UNIVERSITI PUTRA MALAYSIA

ASSESSING THE *IN VITRO* CYTOTOXICITY OF SYNTHESIZED CHITOSAN NANOPARTICLES AGAINST DIFFERENT ORGANIC AND INORGANIC NANOMATERIALS IN HUMAN KIDNEY CANCER CELLS

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By

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ASSESSING THE *IN VITRO* CYTOTOXICITY OF SYNTHESIZED CHITOSAN NANOPARTICLES AGAINST DIFFERENT ORGANIC AND INORGANIC NANOMATERIALS IN HUMAN KIDNEY CANCER CELLS

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One of the biggest concerns regarding the use of nanomaterials for biological and medical applications is its toxicity. A simple way to evaluate nanomaterials' toxicity is by conducting *in vitro* cytoxicity assays. In this study, the synthesis of chitosan nanoparticles (CNP) and the colorimetric MTT assay of CNP along with various organic and inorganic nanomaterials were explored. CNP were synthesised via ionic gelation routes and particle size distribution were analysed using dynamic light scattering (DLS) supplemented with field-emission scanning electron microscopy (FE-SEM) imaging. The 786-O human kidney cancer cell lines were established and treated with various concentrations of CNP, carbon nanotubes (CNT), layered double hydroxides (LDH), solid lipid nanoparticles (SLN), and iron oxide nanoparticles for MTT assay. The morphologies of cells treated with each nanomaterial were also observed. DLS analysis showed that nanoparticles with average size of 67.70 nm were obtained using a formulation of 600 µl of 0.5 mg/ml chitosan solution and 250 µl of 0.7 mg/ml tripolyphosphate (TPP) solution (CNP-F_3) and was further supported by FE-

SEM results. Results from MTT assay showed that cells treated with 1 mg/ml SLN and CNP-F_3 gave the highest cell viability of 49.38% and 39.72%, respectively. Cells treated with 1 mg/ml CNT gave the lowest cell viability of 31.54%. These results were consistent with the observations made on cell morphologies, implying that both organic CNP and SLN were the least toxic. The inorganic nanomaterials tend to be more toxic, with CNT being the most toxic.

Keywords: chitosan nanoparticles, nanobiotechnology, cytotoxicity, MTT assay

Abstrak tesis yang dikemukakan kepada Jabatan Biologi Sel dan Molekul sebagai memenuhi keperluan untuk Ijazah Sarjana Muda Sains (Kepujian) Biologi Sel dan Molekul.

ASSESSING THE *IN VITRO* CYTOTOXICITY OF SYNTHESIZED CHITOSAN NANOPARTICLES AGAINST DIFFERENT ORGANIC AND INORGANIC NANOMATERIALS IN HUMAN KIDNEY CANCER CELLS

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Salah satu kebimbangan dalam penggunaan bahan nano bagi aplikasi dalam bidang biologi dan perubatan adalah ketoksikannya. Satu cara mudah untuk menilai ketoksikan bahan nano adalah dengan melakukan ujian kesitotoksikan *in vitro*. Dalam kajian ini, sintesis dan ujian kolorimetrik MTT bagi partikel nano kitosan (CNP) bersama-sama dengan pelbagai bahan nano organik dan tidak organik telah diteliti. CNP disintesiskan menggunakan kaedah penggelan ion dan distribusi ukuran partikel dianalisis melalui penaburan cahaya dinamik (DLS) disokong dengan analisis pengimejan pancaran medan mikroskopi electron pengimbasan (FE-SEM). Bagi ujian MTT, sel kanser buah pinggang manusia titisan sel 786-O ditetapkan dan dirawati dengan CNP, tiub nano karbon (CNT), hidroksida berganda berlapis (LDH), partikel nano pepejal lipid (SLN), dan partikel nano oksida besi. Morfologi sel yang dirawat dengan setiap bahan nano juga turut diperhatikan. Analisis DLS menunjukkan bahawa partikel nano dengan purata saiz 67.70 nm diperolehi menggunakan formulasi yang mengandungi 600 µl 0.5 mg/ml larutan kitosan dan 250 µl 0.7 mg/ml larutan TPP (CNP-F_3)

dan disokong dengan hasil FE-SEM. Hasil daripada ujian MTT menunjukkan bahawa sel yang dirawat dengan 1 mg/ml SLN dan CNP-F_3 masing-masing memberikan nilai kebolehhidupan sel sebanyak 49.38% dan 39.72%. Sel yang dirawat dengan 1 mg/ml CNT memberikan nilai kebolehhidupan sel paling rendah iaitu 31.54%. Hasil kajian ini konsisten dengan pemerhatian yang dilakukan terhadap morfologi sel, mengimplikasikan bahawa kedua-dua CNP dan SLN yang organik adalah paling kurang toksik. Bahan nano inorganik cenderung untuk bersifat lebih toksik, dan CNT merupakan yang paling toksik.

Kata kunci: partikel nano kitosan, bioteknologi nano, kesitotoksikan, ujian MTT.

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APPROVAL

This thesis was submitted to the Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences and has been accepted as fulfillment of the requirement for the degree of Bachelor of Science (Honours) 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;
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This is to confirm that:

- the research conducted and the writing of this thesis was under supervision.

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Dr. Mas Jaffri Masarudin

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LIST OF ABBREVIATIONS

A	- absorbance
CNP	- chitosan nanoparticles
CNT	- carbon nanotubes
ºC	- degree Celsius
CO_2	- carbon dioxide
DLS	- dynamic light scattering
DMSO	- dimethyl sulfoxide
FBS	- fetal bovine serum
FE-SEM	- field emission-scanning electron microscopy
g	- gram
h	- hour
HCl	- hydrochloric acid
LDH	- layered double hydroxides
min	- minute(s)
mg	- milligram
ml	- millilitre
MTT	- methyl thiazol tetrazolium bromide
MWCNT	- multi-walled carbon nanotubes
NaOH	- sodium hydroxide
NH_2	- amino group
nm	- nanometre
PBS	- phosphate buffered saline
PDI	- polydispersity index
ROS	- reactive oxygen species
rpm	- revolutions per minute
RPMI	- Roswell Park Memorial Institute (media)
SDS	- sodium dodecyl sulphate
SLN	- solid lipid nanoparticles
SWCNT	- single-walled carbon nanotubes
TPP	- (sodium) tripolyphosphate

LIST OF SYMBOLS

%	- percentage
µ	- micro

CHAPTER 1

INTRODUCTION

1.1 Research background

Over the last few years, nanotechnology has started a new revolution with its contribution towards the development of nanomaterials with potential applications across the fields of science, engineering, environment, as well as medicine. Materials at nano scales have been characterized as having unique chemical and physical properties, and are highly sought due to its extremely small size. One method towards classifying nanomaterials is based on whether they are of an organic or inorganic composition. Organic nanomaterials are generally derived from the synthesis using natural and biologically available organic resources. Examples of nanomaterials that fall under this category include dendrimers, lipid-based nanoparticles, and biopolymer-based nanoparticles. In contrast, inorganic nanomaterials are often not derived from organic molecules, but instead from other resources such as metals and carbons (Dutta *et al.,* 2013).

Many of these nanomaterials are being investigated for applications in biological and medical sciences mainly due to their unique characteristics and the ability to function as a delivery vector or carriers of other beneficial molecules including drugs and nucleic acids (De Jong & Borm, 2008). Among the various nanomaterials available, chitosan nanoparticles have garnered heightened interest, mostly for its organic, inert properties. Since it is derived from the polysaccharide polymer chitin, chitosan is readily biodegradable and has low toxicity (Hirano, 1999). However, studying the suitability of nanomaterials application for medical use is obstructed by a lot of uncertainties that surround the issue of nanoparticles' toxicity. Despite the intensive studies being conducted, scientific research and relevant data

currently available are still lacking information on the accurate toxicity of nanomaterials. Further complications arise from the vast amount of nanomaterials available which are unique and distinct from each other, making it difficult to make a direct comparison. Therefore, to overcome this problem, a standardized assay is required to evaluate the toxicity of nanomaterials under a controlled condition.

In this study, chitosan nanoparticles were synthesized based on different parameters to obtain ideally optimum nano-sized particles. Then, in an attempt to achieve a general idea on the cytotoxicity of chitosan nanoparticles in comparison with other organic and inorganic nanomaterials, a cell viability assay was conducted. To further assess the effects of these nanomaterials on cells, a study on the cell morphologies were done. Chapter 2 consists of a review on available literatures that are closely-related to this study. In Chapter 3, the methods used to synthesize chitosan nanoparticles and conduct cell viability assay were thoroughly described. Chapter 4 discusses the results obtained throughout this study and Chapter 5 concludes the outcome of this study and outlines the future work which may be directed from this study.

1.2 Objectives

The objectives of this study are;

1. To synthesize chitosan nanoparticles by ionic gelation method.
2. To evaluate the cytotoxicity of chitosan nanoparticles against different organic and inorganic nanomaterials in 786-O human kidney cancer cell line.
3. To analyze the morphological characteristics of 786-O human kidney cancer cells following treatment with chitosan nanoparticles against different nanomaterials.

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