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SYNTHESIS AND *IN VITRO* DELIVERY OF PLASMID-ENCAPSULATED CHITOSAN NANOPARTICLES IN HUMAN KIDNEY CANCER CELLS AS A VECTOR FOR GENE DELIVERY

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Synthesis and *in vitro* delivery of plasmid-encapsulated chitosan nanoparticles in human kidney cancer cells as a vector for gene delivery

By

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Chitosan has been extensively adopted in biomedical and pharmaceutical research due to its advantageous properties such as biocompatibility, low-toxicity, and biodegradability. These properties suggest chitosan as a robust material for numerous biomedical applications as gene and drug delivery systems. In this study, the synthesis and *in vitro* delivery of plasmid-encapsulated chitosan nanoparticles in human kidney cancer cells (786-O cell line) were reported. A modified ionic gelation system was utilized to synthesize spherical nanoparticles, and its ability for *in vitro* delivery of a plasmid containing a GFP reporter gene was evaluated. CNPs were characterized using Dynamic

Light Scattering to analyze size distribution and polydispersity index, while Field Emission-Scanning Electron Microscopy was used to analyze surface morphology of the nanoparticles. The 786-O human kidney cancer cell line was then established and treated with CNP-pEGFP formulated at different incubation timepoints. Transfection of 786-O cells were performed by using blank media, CNP and plasmid alone and visualized under both bright-field and fluorescent microscope. FESEM images was found to correlate with DLS data, where the optimum nanoparticle size and PDI value was obtained at a chitosan: TPP ratio of 3:1. Transfection results showed that CNP and cells did not readily fluoresce, and successful GFP expression in cells were only achieved with CNP encapsulation. The results of cell transfection at two different time points showed positive GFP expression, suggesting that CNP was potentially an effective delivery system. However, optimizations are required to further increase efficiency of the gene delivery system for subsequent utilization in biomedical and pharmaceutical fields.

Keywords: Nanobiotechnology, CNPs, CNP-pEGFP, gene delivery system

Abstrak tesis yang dikemukakan kepada Jabatan Biology Sel dan Molekul Sebagai memenuhi keperluan untuk ijazah Biologi Sel dan Molekul

SINTESIS DAN PENYAMPAIAN PLASMID-DIKAPSULKAN NANOPARTIKEL KITOSAN *IN VITRO* DALAM SEL-SEL KANSER BUAH PINGGANG MANUSIA SEBAGAI VEKTOR UNTUK PENGHANTARAN GEN

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Kitosan telah banyak diguna pakai dalam penyelidikan bioperubatan dan farmaseutikal kerana ciri berfaedah seperti biocompatibility, ketoksikan rendah, dan biodegredasi. Ciri-ciri ini mencadangkan kitosan sebagai bahan mantap bagi pelbagai aplikasi bioperubatan sebagai system penghantaran gen dan ubatan. Dalam kajian ini, sintesis dan penyampaian *in vitro* plasmid-dikapsulkan nanopartikel kitosan dalam sel-sel kanser buah pinggang manusia (sel 786-O) dilaporkan. Sistem penggelan ionic yang diubahsuai telah digunakan untuk mensintesis nanopartikel sfera, dan keupayaannya untuk

penghantaran *in vitro* plasmid yang mengandungi gen wartawan GFP telah dinilai. CNPs telah dicirikan dengan menggunakan penyerakan cahaya dinamik untuk menganalisis taburan saiz dan indeks polydispersity, manakala mikroskop elektron pengimbas pancaran medan (field emission scanning electron microscope, FESEM) digunakan untuk menganalisis morfologi permukaan nanopartikel. Pertumbuhan sel kanser buah pinggang manusia (sel 786-O) kemudiannya ditubuhkan dan dirawat dengan CNP-pEGFP yang dirumuskan dengan masa inkubasi yang berbeza. Transfection daripada sel-sel 786-O telah dilakukan dengan menggunakan media kosong, CNP dan plasmid sahaja dan digambarkan di bawah mikroskop medan cahaya and mikroskop pendarfluor. Imej FESEM didapati korelasi dengan data DLS, di mana saiz nanopartikel dan PDI yang optimum telah diperolehi pada nisbah kitosan dan TPP 3:1. Keputusan transfection menunjukkan CNP dan sel-sel tidak berkilau sendiri dan ungkapan GFP dalam sel-sel hanya akan dicapai dengan CNP pengkapsulan. Keputusan transfection sel pada dua titik masa yang berbeza menunjukkan ungkapan GFP positif, menunjukkan bahawa CNP adalah berpotensi dalam sistem penyampaian yang berkesan. Walau bagaimanapun, pengoptimuman dikehendaki meningkatkan lagi kecekapan dalam sistem penyampaian gen untuk penggunaan seterusnya dalam bidang bio-perubatan dan farmaseutikal.

kata kunci: Nanobioteknologi, CNPs, CNP-pEGFP, sistem penyampaian gen

Approval

This thesis was submitted to the Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia and has been accepted as fulfllment for the Bachelor of Science (HONS.) Cell and Molecular Biology. The member of the Supervisory Committee was as follows:

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DECLARATION

Declaration by undergraduate student

I hereby confirm that:

- This thesis is my original work;
- Quotations, ilustrations and citations have been duly referenced;
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LIST OF ABBREVIATIONS AND SYMBOLS

A	-absorbance
B	- Beta
o	-degree
C	-Celcius
CO_2	-carbon dioxide
CS	-chitosan
CNP/s	-chitosan nanoparticle/s
DLS	-dynamic light scattering
DNA	-deoxyribonucleic acid
EPR	-enhanced permeability effect
FE-SEM	-field emission-scanning electron microscopy
FITC	-fluorescein isothiocyanate
g	-grams
h	-hour(s)
HCl	-hydrochloric acid
l	-litre
m	-metre
mg	-milligram
min	-minute(s)
ml	-mililitre
NaOH	-sodium hydroxide

nm	-nanometer
PBS	-phosphate buffer saline
PDI	-polydispersity index
PEGFP	-plasmid enhanced green fluorescence protein
PSD	-particle size distribution
RNA	- Ribonucleic acid
rpm	-revolutions per minute
RSV	-respiratory syncytial virus
s	-second(s)
siRNA	-small interfering RNA
TPP	-(sodium) tripolyphosphate
UV	-ultraviolet
%	-percentage
µ	-micro

Chapter 1

Introduction

1.1 Introduction and research background

Over the last decades, radiation, chemotherapy and surgery have become important methods to treat cancer patients. Among these methods, chemotherapy remains the most significant treatment, with anthracyclines being the most potent chemotherapeutic drugs used (Chalasani *et al.*, 2013). The main obstacle faced by current treatment is that the drugs are unable to be delivered efficiently to cancer cells and remain circulated throughout the body. Thus, the treatment often lead to side effects in the patients due to non-specific interaction of the drugs with non-cancerous cells in the body. This has become a constraint for the treatment because the dosage of anthracyclines is limited to a lifetime dose. Due to this reason, the emergence of nanotechnology has arose to enhance the effectiveness of anthracycline drug delivery for the cancer treatment (Masarudin , 2012).

Nanotechnology can be defined as the construction and utilization of devices, materials and systems through the control of matter on the nanometer length scale, which is equal to 1 billionth of a meter. The utilizations of nanotechnology in life sciences is termed as nanobiotechnology (Jain, 2005). Among the application of nanobiotechnology, nanoparticle for drug delivery and biomedical applications are the most significant tools for medical purposes. Since the scale of nanoparticles are relatively small, they can be utilized for the enhanced permeability and retention effect (EPR) of tumor cells to solve

the problem of lack of tumor selectivity for the chemotherapeutic drugs. The nanoparticles are able to exhibit more than 10 to 200 times higher concentrations drugs in tumor cells than that in normal tissues, such as kidney, muscle and heart (Greish, 2010). It means that the nanoparticles help to increase the accumulation of drugs in the tumor tissues.

Basically, nanoparticles can be divided into two categories, which is engineered and nonengineered nanoparticles. Engineered nanoparticles are not naturally existed and invented purposely for application whereas non-engineered nanoparticles are naturally produced (Scalf and West, 2006). Chitosan is one of the appropriate material to be utilized as nanoparticle delivery vector. It is a natural linear polysaccharide encompasses an unbranched chain consisting of β-(1-4)-2-amino-2-deoxy-D-glucopyranose and is formed during the deacetylation of chitin in an alkaline condition. Besides that, this exclusive polysaccharide also been studied because of its biodegradability, biological process, biocompatibility and bioactivity. These characteristics become more popular because do not cause any harmful side-effect to our body as compared with heavy-metal synthesized nanoparticles. In addition, the parameter and payload parameter that we employed will greatly affect the successfulness of the implementation of chitosan nanoparticles. It will be the key for the synthesis of best nanoparticle size. The chitosan nanoparticles can be synthesized by the interaction of amino groups of chitosan backbone with sodium tripolyphosphate (TPP). TPP is a cross-linking agent where this ionic cross-linking with chitosan is beneficial because it is relatively simple and usually made under mild conditions without using organic solvents.

In this thesis, the synthesis and *in vitro* delivery of plasmid-encapsulated chitosan nanoparticles in cells as DNA vector candidates will be studied. The best parameter to

synthesis nanoparticles need to be chosen. Besides that, the ability of delivery of plasmid-encapsulated chitosan nanoparticles in cells as DNA vector candidates is evaluated. Chapter 2 will comprises of the comprehensive literature review of the synthesis of nanoparticles and the delivery system of plasmid-encapsulated nanoparticles in cells. Chapter 3 will includes the methodology for nanoparticles synthesis, the protocol of analysis and delivery of nanoparticles to tumor cells and also the characterization assays. Chapter 4 will covers the results and discussion for the research. Finally, the chapter 5 will conclude the whole research in a nut shell.

1.2 Objectives

The work described in this thesis was designed to achieve the following objectives:

1. To synthesize chitosan nanoparticles by ionic gelation routes using chitosan (CS) and sodium triphosphate, TPP.
2. To encapsulate plasmid DNA into the synthesized chitosan nanoparticles, CNP.
3. To effectively deliver the encapsulated plasmid into human cancer cells.

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