Potato Starch Hybrid Bentonite/Hydroxyapatite Composites as a Potential Materials for Coronary Stent Applications

by

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LIST OF ABBREVIATIONS

AB	Alamar Blue
Al	Aluminum
Al_2O_6	Aluminum oxide
Ca	Calcium
CaO	Calcium Oxide
EDX	Energy dispersive X-ray spectroscopy
Fe_2O_3	Ferric oxide
FTIR	Fourier transform infrared spectroscopy
H	Hydrogen
H_2O	Water
HUVEC	Human Umbilical Vein Endothelial Cells
HA	Hydroxyapatite
K_2O	Pottasium oxide
Mg	Magnesium
Na	Sodium
O	Oxygen
OH	Hydroxide
PNCs	Polymer nanocomposites
PLA	Poly lactic acid
PLGA	Poly lactic co glycolic acid
PSA	Particle size analyzer
SBF	Simulated Body Fluid
SEM	Scanning electron microscopy
Si	Silicon
SiO_2	Silicon dioxide
TEM	Transmission electron microscopy
TGA	Thermogravimetric analysis
XRF	X-ray fluorescence

LIST OF SYMBOLS

Av	Average Number of Cells
C	Cell Concentration
C live	Total Number of Viable Cells
Mi	Initial Mass
Mf	Mass final
m	Mass
v	Volume

Komposit Kanji Kentang Hibrid Bentonit/Hydroxapatit sebagai Bahan Potensi untuk Aplikasi Sten Koronari

ABSTRAK

Penggunaan bahan biodegradasi telah mendapat perhatian yang meluas dalam aplikasi bahan bioperubatan termasuklah sten koronari. Sten biodegradasi telah dibangunkan untuk mengatasi kekurangan stent logam semasa seperti sten restenosis lewat dan pengepungan pembuluh darah secara kekal. Kebanyakan bahan yang digunakan untuk sten biodegradasi adalah polimer sintetik dan logam bio-karat. Walaubagaimanapun, kebimbangan berkaitan polimer sintetik termasuklah kesan jangka panjang di dalam tubuh. Oleh itu, polimer semulajadi telah diperkenalkan untuk mengatasi kekurangan polimer sintetik. Tambahan pula, polimer berasaskan kanji yang boleh diperbaharui secara semula jadi telah menunjukkan potensi yang besar sebagai bahan biorosot. Selain itu, kanji mempunyai sifat yang unik seperti biorosot dan tidak toksik serta bioserasi dimana secara tidak langsung menjadikannya berguna dalam pelbagai aplikasi bioperubatan. Kajian ini didorong oleh pengetahuan semasa yang terhad dalam biobahan berasaskan kanji. Walaupun kanji telah banyak dikaji dalam pelbagai aplikasi bioperubatan, secara amnya aplikasi dalam sten koronari masih dalam perkembangan awal yang memerlukan kajian lanjut. Oleh yang demikian, kajian ini adalah untuk membuat filem komposit kanji kentang melalui teknik larutan tuangan dengan menggunakan hibrid bentonit dan hydroxyapatit (HA) pengisi-nano untuk penambahbaikan sifat mekanikal dan biokeserasian. Filem komposit kanji kentang telah disediakan dengan mencampurkan dua larutan yang berbeza iaitu larutan bentonit/HA dan larutan kanji. Larutan bentonit/HA disediakan terlebih dahulu melalui kaedah campuran mekanikal pada 1000 rpm selama 30 minit, kemudian diikuti dengan campuran sonik selama 30 minit sebelum dicampur dengan larutan kanji. Penyediaan larutan kanji pula melibatkan pencampuran kanji kentang dan gliserol bersama-sama dengan larutan bentonit/HA dalam takungan air oleh pengaduk mekanikal selama 30 minit diikuti dengan pengeringan campuran tadi di dalam ketuhar semalaman bagi membentuk filem komposit kanji. Sebelum diteruskan dengan fabrikasi komposit hibrid kanji kentang, filem kanji/gliserol pada suhu pencampuran yang berbeza dan filem kanji/komposit bentonit pada mulanya dibuat untuk mencari kandungan gliserol, suhu pencampuran dan kandungan bentonit yang optimum. Kesan daripada penambahan gliserol, suhu pencampuran, bentonit dan penambahan HA dalam filem kanji kentang dikaji melalui analisis tensil, fizikal, morfologi dan kumpulan berfungsi. Ujian in vitro menggunakan cecair badan simulasi (SBF) dan penilaian biokeserasian juga dilakukan untuk menilai sifat bioaktiviti dan biokeserasian filem komposit kanji kentang. Keputusan ujian tensil menunjukkan bahawa filem kanji dengan 30 wt% gliserol dan bentonit 15 wt% pada suhu campuran 85 ℃ menunjukkan kekuatan tensil tertinggi, berikutan itu dipilih dalam menghasilkan komposit hibrid kanji dengan pelbagai kandungan HA (1, 5, 10 , 15 dan 20 wt%). Hasil kajian menunjukkan bahawa filem komposit hibrid kanji dengan 5% HA memperoleh nilai kekuatan tensil tertinggi iaitu 4.36 MPa berbanding kanji dan kanji/komposit bentonit. Tambahan pula, bukti daripada kajian scanning electron microscopy (*SEM)* dan transmission electron microscopy

(*TEM)* menunjukkan kadar penyebaran yang lebih baik dan pengukuhan yang mencukupi diperolehi daripada hibrid komposit kanji yang mengandungi 5 wt% HA yang bertepatan dengan keputusan ujian tensil. Sementara itu, ujian in vitro menunjukkan bahawa penyatuan HA dengan kanji/komposit bentonit berjaya menambahbaik bioaktiviti hibrid komposit kanji. Di samping itu, didapati filem kanji yang mengandungi bentonit dan HA yang terdedah kepada *human umbilical vein endothelial cells (HUVEC)* boleh menggalakkan pertumbuhan *HUVEC* pada permukaannya berbanding dengan filem kanji yang menunjukkan kekurangan daya tahan sel. Di antara gabungan hibrid komposit, filem dengan penggantian HA 5 wt% menunjukkan penghasilan biokompatibiliti dan dapat merangsang percambahan sel, yang menjanjikan kesesuaian untuk digunakan dalam aplikasi koronari sten.

Potato Starch Hybrid Bentonite/Hydroxyapatite Composites as a Potential Materials for Coronary Stent Applications

ABSTRACT

The use of biodegradable materials have received great attention in biomedical applications including coronary stent. Biodegradable stents were developed to overcome the limitations of current metallic stents such as late in-stent restenosis and permanently caging the vessel. Most of the materials used in the fabrication of biodegradable stents are synthetic polymers and bio-corrodible metals. However, concern related to synthetic polymers include their long term effects in the body. Thus, natural polymers have been introduced to overcome the limitations of synthetic polymers. Upholding promises, naturally renewable starch based polymers have shown great potential as biodegradable materials. Besides, starch having unique properties such as biodegradable, non-toxic and biocompatible makes them useful for a wide variety of biomedical applications. This study is motivated by a gap in the current knowledge in starch biomaterials. Although starch has been widely researched in various biomedical applications, in general their applications in coronary stent is still in early development that requires further study. Therefore, this study aimed to fabricate potato starch composites film through solution casting technique utilizing hybrid bentonite and hydroxyapatite (HA) nanofillers to enhance mechanical and biocompatibility properties. The potato starch composites films were prepared by mixing two different solutions which is bentonite/HA and starch solutions. The bentonite/HA solution was first prepared by mechanical mixing at 1000 rpm for 30 minutes followed by sonication mixing for 30 minutes prior to mixing with starch solution. Starch solution preparation involved mixing potato starch and glycerol together with bentonite/HA solution in water bath by mechanical stirrer for 30 minutes followed by drying the mixture in an oven overnight to form starch composites film. Prior to potato starch hybrid composites film fabrication, starch/glycerol film at different mixing temperature and starch/bentonite composites film were initially fabricated to find the optimum glycerol, mixing temperature and bentonite content. The effect of glycerol addition, mixing temperature, bentonite and HA addition in potato starch films were evaluated through tensile, physical, morphology and functional group analyses. An in vitro test using simulated body fluid (SBF) and biocompatibility assessment were also performed to evaluate the bioactivity and biocompatibility properties of potato starch composites film. The tensile test results showed that starch film with 30 wt% glycerol and 15 wt% bentonite at 85 ℃ mixing temperature exhibited the highest tensile strength, thus were chosen in fabrication of starch hybrid composites with various content of HA (1, 5, 10, 15 and 20 wt%). Results showed that, starch hybrid composites film with 5 wt% HA demonstrated the highest tensile strength value of 4.36 MPa compared to neat starch and starch/bentonite composites. Furthermore, the evidence from SEM and TEM studies showed better dispersion and sufficient reinforcement obtained from starch hybrid composites containing 5 wt% HA which supported the tensile test result. Meanwhile, the in vitro test shows that the incorporation of HA into starch/bentonite composites improves the bioactivity of the starch hybrid composites. Besides, it was also found that starch film containing bentonite and HA

exposed to human umbilical vein endothelial cells (HUVEC) may promote HUVEC growth on its surface compared to neat starch film which showed lack of cell viability. Among the hybrid combination composites, the film with 5 wt% HA substitution shows biocompatibility and able to stimulate cell proliferation, which suggests suitability and promise to be used in coronary stent applications.

CHAPTER 1 : INTRODUCTION

1.1 Research Background

Biomaterial is defined as a material that interacts with biological systems in order to evaluate, treat, augment, or replace any tissue, organ, or function of the body. (Stanislawska, 2014). Biomaterial can be widely classified into three groups of materials, namely metallic, ceramics and polymeric (Bhat & Kumar, 2012, 2013). These materials have become increasingly attractive in research and development activities since the inception of their widespread use in the biomedical applications as an implant. Indeed, the types and properties of biomaterials are still being examined to satisfy the requirements of artificial implant products. The main purpose developing and characterizing biomaterials suitable for human body implantation is to restore and improve physical performance enhancing survival and quality of life. In 2013, the global market for implantable biomaterials worth was nearly $75.1 billion (USD). This market is expected to grow at 6.7% between 2014 and 2019, resulting in a $79.1 billion and $109.5 billion global market in 2014 and 2019 respectively (Song et al., 2018). These constant demand in global markets have led to the development of cost effective and innovative biomaterials.

Polymers are one of the biomaterials which received greater attention in biomedical applications such as coronary stent. This is due to their versatility properties, particularly easy to be shaped, good mechanical characteristics and their biocompatibility is unmatched by other biomaterials, such as metals (Boni et al., 2018). Numerous synthetic polymers have been develop in the attempt to produced medical