UNIVERSITI PUTRA MALAYSIA

SYNTHESIS, CHARACTERIZATION AND TOXICITY OF INTERCALATED ZINC-LAYERED MATERIAL FOR NANODELIVERY OF SALICYLATE AS AN ANTI-INFLAMMATORY AGENT

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By

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February 2013

Chair: Professor Mohd. Zobir Hussein, PhD

Faculty: Institute of Advanced Technology

The existing organic based-vectors employed for delivery of therapeutic molecules into cells suffer from a number of setbacks ranging from delivery efficiency to severe toxicity. Through fabrication of novel nano-sized inorganic delivery vectors, problems associated with cellular delivery systems are gradually being addressed. The use of layered materials as carriers of a multitude of therapeutic compounds has garnered much interest, particularly due to their ease of synthesis, rich functionality and good biocompatibility. This study sought to develop a new organic-inorganic nanohybrids based on layered double hydroxides (LDH) and zinc layered hydroxides (ZLH) which contains an anti-inflammatory agent, salicylate (SA) and to test the effect of these nanohybrids on viability of cells. The co-precipitation route was utilized for synthesis of Zn/Al LDH containing SA while direct reaction of

aqueous SA with commercial zinc oxide was used for synthesis of ZLH-SA nanohybrids.

Intercalation of SA into LDH and ZLH was confirmed by expansions of the basal spacing recorded in the powder X-ray diffraction analysis (PXRD). The basal spacing for SA-LDH nanohybrid was 15.34 Å, with the SA anions orientated as a tilted bilayer. In SA-ZLH nanohybrid, intercalation resulted in a basal spacing of 15.73 Å, with the SA anions arranged as a monolayer at an angle of 57°. Fourier transform infrared (FTIR) spectra revealed the presence of functional groups characteristic of both the host (LDH/ZLH) and SA in the synthesized nanohybrids. Occupation of SA into LDH and ZLH intergallery was further supported by the CHNS data, in which the anion loading percentage was calculated to be 27.75 % for the former and 29.66 % for the latter. The resulting nanohybrids were further characterized using inductively coupled plasma-atomic emission spectroscopy (ICP-AES), thermogravimetric analyzer, analysis of surface area and porosity (ASAP) and field emission scanning electron microscopy (FESEM).

Methyltriazoltetrazolium (MTT) assay showed that Zn/Al LDH-SA nanohybrids were less toxic than its LDH, with an IC_{50} value of 0.737 mg/ml for the former and 0.420 mg/ml for the latter. On the contrary, ZLH-SA nanohybrids (IC_{50} = 0.052 mg/ml) were more toxic than the free zinc oxide (IC_{50} = 0.942mg/ml). Based on these findings, it was concluded that LDH show an immense potential to be further developed into alternative cellular delivery vectors while ZLH requires an extensive research in order to better ascertain its application in a drug delivery system.

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

SINTESIS, PENCIRIAN DAN KETOKSIKAN BAHAN ZINK BERLAPIS TERINTERKALASI UNTUK NANO-PENGHANTARAN SALISILAT SEBAGAI AGEN ANTI-RADANG

Oleh

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Penggunaan vektor organik yang sedia ada untuk penghantaran molekul-molekul terapeutik ke dalam sel mempunyai pelbagai kelemahan yang merangkumi keberkesanan penghantaran sehinggalah ketoksikan yang serius. Melalui pembuatan vektor bukan organik bersaiz nano, masalah berkaitan sistem penghantaran selular dapat di selesaikan secara berperingkat. Aplikasi bahan berlapis sebagai vector pembawa pelbagai sebatian terapeutik telah mendapat banyak perhatian kerana ia mudah disintesis dan kaya fungsi serta mempunyai biokompatibiliti yang baik. Kajian ini dilakukan untuk menghasilkan nanohibrid organik-bukan organik berasaskan hidroksida berlapis ganda (HBG) dan zink hidroksida berlapis (ZHB) yang mengandungi agen anti-radang yang dikenali sebagai salisilat (SA) dan juga untuk menguji kesan-kesan nanohibrid ini terhadap sel-sel hidup. Kaedah pemendakan telah digunakan untuk mensintesis HBG Zn/Al yang mengandungi SA

manakala tindak balas terus cecair akueus SA dengan zink oksida komersial telah digunakan untuk mensintesis nanohibrid ZHB-SA. Bagi menguji kesan sitotoksisiti nanohibrid yang dihasilkan, ujian metiltriazotetrazolium (MTT) telah dilakukan ke atas sel buah pinggang monyet hijau Afrika (Vero3).

Interkalasi SA ke dalam HBG dan ZHB telah disahkan melalui peningkatan jarak antara ruang seperti yang direkodkan oleh analisis pembelauan sinar-X. Jarak antara ruang bagi HBG-SA dicatatkan pada 15.34 Å manakala bagi ZHB-SA, jarak antara ruangnya adalah 15.73 Å. Molekul SA didapati tersusun secara bi-lapisan condong di dalam HBG-SA manakala di dalam ZHB-SA molekul SA diinterkalasi sebagai mono-lapisan pada posisi 57°. Spektrum FTIR menunjukkan kehadiran campuran kumpulan berfungsi perumah (HBG/ZHB) dan SA di dalam nanohibrid yang dihasilkan. Interkalasi SA ke dalam HBG dan ZHB juga telah dibuktikan oleh data dari analisis CHNS, di mana peratusan pemuatan anion adalah 27.75% bagi HBG-SA dan 29.66% bagi ZHB-SA. Kaedah-kaedah pencirian lain seperti ICP-AES, penganalisis termogravimetri, ASAP dan FESEM juga telah dilakukan ke atas nanohibrid yang dihasilkan.

Ujian sitotoksisiti ke atas sel Vero3 menunjukkan bahawa nanohibrid HBG Zn/Al-SA adalah kurang toksik daripada HBG, dengan nilai IC_{50} sebanyak 0.737 mg/ml bagi HBG Zn/AL-SA dan 0.420 mg/ml bagi HBG. Sebaliknya, nanohibrid ZHB-SA (IC_{50} = 0.052 mg/ml) didapati adalah lebih toksik berbanding zink oksida (IC_{50} = 0.942mg/ml). Berdasarkan penemuan ini, maka dapat disimpulkan bahawa HBG telah menunjukkan potensi yang besar untuk dibangunkan menjadi vektor

penghantaran selular alternatif manakala ZHB memerlukan penyelidikan yang lebih menyeluruh bagi menentukan potensinya untuk digunapakai bagi sistem penghantaran ubat-ubatan.

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I certify that a Thesis Examination Committee has met on 6 February 2013 to conduct the final examination of Munirah binti Ramli on her thesis entitled "Synthesis, Characterization and Toxicity of Intercalated Zinc-Layered Material for Nanodelivery of Salicylate as an Anti-Inflammatory Agent" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

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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:

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Date:

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.

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MUNIRAH BINTI RAMLI

Date: 6 February 2013

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LIST OF ABBREVIATIONS

5-fu	5-fluorouracil
ASAP	Analysis of surface area and porosity
ATCC	American Type Culture Collection
BET	Breuneur, Emmet and Teller
BJH	Barret, Joyner, Hallenda
CHNS	Carbon, Hydrogen, Nitrogen and Sulphur
CMV	Cytomegalovirus
CNT	Carbon nanotube
DMEM	Dulbecco's Modified Eagle's Medium
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
EDTA	Ethylene diamine tetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
FBS	Fetal bovine serum
FESEM	Field emission scanning electron microscope
FTIR	Fourier transform infrared
Gd-DTPA	Gadolinium-diethylene triamine pentaacetic acid
GFP	Green fluorescent protein
IC_{50}	Inhibition concentration at 50% cell viability
ICP-AES	Inductively coupled plasma-atomic emission spectroscopy
IUPAC	International Union of Pure and Applied Chemistry
LDH	Layered double hydroxide
LHS	Layered hydroxide salt
MRI	Magnetic resonance imaging

MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrasodium bromide
MTX	Methotrexate
NSAIDs	Non-steroidal anti-inflammatory drugs
PBS	Phosphate buffered saline
PVA	Polyvinyl alcohol
PXRD	Powder X-ray diffraction
R	Ratio
Rpm	Revolution per minute
SA	Salicylic acid
SAZAL	Zn/Al-NO_3 LDH-SA nanohybrid
SZN	ZLH-SA nanohybrid
TGA-DTG	Thermogravimetric-differential thermogravimetric analysis
ZAL	Zn/Al-NO_3 layered double hydroxide
ZLH	Zinc layered hydroxide

CHAPTER 1

INTRODUCTION

Modern day researchers are constantly experimenting for the best way to tackle a particular scientific problem. While one discipline of study might be able to provide an answer, merging several disciplines together may be more advantageous towards solving the problem, as it allows the issue in question to be investigated from a broader range of perspectives. One of the interdisciplinary fields of study that is worth mentioning is nanobiotechnology, in which nanotechnology; the science of materials of one-billionth of a meter is integrated with biology, chemistry, physical sciences, molecular engineering and biotechnology (Jain, 2012).

Nanotechnology encompasses of fabrication of materials through the control of matter on the nanometer scale (Jain, 2005). Development of nanomaterials like polymeric nanoparticles, dendrimers, nanoshells, magnetic nanoparticles as well as clay minerals and layered double hydroxides for applications in a multitude of industries is progressing at a fast pace due to the increasing need to improve the existing products and systems. In nanobiotechnology, many efforts have been directed towards development of novel nanovehicles for cellular delivery of therapeutics. Collectively termed as inorganic nanoparticles, their usage in drug delivery is garnering considerable interest due to their biocompatibility, biostability (Choy *et al.*, 2008; Choy *et al.*, 2007; Xu *et al.*, 2006b) and readiness to be

functionalized and tailored for specific properties (Choy *et al.*, 2008; Xu *et al.*, 2006b).

Delivery of drugs and other therapeutic compounds like DNA and peptides are currently possible through the use of organic-based vectors such as viruses, cationic lipids and polypeptides. Although viruses are deemed the most effective vectors, their application in clinical trials is suppressed because of costs, severe immunogenic reactions and failure to persist in the host cells (Thomas *et al.*, 2003; Xu *et al.*, 2006b). In addition, application of lipid-based vectors in *in vivo* studies is hampered by their tendency to aggregate in biological fluids (Bergen *et al.*, 2008) while peptide-based vectors may present immunological problems (Luo and Saltzman, 2000). To overcome these impeding factors, development of new and effective delivery vector systems is highly anticipated.

Layered compounds like layered double hydroxides (LDH) and layered hydroxide salts (LHS) are a type of material that can carry negatively-charged moieties within its inorganic layers while maintaining its structural stability (Arizaga *et al.*, 2007). Due to this unique attribute, these compounds may serve as a versatile vector for cellular delivery. To date, these nano-sized materials have been used to contain many bioactive compounds like DNA fragments (Desigaux *et al.*, 2006), functional gene (Tyner *et al.*, 2004), plasmid DNA (Masarudin *et al.*, 2009; Xu *et al.*, 2007b), amino acids (Md Ajat *et al.*, 2008; Aisawa *et al.*, 2006), antibiotics (Silion *et al.*, 2008; Tammaro *et al.*, 2007; Trikeriotis and Ghanotakis, 2007) and various drugs

like methotrexate (Oh *et al.*, 2006), 5-fluorouracil (Choi *et al.*, 2008) and naproxen (del Arco *et al.*, 2004b).

Development of layered material-based nanohybrids for cellular delivery systems are gaining increased attention because of the capability of the vector to protect the therapeutic compounds against physicochemical and biological degradation (Masarudin *et al.*, 2009; Choy *et al.*, 2008) as well as to improve delivery efficiency (Kwak *et al.*, 2004; Choy *et al.*, 2001) and to allow the intercalated compound to be released in a controlled manner (Hussein Al Ali *et al.*, 2011; Perioli *et al.*, 2011; Masarudin *et al.*, 2009). In addition, nanohybrid synthesized from layered material (Hussein *et al.*, 2011) and the layered material itself have exhibited low toxicity on cells (Masarudin *et al.*, 2009; Kwak *et al.*, 2004), an attribute that is very crucial in developing an efficient cellular delivery system.

Salicylic acid, also known as 2-hydroxybenzoic acid, is an active ingredient found in various keratolytic and anti-inflammatory medicines. Intercalation of this organic compound can perhaps alleviate gastric irritation resulted from its ingestion (Mackowiak, 2000) as well as improve its solubility, which is central to release, absorption and bioavailability of the compound (del Arco *et al.*, 2004b). Although intercalation of salicylic acid into LDH has been previously demonstrated (Silion *et al.*, 2009; del Arco *et al.*, 2004b; Tronto *et al.*, 2001), no literature has reported its intercalation into zinc layered hydroxide (ZLH). In addition, effect of the synthesized LDH based-nanohybrids on viability of cells has yet to be investigated.

The present study aimed to synthesize Zn/Al-NO_3 LDH and ZLH nanohybrids containing salicylic acid (SA) and investigate its effect on cell viability. Therefore the objectives set for this study are as follows:

1. to synthesize Zn/Al-NO_3 LDH hosts through co-precipitation method,
2. to intercalate salicylic acid into the synthesized Zn/Al-NO_3 LDH and ZLH hosts,
3. to characterize the hosts and the newly formed SA-LDH/ZLH nanohybrids, and
4. to evaluate cytotoxicity of the hosts and the nanohybrids towards African green monkey kidney (Vero3) cells.

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