PRODUCTION OF TEMPORARY BONE SCAFFOLD REINFORCED WITH OPEFB-CMC FROM OIL PALM EMPTY FRUIT BUNCH

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ABSTRACT

Bone fracture is a common injury because of its nature position that is mostly closest to skin and exposed to excessive compression and depression. Current treatment for bone fracture employs the scaffolding approaches which are specifically positioned for a certain period of time. These allow the defective bones to undergo proper healing processes. However, these scaffolds have two issues that need to be addressed; the material's compatibility and degradability. Previously, there was poor interaction between the Chitosan (CS) and Hydroxyapatite (HA)/nano HA (nHA) phases causing the composite to have poor physico-chemical properties. This research used Carboxymethylcellulose (CMC) as the reinforcement material for CS / HA or nHA composite scaffold. The main objective is to produce CMC from Oil Palm Empty Fruit Bunch (OPEFB) for temporary biodegradable bone scaffold from a combination of CMC, CS and HA/nHA. Series of experiments were done including extracting CMC from the OPEFB, fabricating composite scaffold by a co-solution method followed by freeze-drying approach to produce a porous bone implant. The final procedure was to analyse the CMC and scaffold produced by various analyses and tests including FTIR, SEM, EDX, TGA and compressive-modulus for its mechanical characteristics. The findings indicated that the strength has increased within 32 – 50 kPa with CMC content compared to chitosan scaffold alone which was only recorded at 0.042 – 0.7 kPa. With the additional of Calcium Phosphate the results only recorded from 0.024 kPa until 2 kPa. The composite scaffold was also successfully constructed with lots of pores, allowing the scaffold to demonstrate preferential proliferation and extracellular matrices and generate mineralised bones. The investigation was extended to in-vitro test involving Simulated Body Fluid (SBF) solution to evaluate the biodegradation rate and

the growing of apatite layer during immersion. The implant had exhibited biodegradation feature parallel to new bone formation. The ability in attracting Calcium (Ca) and Phosphate (P) elements for apatite layer development on its surface was also proven with the calculated value of Ca/P ratio that has identical value with the theory, at 1.67.

ABSTRAK

Patah tulang adalah kecederaan biasa kerana kebiasaannya, ianya terletak paling dekat dengan kulit menyebabkan pendedahan yang melampau pada tekanan yang tidak disengajakan. Rawatan terkini untuk patah tulang menggunakan pendekatan perancah yang berada pada kedudukan yang khusus untuk tempoh masa yang tertentu. Ini membolehkan tulang yang rosak untuk menjalani proses penyembuhan semula. Walau bagaimanapun, perancah ini mempunyai dua isu yang perlu ditangani; keserasian bahan dan degradasi. Sebelum ini, wujud interkasi yang lemah di dalam fasa antara Chitosan (CS) dan Hydroxyapatite (HA) / nano HA (nHA) menyebabkan komposit mempunyai ciri-ciri fiziko-kimia yang lemah. Kajian ini menggunakan carboxymethylcellulose (CMC) sebagai pengukuh untuk CS / HA atau nHA perancah komposit. Objektif utama adalah untuk menghasilkan CMC dari Minyak Sawit Tandan Buah Kosong (OPEFB) untuk perancah tulang sementara yang boleh terbiodegradasi sendiri daripada gabungan CMC, CS dan HA / nHA. Beberapa siri eksperimen telah dilakukan termasuk mengekstrak CMC dari OPEFB, merekabentuk perancah komposit dengan kaedah *co-solution* diikuti oleh pendekatan beku-pengeringan untuk menghasilkan implan tulang yang berliang. Prosedur akhir adalah untuk menganalisis CMC dan perancah komposit yang dihasilkan melalui pelbagai analisis dan ujian termasuk FTIR, SEM, EDX, TGA dan mampatan-modulus untuk ciri-ciri mekanikal. Dapatan kajian menunjukkan bahawa kekuatan ini telah meningkat di antara 32-50 kPa bersama kandungan CMC berbanding perancah chitosan sahaja hanya direkodkan pada 0,042-,7 kPa. Dengan tambahan Kalsium fosfat keputusan hanya direkodkan daripada 0,024 kPa sehingga 2 kPa. Perancah komposit ini juga telah berjaya dibina dengan banyak liang, membolehkan sel-sel tulang untuk memulakan percambahan dan matriks extracellular

dan menjana semula tulang yang baru. Siasatan itu telah dilanjutkan kepada ujian in-*vitro* yang melibatkan larutan *Simulated Body Fluid (SBF)*, kaedah untuk menilai kadar biodegradasi dan pertumbuhan lapisan apatite semasa rendaman. Implan tersebut telah menunjukkan cirri-ciri biodegradasi selari dengan pembentukan tulang baru. Keupayaan dalam menarik Kalsium (Ca) dan fosfat (P) elemen untuk pembangunan lapisan apatite di permukaannya juga dibuktikan dengan mengira nisbah Ca/P yang mempunyai nilai yang sama dengan teori, pada 1.67.

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LIST OF SYMBOLS AND ABBREVIATIONS

Abs	Absorbance of the peak sample at a particular wavelength
AGU	Anhydroglucose units
ad	air dried
ASTM	American Society of Testing and Materials
B	Scaffold fabricated from CMC commercial, CS and HA
B40	Scaffold with 40% CMC conventional content, CS and HA
CA	Cellulose Acetate
$CaCl_2$	Calcium Chloride
CED	Cupriethylenediamine
CH_2COOH	Carboxymethyl groups
CH_4O	Methanol
$C_2H_4O_2$	Acetic acid
C_2H_6O	Ethanol
$ClCH_2CO_2H$	Monochloroacetic acid
CMC	Carboxymethyl cellulose
CP	Cellulose Phosphate
CS	Chitosan
D	Scaffold fabricated from CMC conventional, CS and nHA
D40	Scaffold with 40% CMC conventional content, CS and nHA
DMSO	Dimethyl Sulfoxide
DP	Dissolving pulp

DP	*Degree of Polymerization*
DP – O	Oxygen dissolving pulp
DP – OZ	Oxygen-Ozone dissolving pulp
DP – OZP	Oxygen-Ozone-Peroxide dissolving pulp
DS	Degree of Substitution
DTG	Derivative of TGA analysis
E	Scaffold fabricated from CMC improvement, CS and HA
E30	Scaffold with 30% CMC improvement content, CS and HA
F	Scaffold fabricated from CMC improvement, CS and nHA
F40	Scaffold with 40% CMC improvement content, CS and nHA
FTIR	Fourier Transform Infrared
GAGs	Glucosaminoglycans
$(HOCH_2)_3CNH_2$ Tris	Tris-hydroxymethylaminomethane
HA	Hydroxyapatite
HCl	Hydrochloric
HDPE	High Density Polyethylene
H_2O_2	Hydrogen Peroxide
H_2O_{volume}	Volume of water
H_2SO_4	Sulphuric acid
KCl	Potassium Chloride
$K_2HPO_4.3H_2O$	Di-potassium hydrogen phosphate trihydrate
MSCs	Mesenchymal stem cells
M_{avg}	Average moisture content
$MgCl_2.6H_2O$	Magnesium Chloride hexahydrate
$MgSO_4.7H_2O$	Magnesium Sulphate aqueous solution
NaCl	Sodium Chloride
nHA	Nano Hydroxyapatite
$NaHCO_3$	Sodium Carbonate
NMR	Nuclear Magnetic Resonance
NaOH	Sodium Hydroxide
Na_2SO_4	Sodium Sulphate

od	oven dried
OPEFB	Oil Palm Empty Fruit Bunch
OZP	Oxygen-Ozone-Peroxide
PP	Polypropylene
SBF	Simulated Body Fluid
SEM	Scanning Electron Microscope
TAPPI	Technical Association of the Pulp and Paper Industry
TBAF	Tetrabutylammonium Flouride
TCF	Totally Chlorine-Free
TE	Tissue Engineering
TGA	Thermogravimetric Analysis
V	Volume
V_{NaOH}	Volume of aqueous NaOH
$V_{overall}$	Volume of overall liquid
w_a	Initial weight of scaffold (dry weight)
w_b	Weight after dried in an oven
w_d	Dry weight of scaffold before immersed in ethanol
W_o	Weight after immersed in SBF
W_w	Dry weight of scaffold
w_w	Weight of scaffold after immersed in ethanol
XRD	X-Ray diffraction

Greek letters

$\rho_{ethanol}$	Density of ethanol

CHAPTER 1

INTRODUCTION

1.1 Background

Bone is notably created to support and protect various organs in a body. It produces red and white blood cells and also stores minerals for living things; humans and animals. Mechanical functions of bones are for protection where bones protect internal organs. For instance, the skull is protecting the brain and the ribs are protecting the heart and lungs. In addition, bones also provide a structural body frame to keep the body supported.

Dynamically, as referred to the web from The Cleveland Clinic Foundation (2013), bones trigger movement for the body, where it provides a leverage system for skeletal muscles, tendons, ligaments, and joints function together to generate and transfer forces. So, individual body parts or the whole body can be manipulated in three dimensional spaces. It obviously shows that bones are an eventful structure for all living things for survival to execute daily and routine activities.

The characteristics of bones are very interesting and unique. It bends when it receives sudden, unpredictable forces up to its own limitation (Riggs & Melton, 1995). However, bones are prone to impact from unwanted forces. If the forces exerted against a bone exceeded its limit, bones could not withstand the forces and starts to break. This phenomenon occurs as bones are only covered with very thin

skin and less fat surrounding them, hence provide them with little absorption during higher impact events. Despite easily crack problems, bones are able to regenerate and redeveloped (Yamamuro, 1995). The newly generated bones provide the same functions and strength as normal bones. Bone regeneration is a continuous process and happens for an entire life. Unfortunately, the regeneration process decreases slowly with the addition of age.

Bone healing is a complex process. The time required for ossification or process of bone healing are dependent and can be affected by many factors including types of bone fracture and dependent on the patient's age and their nutritional status (Alvarez & Nakajima, 2009) . Since bone healing is a natural process, the period of time taken to cure is of concern. Therefore, several proactive curings are taken to assist the process of bone healing.

Autograft and allograft techniques are frequently used in order to overcome the bone fracture problem. Autograft is a technique of replacing the fractured bone with the healthy bone from the same person. The advantages of autograft are it provides bone cells and growth factors that are essential for healing and bone regeneration, no risk of disease transfer and no risk of rejection (Silber et. al., 2003; Myeroff & Archdeacon, 2011; Oppenheim, Segal & Spitzer, 2002). Despite the advantages of autograft, the patients are required to have double surgical operations from two different sites in the same body host. This caused double pain to the patients as well as increasing the traumatic experiences of the patients (Valliant & Jones, 2011).

As for options, allograft technique is introduced. This technique involves the bone transplant from different host or a bone bank. Allograft provides safer alternative to patients who are at higher risk of complications under anesthesia. The surgeon would not take a long time to harvest and prepare the autograft, complete the reconstruction faster thus avoid having longer period of surgery (Mahony & Jones, 2008).

Synthetic bone graft substitution brings new phenomena in orthopaedic and tissue engineering after more findings were discovered as an effort in curing the bone defect. Moore, Graves & Bain (2001) quoted that a variety of synthetic bone graft substitutes have been developed during the past 30 years with the aim to minimize the risk of postoperative infection and fractures as well as the potential risk of disease

transmission as it is from synthetic origin. Moreover, synthetic bone grafts also contribute in osteoinductive and osteostimulative (osteointegration) (Moore et. al., 2001) which is an essential attribute for bone regeneration stage, offering biodegradable properties, an ample supply for bone substitute and available in a wide range of size and shape. Unfortunately, most synthetic bone grafts do not provide sufficient mechanical strength like ceramics and they are not osteogenic.

Another type of bone treatment is by metallic implants. In this process, metal plates were used rather than the actual bones. Normally, metal plates used were stainless steel and titanium and Cobalt based alloys (Schmutz, Quach-Vu & Gerber, 2008). They show a high corrosion resistance due to their stable passive layer. However, they also have some benefits; superior in mechanical properties such as hardness and stiffness compared to other materials such as polymer and visible during x-ray (Schmutz et. al., 2008). Metallic implants were used in many treatments and were fairly successful, but problems related to stress shielding during post-healing and fatigue and loosening of the implant limit its function. Moreover, second surgery is usually required in order to remove the metallic implant after healing, and it increases the risk of the operation and the expense to the patient (Middleton & Tipton, 2000).

The above treatments have mentioned several benefits and drawbacks of the treatments. It has been a desire for biodegradable implants to be developed that will eventually biodegrade itself. Upon degradation process, ion releases are able to encourage surrounding cells to form new bone formation more rapid at a preferred rate. According to Pilliar et. al. (2001), the controllable rate of new bone formation is necessary in order for the defect site to eventually be replaced by a newly formed natural bone and strong enough to fulfil required load-bearing. The new bone can at least be functional during the early stage of the post - implantation period, before significant bone ingrowth and the replacement has occurred.

Most metallic materials are not biodegradable, which bring polymeric materials more benefits than the metal implants because it eliminates the need for a second operation and can prevent some problems associated with stress shielding. Sundararajan, Ma & Howard (1999), Pilliar et. al. (2001), Langer & Vacanti (1993), Hubbel (1995), Hellman (1997) and Niklason & Langer (1997) have stated that the tissue engineering

approach to repair and regenerate is founded upon the use of polymer scaffolding which serve to support, reinforce and in some cases organize the regenerating tissue. So, the reconstruction of new bone is more effective and well organized.

There is a need for the development of new biodegradable materials to be used in orthopaedics and as scaffolding for hard tissue enginnering (Mano et. al., 1999). Polymers are often used as matrix in bone scaffold composite. For example, lignocellulosic fibers obtained from renewable resources where it is composed from carbohydrate polymers is one of the example of natural polymer. An example of carbohydrate polymer is cellulose. It is the abundant renewable resource that has become of more and more interest as reinforcement in composites. This is because they are biodegradable and harmless for the ecological system. Furthermore, they have promising mechanical properties and are less expensive than conventional synthetic polymers (Zimmermann, Pohler & Geiger, 2004).

1.2 Problem Statement

Bones are important organs to ensure smooth movement for daily activities but it is prone to get fractured since it is surrounded by thin skin and less fat. That makes it easily exposed to get harmed. Bone implant is the second most replaced organ in the body after blood where approximately 2.2 million bone graft procedures are performed worldwide each year (Giannoudis, Dinopoulos & Tsiridis, 2005). Moreover, the estimated cost of these procedures approaches $2.5 billion per year (Desai, 2007). While bone transplantation and tissue reconstruction are highly successful therapies for a variety of bone diseases and fracture problems, a shortage of donor bone tissue limits their application (Jones & Hench, 2001).

Due to the serious circumstances, the vital alternative is to create an implant fabricated from synthetic and also natural sources. Extracellular matrices (ECMs) of hard tissue are composed of organic (collagen type I and small amount of GAGs-glycosaminoglycans) and inorganic phases (mainly nano hydroxyapatite crystals – nHA) (Zhao et. al., 2002).

Nano scale HA is known to own excellent biocompatibility based on its close chemical and crystal resemblance to bone material (Hench, 1998; Suchanek & Yoshimura, 1998; Gomez-Vega et. al., 2000). While that, chitosan (CS) can accelerate the bone formation because of the similarity to GAGs in structure (Seol et. al., 2004; Di, Sittinger & Risbud, 2005; Madihally & Matthew, 1999; Yamane et. al., 2005; Loke et. al., 2000).

However, there is a poor interaction between CS and HA/nHA phases causing the composite to have poor physico-chemical properties. Due to the fact that normally, for interface improvement between HA/nHA and CS, the second organic polymer acts as reinforced phase in HA/nHA-based composite is essential (Jiang, Li & Xiong, 2009b). Carboxymethyl cellulose (CMC) possesses very similar structure to CS structure which creates strong ionic cross-linking action between CMC and CS (Xiao et. al., 2006; Qiu & Li, 2005). This evidence has been supported by Latif, Anwar & Noor (2007) as shown in Fig. 1.1.

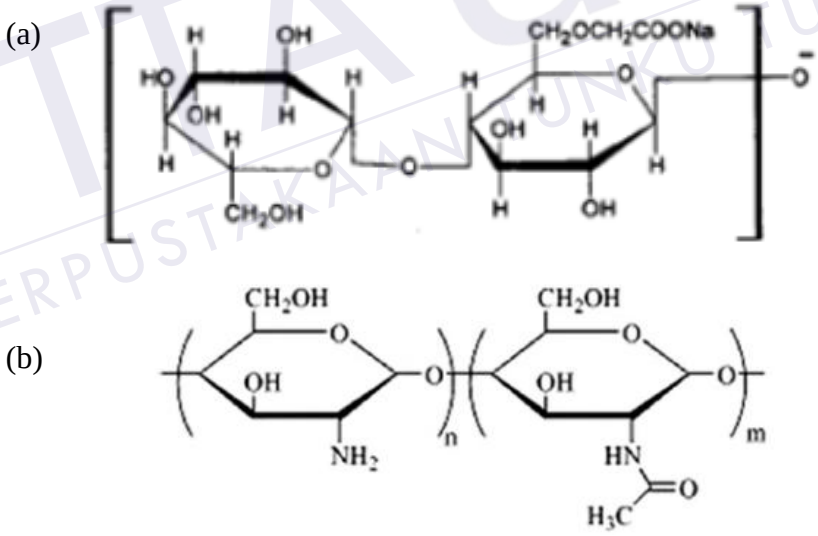

Figure 1.1: The chemical structure of (a) CMC, and (b) Chitosan (Latif et. al., 2007)

Briefly, CMC, also known as cellulose gum, is a cellulose derivative with carboxymethyl groups (-CH_2-COOH). The functional group is bound to some of the hydroxyl groups (-OH) of the glucose monomers that make up the cellulose backbone.

The availability of CMC sources is undoubted. In this research, it was extracted from Oil Palm Empty Fruit Bunch (OPEFB). Empty Fruit Bunch (EFB) from palm oil waste is a potential raw material. This is because palm oil has made an impressive and sustained growth in the global market over the past four decades, and it is projected that in 2016-2020, the average annual production of palm oil in Malaysia will reach 15.4 million tonnes (Teoh, 2000; Abdullah & Sulaiman, 2013).

Sulaiman et. al. (2010) indicated that large amount of oil palm residues that can be re-utilised were dumped. This resulted in millions of ringgit energy value wasted each year with approximate loss of about 6,379 million ringgit (Sulaiman et. al., 2010). Due to the environmental concerns over properly disposing the waste, OPEFB could be converted into useful material in biomedical engineering.

Therefore, a novel approach of the composite with the additional of CMC as a natural polymer in order to reinforce CS and HA was created to address the limitations of the previous sample. For the scaffold to integrate with surrounding tissue, it should imitate the structure and morphology of the natural bone tissue (Stevens et. al., 2007). Thus, there is strong ionic cross-linking action between CMC and chitosan and it is able to produce better composite for bone scaffold. The strong ionic cross-linking between CMC and chitosan is possible to occur because chitosan is a cationic polymer whereas CMC is an anionic polymer where by their combination, a strong ionic bond is created to produce stronger composite.

1.3 Objective

The aim of this research is to produce CMC from OPEFB as biomaterial for temporary bone scaffold reinforced with chitosan and HA/nHA. In order to achieve the aim, several objectives have been highlighted as follow:

(1) To evaluate and analyse the performance of the OPEFB-CMC as the reinforcement material to strengthen chitosan and HA/nHA, as a porous composite scaffold,

(2) To investigate the strength of composite by compression test and physical characteristics,

(3) To evaluate the degradation time, apatite layer formation, porosity measurement and swelling ability through in-vitro test simulation.

1.4 Scope of Research

The scope of this research includes:

(1) To produce CMC that was synthesized from oil palm waste, the empty fruit bunch. It was chosen because it dissolved easily in water because in order to utilize cellulose widely in any application, cellulose must be converted to soluble derivatives. The fabrication process is also at lower cost, easy and safe to produce. Analyses involved are FTIR and XRD.

(2) To produce porous scaffold fabricated from natural polymer and HA/nHA with the attendance of chitosan for better physico-chemical properties.

(3) To investigate the mechanical properties and focus only on compression test in evaluating the effectiveness of CMC as a potential material in bone scaffold. The analysis involved is TGA analysis.

(4) To analyse the morphology of the scaffold including its porosity content either at the surface or inside the scaffold. FTIR, SEM and EDX analyses will be implemented to examine this.

(5) To simulate the biodegradation rate of bone scaffold and the growing of apatite layer by immersion of samples in Simulated Body Fluid (SBF) liquid for in-vitro test.

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