Fabrication and characterization of tissue engineering scaffold based of nanohydroxyapatite - local rice starches

By

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LIST OF ABBREVIATIONS

3D	3 Dimension
ASTM	American Standard Testing Material
ECM	Extracellular Matrices
EEA	Ethylene-Co-Acrylic Acid
FTIR	Fourier Transform Infrared
GA	Glutaraldehyde
HA	Hydroxyapatite
HIV	Human Immunodeficiency Virus
JCPDS	Joint Committee on Powder Diffraction Standard
NaCl	Sodium Chloride
nHA	Nano Hydroxyapatite
PLGA	Polylactic-Co-Glycolic Acid
PLLA	Poly-L-Lactic Acid
PNA	Performance Network Analyzer
PVA	Poly Vinyl Alcohol
SCA	Cellulose Acetate
SEM	Scanning Electron Microscope
SPLA	Poly Lactic Acid
UTS	Ultimate Tensile Strength
XRD	X-Ray Diffraction

LIST OF SYMBOLS

cm^{-1}	Reciprocal wavelength
cm^3	Centimeter cube
g	Gram
g/cm^3	Gram per centimeter cube
GHz	Gigahertz
kV	Kilo Volt
ml	Milliliter
mm	Millimeter
MPa	Mega pascal
W	Weight
wt%	Weight percentage
w/w	Weight per weight
µm	Nano meter
%	Percent
%E	Elongation at break
ε	Porosity
ε*	Relative permittivity
ε'	Dielectric constant
ε''	Dielectric loss
ºC	Degree celcius
ρ	Density

º	Degree
º/min	Degree per minute

Fabrikasi dan Pencirian Perancah Kejuruteraan Tisu Berasaskan HidroksiapatitNano-Kanji Beras Tempatan

ABSTRAK

Perancah tisu tulang telah diteroka sejak beberapa dekad yang lalu dalam menyelesaikan isu kegagalan fungsi tulang dan trauma. Kanji adalah polimer semula jadi yang telah digunakan sebagai biobahan dan calon yang sesuai untuk membina perancah kerana terdapat banyak sumber kanji di Malaysia. Kanji-kanji dari sumber tempatan mampu menyumbang secara berbeza disebabkan oleh struktur mereka yang bergantung kepada kandungan amilosa, interaksi di antara granul, keupayaan untuk mengembang dan kelarutan di mana perbezaan-perbezaan ini mungkin disebabkan oleh asal-usul botaninya. Di Malaysia sendiri, terdapat pelbagai sumber kanji beras. Belum ada lagi penyelidikan yang telah dibuat berkenaan aplikasi kanji beras Malaysia sebagai perancah tisu tulang. Kanji beras Balik Wangi, Bubuk Wangi, dan Bario telah digunakan untuk membina perancah tulang melalui teknik acuan pelarut dan pelarutan garam. Ketiga-tiga jenis kanji beras asalnya ditanam di Sarawak, Malaysia. Daripada kajian ini, keliangan dan ketumpatan diperiksa melalui ujian anjakan cecair dimana keliangan akan meningkat apabila peratusan kanji meningkat. Manakala, sifat dielektrik pula diperolehi dari nilai kehilangan dielektrik dan nilai berterusan dielektrik dimana ia adalah satu lagi cara pengukuran tidak langsung dalam mengkaji keliangan perancah. Keliangan mempunyai nilai dieletrik yang sama dengan nilai dieletrik udara di mana udara diwakili oleh keliangan perancah tisu tulang. Sementara itu, morfologi dan struktur mikro telah dinilai dengan menggunakan Mikroskop Elektron Pengimbas (SEM) dan menunjukan peningkatan saiz keliangan apabila peratusan kanji meningkat. Modulus young pula diperolehi untuk mengkaji kekuatan mekanikal perancah dimana perancah tulang berasakan kanji beras Balik Wangi-nano Hidrosiapatasi mempunyai nilai modulus young paling tinggi. Manakala, interaksi campuran kanji dan hidrosiapatasi dianalisis melalui Fourier Transform Infrared Spektroskopi (FTIR). Akhir sekali, struktur penghabluran untuk semua perancah berasas beras kanji dan nHA dikaji melalui analisis pembelauan X-Ray dan secara umumnya faktor pengkristalan perancah didominasi oleh pengkristalan hidroksiapatit.

Fabrication and Characterization of Tissue Engineering Scaffold Based of Nanohydroxyapatite-Local Rice Starches

ABSTRACT

Bone tissue scaffold had been ventured for over the decades as a solution for bone failure and trauma. Starch is the most common natural polymer that has been used as a biomaterials and a good candidate for scaffold fabrication as there are many resources of starch in Malaysia. Native starches may contribute differently in their structure in terms of the amylose content, interactions between granules, swelling ability and solubility in which those differences can be mainly due to the botanical origin. In Malaysia itself, there are various resources of rice starches. No research yet has been performed on the application of Malaysian rice starch as a bone tissue scaffold. Balik Wangi, Bubuk Wangi, and Bario rice starches were used to fabricate the scaffold via solvent casting and salt leaching technique. These three types of rice starch originally from Sarawak, Malaysia. From the study, the porosity and the density were examined via liquid displacement test and it was found the higher the starch percentage results higher porosity percentage. Here, the dielectric properties were obtained via the values of dielectric constant and dielectric loss which are another type of indirect measurement to study the porosity of the scaffolds. The porosity has the value of dielectric constant and loss air matrix whereby this air matrix is represented by the pore in the scaffold. The morphologies and microstructures were evaluated by using Scanning Electron Microscopic (SEM) and showed the increasing pore size as increasing the starch percentage. The Young's modulus is obtained to study the mechanical strength of the scaffolds where Balik Wangi rice starch-nHA scaffolds have the highest value of young's modulus due to fibre content in the rice. Whereas, the interaction of the starch and hydroxyapatite blends were analyzed via Fourier Transform Infrared Spectroscopy (FTIR). Lastly, the crystallinity structure for all the rice starch-nHA scaffolds is studied via the X-Ray diffraction analysis and basically hydroxyapatite dominate the crystalinity structure of the scaffold.

CHAPTER 1

INTRODUCTION

1.1 Background history

Hydroxyapatite is known to have similar chemical composition, mineral phase, and crystallinity of natural bone (Frohbergh *et al.,* 2012; Wiria, Tay, & Ghassemieh, 2013; Mcnamara *et al.,* 2014) which majorly exhibit high osteoconduction and osteoinduction when implanted in the human body (Sarath Chandra *et al.,* 2015). Unfortunately, the nature of hydroxyapatite is brittle and low in mechanical properties which limits its application (Fanovich, Castro, & Lo, 1999; Cipreste & Sousa, 2014; Sarath Chandra *et al.,* 2015) beside that, hydroxyapatite alone is not mechanically competent to support various applications in biomedical engineering (Cipreste & Sousa, 2014).

The starch is recognized to have favorable criteria such as biocompatible, low in cost, good biodegradable term, and non toxic (A. Rodrigues & Emeje, 2012; Xie *et al.,* 2013). Polymers formulated from starch have been widely applied in biomedical application such as for bone replacement, bone cement, and bone tissue engineering (Sundaram, Durance, & Wang, 2008). Charoenrein *et al.* stated that different starches would exhibit different criteria and in order to improve the textural qualities of starch, the incorporation of starch with different botanical sources had been performed (Charoenrein & Preechathammawong, 2012). The differences in starch behaviour is mainly due to the concentration, mixing ratio, amylose content, interactions between granules, swelling power and solubility, and granule characteristics (Seetapan *et al.,* 2015). Considering the

Sarawakian rice has numerous varieties and differs in term of botanical resource, it had been used as reinforcement to hydroxyapatite for bone tissue scaffold. The properties of the newly fabricated scaffolds were characterized in detail including their mechanical and electrical properties.

1.2 Problem statement

Previously, scaffold was fabricated by using single phase of material which means it is not mixed together with other type of biomaterials which is meant to improve the porosity of the scaffolds which is vital for the cell generation (Ito, Honda, & Ueda, 2003). The problem of ceramic scaffolds are due to their brittleness which had restricted the performance of the scaffolds (Kang *et al.*, 2011). Thus, an addition of other biomaterials to the scaffolds' composition may eliminate or reduce the brittleness of the scaffold. Starch is one the natural biomaterials that should be considered as a promising material for the biocomposite scaffolds fabrication due to its low cost, wide availability, and biocompatible to enhance the mechanical properties of ceramic material such as hydroxyapatite (HA) (Mishra *et al.*, 2011). Starch is a natural material which is able to control the adhesion of stem cell and promoting growth through inherent binding sites (Chien & Shah, 2012). Besides, the polar nature of the starch enhances the strong adhesion between HA and starch (M.S. Sadjadi, M.Meskinfam, 2015). Aforementioned, different botanical sources of starch have different properties (Charoenrein & Preechathammawong, 2012) whereby the differences are mainly due to the concentration, mixing ratio, amylose content, interactions between granules, swelling power and solubility, and granule characteristics (Seetapan *et al.*, 2015). Thus, this may improve the materials and mechanical properties of the scaffold without or with lesser additives. Starch comprised of two component which are amylose and amylopectin (Tomka, 1991) whereas the percentage of amylose and amylopectin are differ regarding the type of natural starch. In addition, amylopectin is contribute to crystallinity structure in starch.

Previously, scaffolds had been fabricated by starches such are maize, wheat, and potato. However, it would be interesting to observe the characteristics of the scaffold made from native rice starches since they may introduce new properties to scaffolds due to their different fiber, amylose, amylopectin and other substances unique to the type of rice. Malaysia is rich with her paddy fields which offer many variants and species of rice. For instance, the Balik Wangi rice is claimed to have high fiber content and this may affect the mechanical strength of the starch structure.

Few dielectric properties studies were conducted on various types of starches including tapioca, corn, wheat, rice, waxymaize, amylomaize and also basmathi rice but none are performed especially on the scaffold fabricated from starch (Ahmed *et al.*, 2007; Ndife & S, 1998). Thus, it is hope that by venturing the dielectric properties of scaffold to analyze its porosity may contribute to the new method in examining the porosity in scaffolds. In addition, it is hope that the Malaysian rice starch used in this research may able to produce rice starch-nHA scaffolds with superior materials and mechanical properties.

1.3 Objectives

- To fabricate tissue engineering scaffolds based on the rice starch from Malaysian native origin.
- To investigate the variations of the scaffolds' mechanical strength due to the ratio and the type of rice used in this study.
- To measure the dielectric properties of scaffold.

1.4 Scope

The scope of this study is to reinforce hydroxyapatite with native starch originated from Sarawak, Malaysia. The type of the rice starches used are Balik Wangi rice, Bubuk Wangi rice, and Bario rice. Solvent casting and particulate leaching is used as the method to fabricate the bone tissue scaffold. Here, sodium chloride (NaCl) is chosen as the porogen agent to create pores in the scaffold. A series of characterizations were performed which include the dielectric measurement is performed to quantify the dielectric value of the scaffold and this will be used later to measure the porosity indirectly. Scanning Electron Microscope (SEM) is used to observe the morphology and to measure the pores size. The mechanical testing (compression) was done to obtain the young's modulus of the scaffolds. The Fourier Transform Infrared Spectroscopy (FTIR) is to get the information about the group interactions between hydroxyapatite (HA) and starch. Last but not least, X-Ray Diffraction is to observe the crystallinity structure of the scaffolds.

1.5 Thesis organization

This thesis reported the fabrication of rice starch-nHA composite scaffold and its characterization. This thesis consists of five chapters which are introduction, literature review, methodology, result and discussion and lastly, conclusion.

Chapter 1 reviews the overall introduction of this research such background of studies, problem statements, scope, and objectives.

Chapter 2 covers on the literature review of the journals and articles regarding the scaffold and its characterization that have been done by the previous studies.

Chapter 3 covers the methodology for the project. Here, method via solvent casting and salt leaching is done to fabricate rice starch-nHA composite scaffold is explained in details.

Chapter 4 covers the result and discussion. The result and discussion is on the liquid displacement analysis, the dielectric properties analysis Scanning Electon Microscopic (SEM) analysis, Young's modulus analysis, Fourier Transform Infrared (FTIR) analysis and last but not least is the X-Ray Diffraction (XRD) analysis.

Chapter 5 is the overall summary and conclusion on this research and suggestions for the possible future works.

CHAPTER 2

LITERATURE REVIEW

2.1 Bone disease and tissue engineering

Bone disease and trauma had inspired several techniques invented in orthopedics to treat these issues. For instance, in traumatic lesions of cartilage and bone, microfracturing technique is used to overhaul the articular cartilage, and autologous chondrocyte implantation is applied as cell therapy. In the late 1990's, researches on reconstruction and re-establishment of organs and tissues are established by understanding the growth factor and the applications of stem cells. These had contributed to the advancement of the field of tissue engineering and regenerative medicine (Cancedda *et al.*, 2003).

Autografts, allografts, and xenografts were applied as a strategy in bone tissue engineering. Autografts is transferring of tissue graft from one part to another part in the same individual body. It demonstrates no adverse immunologic effect due to sufficient osteogenic capacity; hence new bone generation will take into action. Unfortunately, autografts has several limitations. For instance, autografts' is relatively scarce due to its accessibility where it can cause morbidity to the donor site and this may lead to lesser tissues that can be available for harvest (Frohbergh *et al.*, 2012). Besides, it requires second surgical procedure and this would give serious pain to the patient (O'Brien, 2011). Furthermore, this may inflict nerve damage, and infection at the implantation site.

Allograft on the other hand is transferring of tissue graft to the recipient which is same species but identically different. It promotes certain advantages such as no need for additional surgery, able to accommodate the deficiency of bone, and higher availability. However, in comparison to autografts, allografts and xenografts are more immunogenic and less osteoinductive which may lead to non-merging with the host tissue (Van Blitterswijk *et al.*, 2008). In addition, the possibility of HIV and hepatitis transmission is high if allografts is used (Sopyan *et al.*, 2007). Furthermore, xenografts might impose zoonotic related disease transmissions which hinder its application. In response to the issues of these bone grafts, artificial substitute for tissue engineering scaffolds using engineering materials such as metals, ceramics and polymers had been considered.

2.2 Tissue engineering scaffold

Bones tissue engineering is one of the approaches to reconstruct the natural bone which were defected due to disease, trauma, or surgery (Azami, Moztarzadeh, & Tahriri, 2009). Materials which are biodegradable upon time and do not impose immunologic effects are favorable. For instance, metallic implants might posed the risk of toxicity towards patient due to corrosion (Park J.B., 1992). Metallic material is stiffer hence demonstrate better modulus value compared to the natural bone. Unfortunately due to this fact, stress shielding effect existed and this will cause osteoporosis and thinner new bone tissue during healing process thus causing the bone fracture to be high (Matsusue Y *et al.*, 1992).

Scaffold is commonly produced from biocomposites which serves as temporary or permanent artificial Extracellular Matrices (ECM) to accommodate cells and support the 3D tissue regeneration. Extracellular Matrices (ECM) is a blend of macromolecules (protein) around the cells which acts as space filler. Ideally, scaffold acts as template for the tissues growth in 3D and the structure of biocomposite scaffold should have an interconnected macroporous network specialized for vascularisation, nutrient delivery, and tissue ingrowth (Spurlin *et al.,* 2009).

Scaffolding in tissue engineering imposed several advantages such as the capability of host cells colonization on the scaffold at specific site area of implantation and minimize the surgery needed (Costa-Pinto, Reis, & Neves, 2011). Hence, the biomaterials have to be biodegradable in order to match the period of new matrix production (Leong, 2008). Starch is one of the biomaterials which is known to be biocompatible, low in cost, good biodegradability and non-toxic (A. Rodrigues & Emeje, 2012; Xie *et al.,* 2013). Besides that, there is a wide application of starch in biomedical field especially for bone replacement, bone cement, and bone tissue engineering (Sundaram *et al.,* 2008).

2.3 The mechanical properties of the bone tissue scaffolds

In tissue engineering, the mechanical properties of the tissue scaffold need to be considered while scaffolding. Basically, scaffolds need to exhibit the same mechanical properties with the implanted site but, it need also to be strong enough to allow surgical handling during implantation (O'Brien F, 2011). For the respective host tissue, it should match with the post-processing properties so that it will achieve the maximum performance of intrinsic mechanical properties (Leong, 2008). For the human bone, it can be divided into two categories which are cortical and trabecular bone (Yaszemski *et al.,* 1996) which is also known as spongy bone (Gibson, L.J., and Ashby, 1997). These bones differ in term of its porosity, for instance, the porosity for the cortical bone is less than 30% (McCalden *et al.,* 1993). The porosity of the cortical bone is about 5%-10% (U. Vidyarthi, P. Zhdan, 2007) while the porosity of the trabecular bone is about 70%-95% where the porosity of the end of the long bone is about 50%-90%. So, the cortical bone are more stronger compared to trabecular bone as it has higher porosity (U. Vidyarthi, P. Zhdan, 2007; Volokhina *et al.,* 2003). Porosity in scaffolds highly affected its mechanical strength whereby the scaffolds with high porosity are more fragile compared to the less porous scaffolds. Thereby, it's crucial in maintaining connecting porosity and mechanical strength of scaffolds at equilibrium (Mallick, Tripathi, & Srivastava, 2015). Thus, researchers had explored the possibility of fabricating porous scaffolds by using polymers, ceramics, composites, and metals. The density of the bioceramic materials resembles the strength of cortical bone while different polymers mimic the strength of cancellous bone. Nevertheless, scaffolds based of ceramic polymer composites are weaker in mechanical strength compared to the real bone. Even though porous metallic scaffolds comply the mechanical criteria of bone, it is fail to furnish the tissue integration at implantation site besides engender the metal ion leaching conflict (Scaffolds & Kadeval, 2014). Table 2.1 below shows the young's modulus of the scaffolds fabricated by several types of materials.

Table 2.1: Modulus value for scaffolds with different types of material

Biocomposite		Modulus (MPa)	Reference
Hydroxyapatite	PLLA	10 to 14	(Velasco *et al.,* 2015)
	PLGA	2 to 7.5	
	Collagen	0.44 to 2.82	
	Chitosan	1.9 to 14.4	(T. Rodrigues, Silva, & Malafaya, 2006a)

2.4 Starch

2.4.1 General application of starch

One of the material which has excellent biocompatibility, low in cost and great degradability is starch (M. Li *et al.*, 2015). Starch is also suggested for further application in bone tissue engineering scaffold as it has sufficient mechanical properties (A. Rodrigues & Emeje, 2012). Starch could be used in large scale especially as a biomaterial. J.W. Lawton *et al.* investigated the values of ultimate tensile strength (UTS), percent elongations at break (%E), tear resistance, and impact strength of the starch film. The film comprised of 41% starch, 41% PVA, 15% glycerol, and 3% of poly (ethylene-co-acrylic acid) EEA. Different types of starches were used by Lawton *et al.* which are normal corn starch, waxy corn starch, high amylose corn starch (50% amylose and 70% amylose), wheat starch, potato starch, and tapioca starch. The result shows that as the relative humidity increased, the percent of elongation (%E) had increased and the ultimate tensile strength (UTS) was decreased. Whereas, the starch with high content of amylose has better stability on the percent of elongation in regards to various values of relative humidity. Apparently, corn starch with high amylose promotes better properties in comparison to other types of starches.

Bijan Nasri-Nasrabadi *et al.* had prepared scaffolds made from starch/cellulose nanofibers using salt leaching and film casting technique. Ideally, scaffold should attain favorable properties such as pertinent porosity for cell migration, hydrophilicity, and degradable period of scaffold. Cellulose was added to induce these demands where the cellulose is from the extracted rice straw with 70% of diameter and in the range 40nm to 90nm. Here, the water uptake was improved after adding 10% cellulose nanofibers. Besides that, scaffolds have the ability to enhance the young’s modulus and tensile strength which directly enhance its mechanical properties. Good mechanical properties