UNIVERSITI PUTRA MALAYSIA

DEVELOPMENT OF COCKLE SHELL-BASED NANOCOMPOSITE BIOMATERIAL BONE PASTE AND ITS EFFECTIVENESS FOR BONE REPAIR IN RABBIT MODEL

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By

KH. NURUL ISLAM

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

January 2013

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DEDICATION

To my late parents who are in the garden of heaven for eternal peace

To my relatives

To my three sisters, two wives, four daughters and only one son Kh. Omar Faruque

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

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Chairman: **Professor Md. Zuki Bin Abu Bakar@ Zakaria, PhD**

Faculty: **Veterinary Medicine**

This study revealed the development of paste from cockle shell-based calcium carbonate nanoparticles and *in vivo* evaluation using a rabbit model. Calcium carbonate and its polymorphs from cockle shells (*Anadara granosa*) were characterized using variable pressure scanning electron microscope (VPSEM), a transmission electron microscope (TEM), an energy dispersive X- ray analyzer (EDXA), X-ray diffraction (XRD) and Fourier transmission infrared spectroscopy (FT-IR). Rod-like aragonite crystals of cockle shell powders were observed by both SEM and TEM. The EDXA results showed that the cockle shells contained more calcium and carbon than the commercial calcium carbonate. The FT-IR analyzes revealed the presence of carbonate groups in cockle shell powders. The FT-IR analyzes also showed the presence of aragonite in cockle shell powders. The FT-IR and XRD analyzes showed that the cockle shell powders contained aragonite. The

cockle shell powders were formed with good quality calcium carbonate and contained calcium carbonate in the aragonite phase.

The calcium carbonate aragonite nanoparticles were synthesized from micron sized cockle shell powders. The method involves a simple mechanical stirring of the micron-sized cockle shells powders in the presence of a non-toxic and non-hazardous biomineralization catalyst, dodecyl dimethyl betaine (BS-12). The method produced rod-shaped aragonite nanoparticles with the diameter of 20-30 nm with good reproducibility and without any additional impurities. This was confirmed by a combined analysis of variable pressure scanning electron microscopy (VPSEM), transmission electron microscopy (TEM), Fourier transmission infrared spectroscopy (FTIR), Thermogravimetric analyzer (TGA), X-ray powder diffractometer (XRD) and an energy dispersive X-ray analyzer (EDX). The method should find potential applications in the industry for large scale synthesis of aragonite nanoparticles at low cost from an abundant natural resource such as cockle shells. The calcium carbonate nanoparticles in the aragonite phase were synthesized from the cheap and naturally abundant cockle shells.

The pastes were developed from the cockle shell-based calcium carbonate nanoparticles. The composite pastes were used as bone repair in surgical applications. The cockle shell-based calcium carbonate nanoparticles were mixed with chitosan solution containing 2% acetic acid in a 250 ml glass beaker. The paste mixture was mixed using a multi-system hot plate mechanical stirrer with magnetic stirrer bar. The mixture clumped together and gave a homogeneous mesh like appearance. The paste was characterized by the Field Emission Scanning Electron

Microscopy (FESEM), an Energy Dispersive X-ray Analyzer (EDXA), a Fourier transform infrared (FT-IR) spectrophotometer**,** an X-ray Powder Diffractometer (XRD)**,** a thermogravimetric Analyzer (TGA), an Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) and Phosphate Buffer Saline Medium (PBS). The biocompatible and bioabsorbable pastes were developed from cockle shell-based nano calcium carbonate.

The developed paste was evaluated *in vivo* using a rabbit model. The paste was compared functionally from the pastes of cockle shell-based micron sized calcium carbonate and commercial calcium carbonate respectively. Twelve rabbits which were divided into three groups (n=4) were used for the *in vivo* evaluation. The first, second and third groups were used for the paste of cockle shell-based nano calcium carbonate, cockle shell-based micron sized calcium carbonate and commercial calcium carbonate, respectively. One bone hole with 5 mm diameter was created on the medial surface of the proximal extremity of both left and right tibia. The left hole was left empty and acted as negative control while the right hole was treated with the paste implantation. After implantation, the paste effectiveness was evaluated by radiographic, biochemical, gross and histological examination. The dynamic cockle shell-based nanocomposite biomaterial bone paste showed excellent bone healing performance in the right bone holes as compared to the micron sized cockle shell-based calcium carbonate paste, commercial calcium carbonate paste and many previously prepared bone pastes. This novel bone paste can be potential for biomaterial industry, human and veterinary medicine.

Key words: Cockle shells, nano calcium carbonate, nanocomposite biomaterial bone paste, *in vivo* study, rabbits.

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

PEMBANGUNAN PES NANOKOMPOSIT DAN KEBERKESANANNYA UNTUK DALAM PEMBAIKAN TULANG

Oleh

KH. NURUL ISLAM

Januari 2013

Pengerusi: Profesor Md. Zuki Bin Abu Bakar @ Zakaria, PhD

Fakulti: Perubatan Veterinar

Kajian ini mendedahkan pembangunan pes daripada kulit kerang berasaskan nanopartikel kalsium karbonat dan penilaian *in vivo* menggunakan model arnab. Kalsium karbonat dan Polimorf daripada kulit kerang (*Anadara granosa*) telah diciri menggunakan pengimbas mikroskop elektron pelbagai tekanan (VPSEM), mikroskop transmisi elektron (TEM), pengimbas serakan tenaga sinar-X (EDX), pembelauan sinar-X (XRD) dan spektroskopi penghantaran Fourier inframerah (FT-IR). Rod kristal aragonite dari serbuk kulit kerang telah diperhatikan dibawah kedua-dua SEM dan TEM. Keputusan EDXmenunjukkan kulit kerang mengandungi lebih banyak kalsium dan karbon daripada kalsium karbonat komersial. Analisa FT-IR mendedahkan kehadiran kumpulan karbonat di dalam kulit kerang. Analisa FT-IR juga menunjukkan kehadiran aragonite di dalam kulit kerang. Analisa XRD menunjukkan bahawa serbuk kulit kerang mengandungi aragonite. Serbuk kulitkerang telah dibentukdaripada kalsium karbonat yang berkualiti baik dan

mengandungi kalsium karbonat fasa aragonite.Nanopartikel aragonite kalsium karbonat telah disintesis daripada serbuk kulit kerang bersaiz mikron. Aragonite adalah salah satu polimorf kurang biogenik kalsium karbonat dan digunakan secara meluas sebagai bahan bio untuk pembaikan tulang patah, pembangunan sistem awal penyampaian dadah, dan perancah tisu. Kaedah ini melibatkan pengisaranmekanikal serbuk kulit bersaiz mikron dengan kehadiran pemangkin biomineral bukan toksik dan tidak merbahaya, dodesil betaine dimetil (BS-12). Kaedah ini menghasilkan nanopartikel aragonite berbentuk rod dengan penghasilan semula yang baik dan tanpa sebarang kekotoran tambahan. Ini telah disahkan melalui analisa gabungan pengimbas mikroskop elektron pelbagai tekanan (VPSEM), penghantar mikroskop elektron (TEM), spektroskopi Fourier penghantaran inframerah (FTIR), Termogravimetri penganalisis (TGA), spektroskopi pembelauan sinar-X (XRD) dan tenaga serakan X-ray penganalisis (EDX). Kaedah ini boleh menjadi aplikasi yang berpotensi di dalam industri bagi sintesis nanopartikel aragonite berskala besar darisumber semula jadi seperti kulit kerang dengan kos yang rendah.Nanopartikel kalsium karbonat di dalam fasa aragonite telah disintesis daripada kulit kerang yang murah dan banyak terdapat secara semulajadi.

Pes biokomposittelah dibangunkan dari kulit kerang berasaskan nanopartikel kalsium karbonat. Pes ini telah digunakan sebagai pembaikan tulang di dalam aplikasi pembedahan. Cengkerang kerang berasaskan nanopartikel kalsium karbonat telah dicampur dengan larutan kitosan yang mengandungi 2% asid asetik di dalam bikar kaca 250 ml. Campuran pes dicampur dengan menggunakan pengacau mekanikal plat panas pelbagai sistem dengan bar pengacau magnet. Campuran tersebut berkelompok bersama-sama dan memberikan penampilan seperti jaringan homogen.

Pes dicirikan oleh Pelepas Bidang Mengimbas Mikroskop Elektron (FESEM), Sebaran Tenaga Analisis Sinar-X (EDX), Jelmaan Fourier Inframerah (FT-IR) spektrofotometer, X-ray Difakometer (XRD), Termogravimetri Analisa (TGA), Plasma Gandingan Induktif Atom Pelepasan Spektrometer (ICP-OES) dan Fosfat Penimbal Saline (PBS). Pes biokomposit setanding dan bioserapan telah dibangunkan daripada kulit kerang berasaskan nano kalsium karbonat.

Pes yang telah dibangunkan telah dinilai secara *in vivo* menggunakan model arnab. Pestelah dibandingkan fungsinya dari pes kulit kerang masing-masing berdasarkan kalsium karbonat bersaiz mikron dan kalsium karbonat komersial. Dua belas arnab dibahagikan kepada tiga kumpulan (n = 4) telah digunakan di dalam penilaian*in vivo*. Kumpulan pertama, kedua dan ketiga masing-masing telah digunakan untuk pes kulit kerang nano berasaskan kalsium karbonat, kulit kerang berasaskan kalsium karbonat bersaiz mikron dan kalsium karbonat komersial. Satu lubang tulang dengan diameter 5 mm telah dibuat pada permukaan medial hujung proksimal kedua-dua kiri dan kanan tulangtibia. Lubang kiri dibiarkan kosong dan bertindak sebagai kawalan negatif manakala lubang kanan telah dirawat dengan pes. Selepas implantasi, keberkesanan pes dinilai melalui pemeriksaan radiografik, kasar dan histologi. Pes dinamik kulit kerang nano berasaskan kalsium karbonat menunjukkan penyembuhan tulang yang amat baik di dalam lubang tulang kanan berbanding kulit kerang berasaskan pes kalsium karbonat bersaiz mikron dan pes kalsium karbonat komersial.

Kata kunci: kerang kerang, nano kalsium karbonat, pes tulang biobahan nanokomposit, kajian *in vivo*, arnab

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I certify that a Thesis Examination Committee has met on 24 April to conduct the final examination of Kh. Nurul Islam on his thesis entitled "Development of Cockle Shell Based NanocompositeBiomaterial Bone Paste and Its Effectiveness for Bone repairin Rabbit Model" 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 Doctor of Philosophy.

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

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DECLARATION

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

KH. NURUL ISLAM

Date: 24 /04/ 2013

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