



**UNIVERSITI PUTRA MALAYSIA**

***HEPATITIS B CAPSID AS A DELIVERY VEHICLE INTO MAMMALIAN  
CELLS***

**LEE KHAI WOUI**

**FBSB 2012 36**

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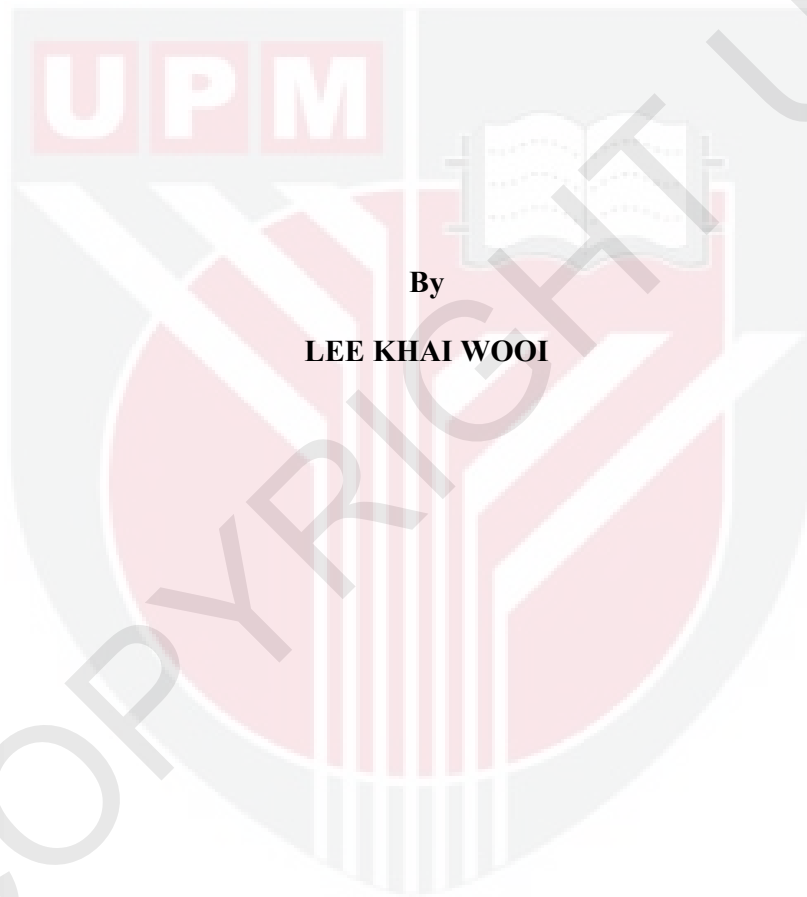


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CELLS**



By

**LEE KHAI WOUI**

**This Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,  
in Fulfilment of the Requirements for the Degree of Doctor of Philosophy**

**August 2012**

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

**HEPATITIS B CAPSID AS A DELIVERY VEHICLE INTO MAMMALIAN CELLS**

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**August 2012**

**Chair: Professor Tan Wen Siang, PhD**

**Faculty: Biotechnology and Biomolecular Sciences**

The recombinant hepatitis B virus (HBV) core antigen (HBcAg) expressed in *Escherichia coli* self-assembles into icosahedral virus-like particles (VLPs) of about 35 nm. It possesses a range of surface chemistries for recombinant DNA alteration and chemical modification to display cell-targeting signals on its surface. The HBcAg nanoparticles could serve as a potential nano-container to package therapeutic molecules for drug and gene delivery. In this study, the association, dissociation and the molecule packaging properties of the C-terminally truncated HBcAg (tHBcAg) were studied with protein denaturants. Light scattering analysis and transmission electron microscopy (TEM) revealed that the dissociated tHBcAg was able to re-associate into nanoparticles when the denaturants were removed. In order to evaluate the potential of the nanoparticles in capturing molecules, the green fluorescent protein (GFP) was used as a model for encapsidation. Data showed that

the GFP molecules were packaged and remained active in the tHBcAg nanoparticles after the dissociation and re-association steps.

The insertion of foreign peptides at the N-terminal end of HBcAg displays the inserts on the surface of the nanoparticles. In order to develop the tHBcAg nanoparticles as a cell specific delivery system, the HBV preS1<sub>1-108</sub> region was employed as a ligand to target the human hepatocytes. The preS1<sub>1-108</sub> region was fused to the N-terminal end of the His<sub>6</sub>HBcAg (preS1His<sub>6</sub>HBcAg) via recombinant DNA technique. However, the fusion protein was insoluble in *E. coli* and did not form VLPs. A method to reconstruct and to display the preS1 on the tHBcAg nanoparticle was established by dissociating an appropriate molar ratio of the tHBcAg to the preS1His<sub>6</sub>HBcAg in urea. Gold immuno-TEM showed that the subunit mixture re-assembled into icosahedral nanoparticles, displaying the preS1 ligand on the surface of VLPs. In order to assess the application of the reconstructed VLPs (rVLPs) as a delivery vehicle, fluorescein molecules were cross-linked on the tHBcAg of the rVLPs and tested on hepatocytes *in vitro*. Fluorescence microscopy revealed that the fluorescent rVLPs were internalised into HepG2 cells, efficiently.

The peptide, SLLGRMKGA has been shown to bind and chemically cross-link the aspartic acid residues (D<sub>64</sub> and D<sub>78</sub>) or glutamic acid residue (E<sub>77</sub>) at the tips of tHBcAg nanoparticles. The following study reports an extension of this idea as a universal 'nano-glue' to display different cell-internalising peptide (CIP) at the tips of tHBcAg nanoparticles. As a model of our studies, peptide ligand that internalised HeLa cells were selected from a random 12-mers peptide library displayed on

filamentous M13 phage. Immuno-fluorescence microscopy showed that the isolated M13 clones, displaying HTLQIPQHATSF, KLHISKDHIYPT and THASKNTSYFTV internalised HeLa cells specifically. The isolated peptide (KLHISKDHIYPT) was co-synthesised with the 'nano-glue' sequence (KLHISKDHIYPTGGGSLLGRMKGA; JG24) and conjugated chemically to the tHBcAg nanoparticles using cross-linkers (EDC and sulfo-NHS). Transmission electron microscopy (TEM) revealed that the tHBcAg nanoparticles remained intact after the conjugation. Interestingly, when the peptide was conjugated to the fluorescein labelled tHBcAg (FtHBcAg) nanoparticles, the nanoparticles internalised HeLa cells and the fluorescent was observed under a fluorescence microscope. tHBcAg nanoparticles were later used to packaged fluorescent oligonucleotide, conjugated with cell-targeting peptide and added to HeLa cells. Fluorescent microscopy revealed the delivery of the oligonucleotide into the cells.

The tHBcAg nanoparticles could serve as a potential nano-vehicle to package molecules and to target various cells specifically by displaying different cell specific ligands at the N-terminal region or having the CIPs conjugated chemically at the tips of tHBcAg nanoparticles.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**KAPSID HEPATITIS B SEBAGAI KENDERAAN PENGHANTARAN KE  
DALAM SEL-SEL MAMALIA**

Oleh

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Antigen teras (HBcAg) rekombinan virus hepatitis B (HBV) yang diekspres dalam *Escherichia coli* dapat berinteraksi sesama sendiri menjadi kapsid icosahedral dengan ukuran lebih kurang 35 nm. Kapsid tersebut mempunyai permukaan untuk modifikasi DNA rekombinan dan pengubahsuaian secara kimia demi mempamerkan isyarat-isyarat sasaran sel pada permukaannya. Partikel-partikel nano HBcAg tersebut sesuai digunakan sebagai bekas-nano yang berpotensi dalam pembungkusan molekul-molekul terapeutik untuk kegunaan penghantaran gen dan ubat. Dalam kajian ini, sifat-sifat persatuan, penceraian dan pembungkusan molekul HBcAg yang dipendekkan di terminal-C (tHBcAg) telah dikaji dengan penyahasli-penyahasli protein. Analisis penaburan cahaya dan mikroskopi penyiaran elektron (TEM) mendedahkan bahawa tHBcAg yang tercerai tersebut dapat bersatu semula kepada partikel-partikel nano aslinya selepas penyahasli-penyahasli disingkirkan. Demi menilai potensi partikel-partikel nano tersebut dalam pembungkusan molekul-

molekul, protein berpendarfluor hijau (GFP) telah digunakan sebagai model dalam pembungkusan. Data menunjukkan bahawa molekul-molekul GFP terbungkus dan kekal aktif di dalam partikel-partikel nano tHBcAg selepas langkah-langkah perceraian dan penghimpunan semula.

Penyisipan peptida-peptida asing di bahagian terminal-N HBcAg mempamerkan sisipan-sisipan tersebut pada permukaan partikel-partikel menyerupai virus (VLPs). Dalam kajian ini, bahagian preS1<sub>1-108</sub> telah digunakan sebagai ligan sasaran sel-sel hati manusia. Bahagian preS1<sub>1-108</sub> tersebut telah dicantumkan pada hujung terminal-N His<sub>6</sub>HBcAg (preS1His<sub>6</sub>HBcAg) melalui teknik rekombinan DNA. Akan tetapi, hasil protein rekombinan tersebut tidak larut di dalam *E. coli* dan tidak membentuk VLPs. Satu kaedah untuk pembinaan semula dan pempameran preS1 pada partikel-partikel nano telah ditubuhkan dengan penceraian nisbah molar tHBcAg kepada preS1His<sub>6</sub>HBcAg yang bersesuaian dalam larutan urea. TEM-imuno emas menunjukkan bahawa campuran subunit-subunit tersebut dapat berhimpun semula menjadi partikel-partikel nano icosahedral serta mempamerkan ligan preS1 tersebut di permukaan VLPs. Dalam kajian aplikasi VLPs yang terbina semula (rVLPs) tersebut untuk kegunaan sebagai kenderaan penghantaran, tHBcAg di dalam rVLPs tersebut telah dilabelkan dengan molekul-molekul “fluorescein” dan diuji dengan sel-sel hati manusia secara *in vitro*. Mikroskopi berpendarfluoran menunjukkan bahawa rVLPs berpendarfluor tersebut dapat menembusi sel-sel HepG2 dengan berkesan.

Sebelum ini, kajian telah menunjukkan bahawa peptida SLLGRMKGA dapat mengikat dan disambung-silang secara kimia pada residu-residu asid aspartik (D<sub>64</sub>



and D<sub>78</sub>) atau residu asid glutamic (E<sub>77</sub>) di hujung-hujung partikel nano tHBcAg. Di sini, kami melaporkan satu unjuran tersebut sebagai 'glu-nano' umum untuk mempamerkan pelbagai peptida penembusi sel (CIP) pada hujung-hujung partikel nano tHBcAg. Sebagai satu model dalam kajian tersebut, ligan peptida yang menembusi sel-sel HeLa telah dipilih dari satu perpustakaan peptida rawak 12-mer yang terpamer pada faj M13. Mikroskopi-imuno berpendarfluoran menunjukkan bahawa klon-klon M13 terpencil yang memaparkan peptida-peptida HTLQIPQHATSF, KLHISKDHIYPT dan THASKNTSYFTV yang sewajarnya dapat menembusi secara khususnya ke dalam sel-sel HeLa. KLHISKDHIYPT disintesis bersama dengan urutan 'glu-nano' (KLHISKDHIYPTGGGSLGRMKGA; JG24) dan dikonjugasikan secara kimia pada partikel-partikel nano tHBcAg dengan menggunakan penyambung-silang (EDC dan sulfo-NHS). Permerhatian TEM mendedahkan bahawa partikel-partikel tHBcAg tersebut dapat mengekalkan kesempurnaannya selepas proses konjugasi. Apabila peptida JG24 dikonjugasikan pada partikel-partikel nano tHBcAg yang dilabel dengan fluorescein, partikel-partikel nano tersebut menembus ke dalam sel-sel HeLa dan pendarfluoran sel-sel tersebut dapat diperhatikan di bawah mikroskopi berpendarfluoran. Selepas itu, partikel-partikel nano tHBcAg digunakan untuk membungkus oligonukleotida berpendarfluor, konjugasi dengan peptide-peptida sasaran sel, dan ditambahkan dalam sel-sel HeLa. Mikroskopi berpendarfluoran menunjukkan penghantaran oligonukleotida-oligonukleotida ke dalam sel-sel tersebut.

Partikel-partikel nano tHBcAg dapat digunakan sebagai kenderaan-nano yang berpotensi untuk membungkus molekul-molekul dan mensasar ke pelbagai sel yang

tertentu dengan pempameran ligan-ligan sel yang khusus sama ada di bahagian terminal-N ataupun secara konjugasi kimia di puncak-puncak partikel nano tHBcAg.



## ACKNOWLEDGEMENTS

I would like to express my deepest gratitude and respect to my supervisor, Prof. Dr. Tan Wen Siang for his professional competence and guidance throughout the research period. He treated me, genuinely, as part of his extended family members. My sincere appreciations are also extended to my co-supervisors, Prof. Dr. Tey Beng Ti, Dr. Ho Kok Lian and Dr. Bimo A. Tejo for their excellent advices and constructive suggestions, pushing me forward into the limelight while standing back in the shadows themselves.

I am thankful to Prof. Sir K. Murray, Dr. C.S. Tan and Dr. W.B. Yap for providing plasmid pR1-11E, pRSETGFP and pHis- $\beta$ -L-HBcAg, respectively. Many thanks to Dr. M.Y.T. Ng, Dr. S.T. Ong and Ms. C.Y. Teo for technical supports and guidance; staff members and laboratory assistants from Department of Microbiology and Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia (UPM), for their help and assistance; research funding from FRGS Grant # 01-01-07-161FR and the Science Fund # 03-01-04SF0015 for supporting the study; and Graduate Research Fellowship (GRF) from UPM for my financial sponsorship.

Deepest affection is also due to my friends, especially, Rattana, Eddie Chia, Kah Fai, Narcisse, Azira, Kie Hie, Yong, Goh, Kam Yee, Gracie, Chare Li, Shamala and Chuan Loo, who have helped and supported me spiritually! I have gained a lot of invaluable scientific skills and advices from them to work my very best throughout my Ph.D. candidature. I enjoyed doing experiments with them very much!

Finally, my earnest love is to my caring Papa and Mama, my sister and brother in law, and my little brother for their sacrifices, supports, encouragements and love. Special thanks to my best friends, June, Alex, Mei Ling, Mei Fun and Ka Heng. I could not have done this without their constant care and support.



I certify that an Examination Committee has met on **August 2012** to conduct the final examination of **Lee Khai Wooi** on his degree thesis entitled “**Hepatitis B capsid as a delivery vehicle into mammalian cells**” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The committee recommends that the student be awarded the Degree of Doctor of Philosophy.

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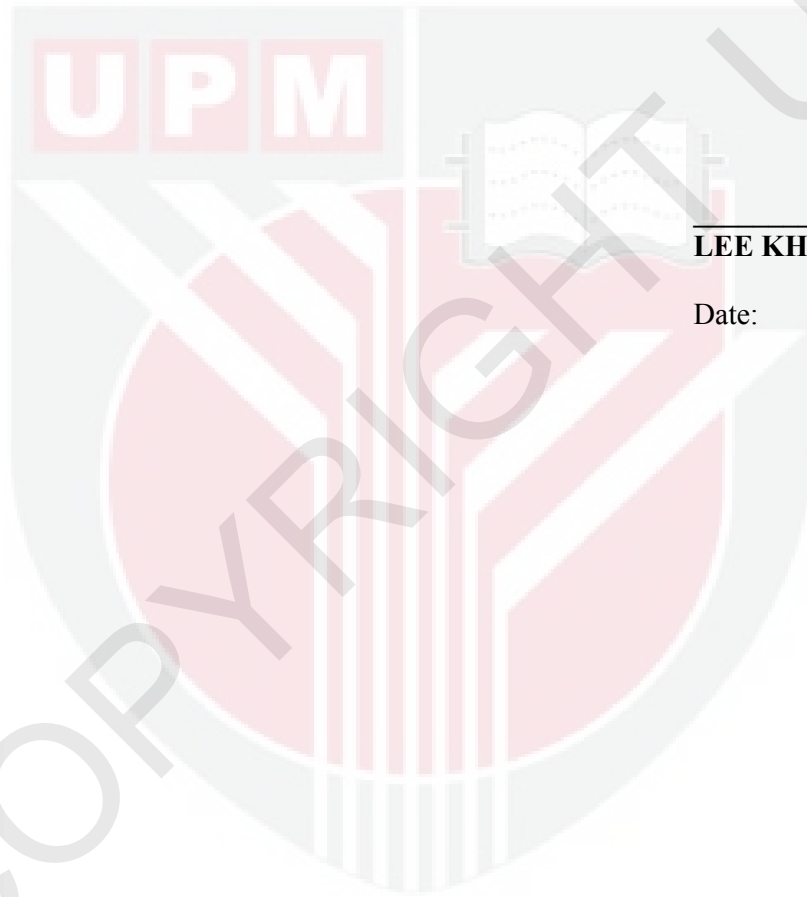
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## DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.



**LEE KHAI WOUI**

Date:

## TABLE OF CONTENTS

	<b>Page</b>
<b>ABSTRACT</b>	ii
<b>ABSTRAK</b>	v
<b>ACKNOWLEDGEMENTS</b>	ix
<b>APPROVAL</b>	xi
<b>DECLARATION</b>	xiii
<b>LIST OF TABLES</b>	xviii
<b>LIST OF FIGURES</b>	xix
<b>LIST OF ABBREVIATIONS</b>	xxii
<b>CHAPTER</b>	
<b>1 INTRODUCTION</b>	<b>1</b>
<b>2 LITERATURE REVIEW</b>	
2.1 Hepatitis B virus (HBV)	6
2.1.1 Hepatitis B core antigen (HBcAg)	8
2.1.2 Hepatitis B surface antigen (HBsAg)	12
2.2 Green fluorescent protein (GFP)	14
2.3 Effects of protein denaturants on protein folding	16
2.4. Dynamic light scattering (DLS)	17
2.5. Phage display technology	20
2.5.1. Selection of cell-targeting ligand	21
2.5.2. Selection of HBV capsid binding ligand	22
2.6 Chemical cross-linking	23
2.7. The potential of HBV capsid to serve as a universal nanocarrier	24
2.7.1. Platform for antigen presentation via genetic modification	25
2.7.2. Surface chemistry and its potential for chemical modification	30
2.7.3. Application of viral nanoparticles, viral and bacterial vectors as a cell delivery system	32
<b>3 GENERAL MATERIALS AND METHODS</b>	
3.1 Materials	35
3.1.1 Media, buffers and solutions	35
3.1.2 Bacteria strains, plasmids and vectors information	37
3.2 General methods	
3.2.1 Protein expression and protein extraction	38
3.2.1.1 The production of tHBcAg	38
3.2.1.2 Cell disruption	39



3.2.2	Protein purification	39
3.2.2.1	Sucrose density gradient ultracentrifugation	39
3.2.2.2	Immobilised nickel affinity chromatography (IMAC; HisTrap™ HP/FF)	40
3.2.3	Analytical procedure	40
3.2.3.1	Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)	41
3.2.3.2	Western blot analysis	42
3.2.3.3	The Bradford assay	42
3.2.3.4	Protein purity determination	43
3.2.3.5	Transmission electron microscopy (TEM)	43
3.2.3.6	Native agarose gel electrophoresis	44
3.2.3.7	Enzyme-linked Immunosorbent Assay (ELISA)	44
3.2.4	Molecular cloning techniques	45
3.2.4.1	Plasmid extraction (alkaline lysis method)	45
3.2.4.2	Quantitation of DNA	46
3.2.4.3	Digestion of insert and vector with restriction Endonucleases	47
3.2.4.4	Ligation	47
3.2.4.5	Competent cells preparation	48
3.2.4.6	Transformation	48
3.2.4.7	DNA sequencing	49
3.2.5	Fluorescein labelling of the tHBcAg	49
3.2.6	General techniques for mammalian tissue culture	50
3.2.6.1	Thawing the cells	50
3.2.6.2	Subculturing the cells	51
3.2.6.3	Preserving the cells	51
3.2.6.4	Counting the cells	52

#### 4

### **RECOMBINANT HEPATITIS B VIRUS CORE NANOPARTICLES: ASSOCIATION, DISSOCIATION AND ENCAPSIDATION OF GREEN FLUORESCENT PROTEIN**

4.1	Introduction	53
4.2	Materials and methods	
4.2.1	Expression and purification of tHBcAg	55
4.2.2	Expression and purification of green	55

	fluorescent protein (GFP)	
4.2.3.	Dynamic light scattering (DLS) analysis	56
4.2.4	Dissociation of tHBcAg nanoparticles	57
4.2.5	Association of tHBcAg nanoparticles	57
4.2.6	Stability of tHBcAg nanoparticles at different temperatures	58
4.2.7	Encapsulation of green fluorescent protein by tHBcAg nanoparticles	58
4.3	Results	
4.3.1	The effect of GdnHCl on tHBcAg nanoparticles	60
4.3.2	Dissociation and association of tHBcAg nanoparticles in urea	63
4.3.3	Heat treatment on tHBcAg nanoparticles	66
4.3.4	Encapsulation of GFP by tHBcAg nanoparticles	67
4.4	Discussion and conclusions	73
<b>5</b>	<b>DELIVERY OF CHIMERIC HEPATITIS B CORE NANOPARTICLES INTO LIVER CELLS</b>	
5.1	Introduction	77
5.2	Materials and methods	
5.2.1	Construction of recombinant plasmids encoding the preS1His <sub>6</sub> HBcAg	78
5.2.2	Expression and purification of tHBcAg	81
5.2.3	Expression and purification of preS1His <sub>6</sub> HBcAg	81
5.2.4	Incorporation of the preS1His <sub>6</sub> HBcAg into tHBcAg nanoparticles	82
5.2.5	Western blot analysis of the preS1His <sub>6</sub> HBcAg+tHBcAg chimeric particles	83
5.2.6	Enzyme-linked Immunosorbent Assay	84
5.2.7	Transmission electron microscopy (TEM)	85
5.2.8	Colloidal gold immunoelectron microscopy	85
5.2.9	Fluorescein labeling of the tHBcAg	85
5.2.10	Incorporation of preS1His <sub>6</sub> HBcAg into FtHBcAg nanoparticles	86
5.2.11	Fluorescence microscopy	86
5.3	Results	
5.3.1	Construction and expression of preS1His <sub>6</sub> HBcAg	87
5.3.2	Incorporation of the preS1His <sub>6</sub> HBcAg into tHBcAg nanoparticles	92
5.3.3	Liver cells translocation assay	97
5.4	Discussion and conclusions	101

<b>6</b>	<b>NANO-GLUE: AN ALTERNATIVE WAY TO DISPLAY CELL-INTERNALISING PEPTIDE (CIP) AT THE SPIKES OF HBV CORE NANOPARTICLES FOR CELL-TARGETING DELIVERY</b>	
6.1	Introduction	105
6.2	Materials and methods	
6.2.1	Selection of cell-internalising peptides	107
6.2.2	Immuno-fluorescence microscopy	108
6.2.3	Peptide synthesis	109
6.2.4	Preparation of tHBcAg nanoparticles	110
6.2.5	Chemical cross-linking of KLHISKDHIYPTGGGSLLGRMKGA peptide to tHBcAg nanoparticles	110
6.2.6	Chemical cross-linking of CIP (KLHISKDHIYPT) to tHBcAg nanoparticles	111
6.2.7	Transmission electron microscopy (TEM)	111
6.2.8	Fluorescein labeling of the tHBcAg nanoparticles	111
6.2.9	Delivery of fluorescein labeled tHBcAg (FtHBcAg) nanoparticles into HeLa cells	112
6.2.10	Delivery of fluorescein labelled oligonucleotides by tHBcAg nanoparticles	112
6.3	Results	
6.3.1	Selection of HeLa cell-internalising peptides	113
6.3.2	Cell-internalising activity of free peptide	117
6.3.3	Chemical cross-linking of CIP via nano-glue	118
6.3.4	Conjugation of fluorescein and JG24 peptide on tHBcAg nanoparticles	122
6.3.5	Delivery of fluorescent tHBcAg (FtHBcAg) nanoparticles into HeLa cells	123
6.3.6	Packaging and delivery of fluorescent oligonucleotides into cells	124
6.4	Discussion and conclusions	126
<b>7</b>	<b>GENERAL DISCUSSION AND RECOMENDATIONS FOR FUTURE WORK</b>	<b>130</b>
7.1	General discussion	130
7.2	Recommendations for future work	135
	<b>REFERENCES</b>	<b>136</b>
	<b>APPENDICES</b>	<b>154</b>
	<b>BIODATA OF STUDENT</b>	<b>161</b>
	<b>LIST OF PUBLICATIONS</b>	<b>162</b>