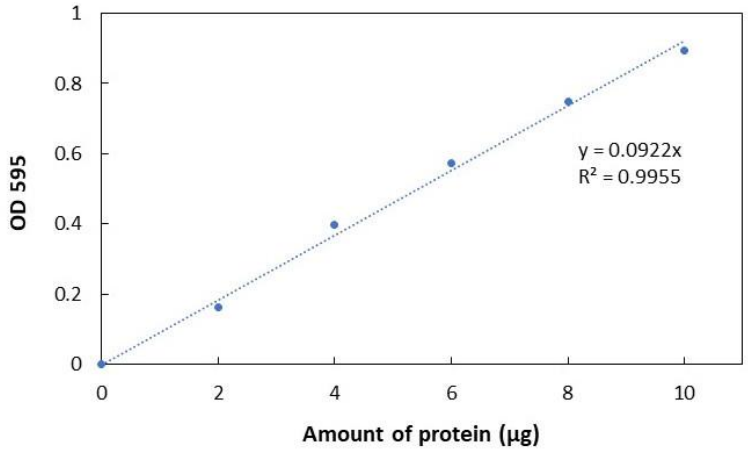
Zhao, Y., Wang, C., Wang, L., Yang, Q., Tang, W., She, Z., & Deng, Y. (2012). A frustrating problem: Accelerated blood clearance of PEGylated solid lipid nanoparticles following subcutaneous injection in rats. *European Journal of Pharmaceutics and Biopharmaceutics*, *81*(3), 506–513.

Zielińska, A., Carreiró, F., Oliveira, A. M., Neves, A., Pires, B., Venkatesh, D. N., Durazzo, A., Lucarini, M., Eder, P., Silva, A. M., Santini, A., & Souto, E. B. (2020). Polymeric nanoparticles: Production, characterization, toxicology and ecotoxicology. *Molecules*, *25*(16), 3731.

APPENDICES

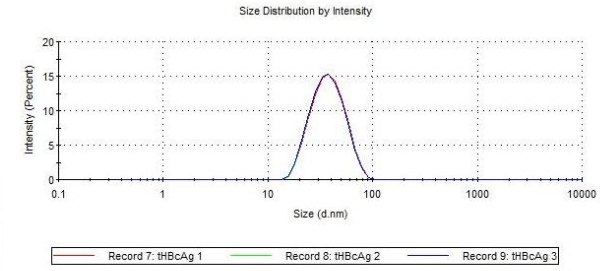
Appendix A

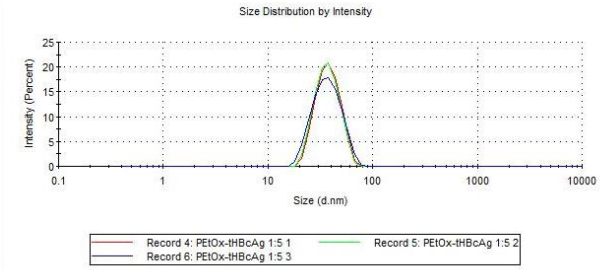
The standard curve for the Bradford assay

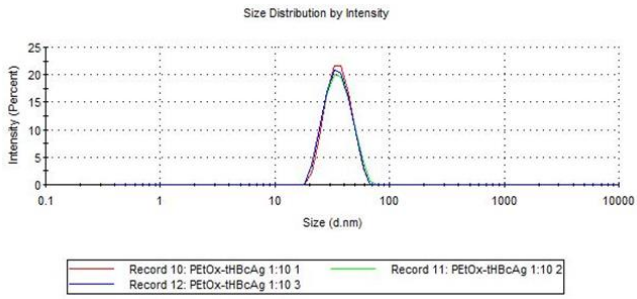


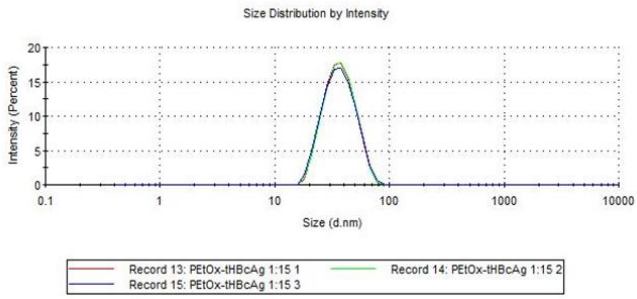
Appendix B

The size distribution graphs of HBcAg and PEtOx-HBcAg VLNPs



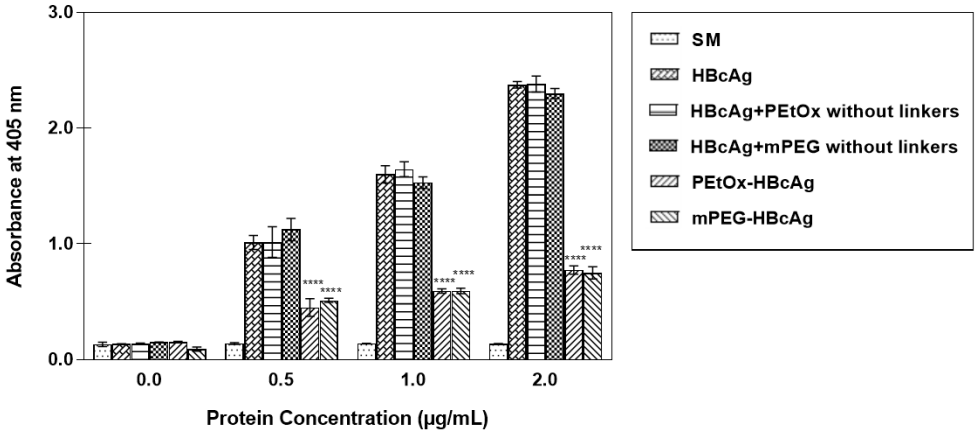






Appendix C

ELISA analysis of HBcAg, PEtOx-HBcAg, and mPEG-HBcAg VLNPs



BIODATA OF STUDENT

Fam See Yee was born on 31st May 1995 in Malacca and raised in Negeri Sembilan. In July 2017, she graduated in BSc (Hons) Biomedical Sciences with a second upper class from The University of Nottingham Malaysia Campus. After graduation, she furthered her study in Master of Science (MSc) in Nanobiotechnology at the Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia (UPM). During her Master's study, she was a recipient of Graduate Research Fellowship (GRF) from UPM.

LIST OF PUBLICATIONS

Fam, S. Y.; Chee, C. F.; Yong, C. Y.; Ho, K. L.; Mariatulqabtiah, A. R.; Lau, H. Y.; Tan, W. S. (2019). Shielding of hepatitis B virus-like nanoparticle with poly(2-ethyl-2-oxazoline). *International Journal of Molecular Sciences*, *20*(19), 4903.

Fam, S. Y.; Chee, C. F.; Yong, C. Y.; Ho, K. L.; Mariatulqabtiah, A. R.; Tan, W. S. (2020). Stealth coating of nanoparticles in drug delivery system. *Nanomaterials*, *10*(4), 787.

UNIVERSITI PUTRA MALAYSIA

STATUS CONFIRMATION FOR THESIS / PROJECT REPORT AND COPYRIGHT

ACADEMIC SESSION : Second Semester 2020/2021

TITLE OF THESIS / PROJECT REPORT :

SHIELDING OF VIRUS-LIKE NANOPARTICLES OF HEPATITIS B CORE ANTIGEN BY POLY(2-OXAZOLINE) FOR REDUCED ANTIGENICITY

NAME OF STUDENT: FAM SEE YEE

I acknowledge that the copyright and other intellectual property in the thesis/project report belonged to Universiti Putra Malaysia and I agree to allow this thesis/project report to be placed at the library under the following terms:

1. This thesis/project report is the property of Universiti Putra Malaysia.
2. The library of Universiti Putra Malaysia has the right to make copies for educational purposes only.
3. The library of Universiti Putra Malaysia is allowed to make copies of this thesis for academic exchange.

I declare that this thesis is classified as :

*Please tick (√)

☐	**CONFIDENTIAL**	(Contain confidential information under Official Secret Act 1972).
☐	**RESTRICTED**	(Contains restricted information as specified by the organization/institution where research was done).
☐	**OPEN ACCESS**	I agree that my thesis/project report to be published as hard copy or online open access.

This thesis is submitted for :

☐ **PATENT** Embargo from _____ (date) until _____ (date)

Approved by:

_____
(Signature of Student)
New IC No/ Passport No.:

Date :

_____
(Signature of Chairman of Supervisory Committee)
Name:

Date :

[Note : If the thesis is CONFIDENTIAL or RESTRICTED, please attach with the letter from the organization/institution with period and reasons for confidentially or restricted.]