REINFORCEMENT AND OPTIMIZATION OF HYDROXYETHYL CELLULOSE / POLY (VINYL ALCOHOL) WITH CELLULOSE NANOCRYSTAL AS A BONE TISSUE ENGINEERING SCAFFOLD

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I hereby declare that I have checked this thesis and in my opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Master of Science.

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

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ABSTRAK

Bahan bio adalah istilah perubatan yang digunakan untuk menggambarkan semua sumber semulajadi atau sintetik seperti polimer yang berguna dalam pengenalan tisu hidup sebagai sebahagian daripada peranti perubatan atau implan tanpa menyebabkan sebarang tindak balas imun. Selulosa telah diterokai secara meluas sejak beberapa dekad sebagai salah satu daripada bahan bio yang digunakan dalam aplikasi kejuruteraan tisu kerana sifatnya yang unik yang kos rendah, biokompatibilitas yang baik dan sifat mekanikal yang baik. Penyediaan kristal nano selulosa (CNC) dari pulpa selulosa adalah cara alternatif untuk memenuhi permintaan CNC. Dalam kejuruteraan tisu, penggantian atau regenerasi tulang yang rosak adalah satu cabaran utama dalam pembedahan ortopedik.Oleh itu, reka bentuk kejuruteraan tisu tulang berasaskan perancah untuk mengatasi kecacatan tulang ini. Laporan ini terdiri daripada dua bahagian. Bahagian pertama adalah mengenai fabrikasi dan pencirian CNC manakala bahagian kedua adalah fabrikasi dan pencirian perancah termasuk degradasi secara vitro dan kajian hidup sel. Dalam kerja-kerja ini, CNC yang dihasilkan daripada tandan buah kosong (EFB) berjaya dihasilkan oleh hidrolisis asid. Pulpa selulosa dipanaskan pada suhu 85 °C dalam 65 % asid sulfurik. Pulpa selulosa telah dicairkan, sonikasi, dan kemudian mengalami pengeringan beku untuk mendapatkan CNC. CNC bertindak sebagai pengisi nano dalam perancah dan akan dicirikan secara fizikal, kimia dan termal dicirikan dengan menggunakan mikroskop elektron pengimbasan pelepasan medan (FESEM), keseluruhan reflektansi dilemahkan-Fourier mengubah spektroskopi inframerah (ATR-FTIR) dan calorimetry scanning differential (DSC). Keputusan FESEM menunjukkan bahawa CNC muncul dalam bentuk sfera dengan dimensi zarah dalam diameter 5 hingga 30 nm. Spektrum penyerapan CNC muncul dalam kumpulan tertentu yang berada pada 1045, 1346, 1637, 2903, dan 3391 cm^{-1}. Termometer DSC menunjukkan bahawa suhu lebur, T_m berlaku pada 336.4 °C, manakala suhu peralihan kaca, T_g ialah 43.5 °C. Seterusnya, perancah tiga dimensi (3D) HEC / PVA dan HEC / PVA / CNC telah berjaya direka oleh teknik pengeringan beku. HEC (5 %berat) dan PVA (15 %berat) telah dibubarkan dan dicampur pada nisbah 50:50 dan digabungkan dengan pelbagai kepekatan CNC (1, 3, 5 dan 7 %berat). Ciri-ciri morfologi, mekanikal dan terma perancah dicirikan oleh SEM, ATR-FTIR, DSC, thermogravimetric (TGA), dan mesin tegangan sejagat (UTM). Tingkah laku perancah scaffolds dicirikan oleh satu siri analisis termasuk nisbah pembengkakan, penurunan berat dan perubahan pH. Sementara itu, kajian sitotoksisiti pada kedua-dua bahan bio perancah dilakukan dengan menggunakan sel osteoblast janin manusia (hFOB) menggunakan ujian MTT dan kajian morfologi perancah sel. Gabungan HEC/PVA dengan CNC dipamerkan kefungsian yang lebih baik yang mengakibatkan penurunan ukuran liang rata-rata dan terdapat sedikit perubahan dalam struktur kimia seperti yang ditentukan oleh spektrum FTIR. Kajian termal menunjukkan bahawa suhu lebur perancah HEC/PVA/CNC beralih kepada nilai yang lebih tinggi. Selain itu, dapat dilihat bahawa penambahan CNC menghasilkan peningkatan tekanan muktamad (dari 0.18 hingga 0.92) dan tegangan muktamad (dari 5.83 hingga 11.03). Oleh itu, ia menawarkan prestasi mekanikal yang sangat baik. Kajian kultur sel mendedahkan bahawa sel-sel hFOB dapat melekat dan menyebar ke semua perancah dan menyokong perekatan sel dan percambahan sel. Kepekatan optimum CNC yang paling baik didapati pada 3 dan 5 %berat dan tambahan CNC mengurangkan daya tahan sel. Oleh kerana ciri-ciri bio serasi dan terbiodegradasi, perancah yang sangat baru ini boleh menghasilkan matriks perancah alternatif yang menjanjikan untuk pertumbuhan semula kejuruteraan tisu tulang.

ABSTRACT

Biomaterial is a medical terminology that is used to describe all natural or synthetic resources such as polymers that are useful in the introduction of living tissue as part of medical devices or implants without causing any adverse immune rejection reactions. Cellulose has been extensively explored over many decades as one of the biomaterials used in tissue engineering applications due to their unique properties which are low cost, good biocompatibility and good mechanical properties. Preparation of cellulose nanocrystals (CNC) from cellulose pulp is an alternative way to fulfil the demand for CNC. In tissue engineering, replacement or regeneration of damaged bone is a major challenge in orthopaedic surgery. Hence, scaffold-based bone tissue engineering is designed to overcome these bone defects. This report is comprised of two parts. The first part is about the fabrication and characterization of CNC while the second part is the fabrication and characterization of scaffolds including in vitro degradation and cell culture studies. In this present work, CNC produced from empty fruit bunch (EFB) was successfully fabricated by acid hydrolysis. Cellulose pulps were heated at 85 °C in 65 % of sulphuric acid. The cellulose suspension was diluted, centrifuged, sonicated, and then freeze-dried to obtain the CNC. The CNC acted as nanofillers in scaffolds and was physically, chemically and thermally characterized by using field emission scanning electron microscope (FESEM), attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) and differential scanning calorimetry (DSC). FESEM results showed that CNC appeared in a spherical shape with particle dimensions in the range of 5 to 30 nm in diameter. The absorption spectra of CNC appeared in specific bands which were at 1045, 1346, 1637, 2903, and 3391cm^{-1}. DSC thermograms shows that the melting temperature, T_m was at 336.4 °C, while the glass transition temperature, T_g was 43.5 °C. Next, a porous three-dimensional (3D) scaffold of HEC/PVA and HEC/PVA/CNC were successfully fabricated by freeze-drying technique. HEC (5 wt%) and PVA (15 wt%) were dissolved and blended at a ratio of 50:50 and incorporated with various concentrations of CNC (1, 3, 5 and 7 wt%). The morphology, mechanical and thermal properties of scaffolds were characterized by SEM, ATR-FTIR, DSC, thermogravimetric analysis (TGA), and universal tensile machine (UTM). The degradation behaviours of scaffolds were characterized by a series of analyses including swelling ratio, weight loss and pH changes. Meanwhile, cytotoxicity studies on both porous scaffold biomaterials were carried out by utilizing human fetal osteoblast (hFOB) cells using MTT assays and cell-scaffold morphological study. HEC/PVA incorporated with CNC exhibited superior functionality which resulted in decreased average pore size and there were some slight changes in the chemical structure as determined by FTIR spectra. Thermal studies revealed that the melting temperatures of HEC/PVA/CNC scaffold were slightly shifted to a higher value. Furthermore, it can be seen that the addition of CNC resulted in increases in the ultimate tensile stress (from 0.18 to 0.92) and ultimate tensile strain (from 5.83 to 11.03). Hence, it offers a very good mechanical performance. The cell culture study revealed that the hFOB cells were able to attach and spread on all scaffolds and supported the cell adhesion and proliferation. The optimum concentration of CNC was at 3 and 5 wt% while further addition of CNC reduced the cell viability. Due to its biocompatible and biodegradable properties, these newly developed highly porous scaffolds may provide a promising alternative scaffolding matrix for bone tissue engineering regeneration.

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