Preliminary Analysis Using Dielectric Properties to Characterize nHA/starch Based Scaffolds

by

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LIST OF ABBREVIATIONS

nHA	nano-Hydroxyapatite
NaCl	Sodium Chloride
$NaHCO_3$	Sodium Bicarbonate
FESEM	Field Electron Scanning Electron Microscope
IR	Infrared
XRD	X- ray Diffraction
PNA	Performances Network Analyzer
PCL	Polycaprolactone
SEVA-C	Poly (ethylene –co-vinyl alcohol)
PLGA	Poly (lactic – co- glycolic acid)
PZT	Lead Zirconate Titanate
GA	Glutareldehyde
VNA	Vector Network Analyzer
MUT	Material Under Test
KBr	Potassium Bromide
FTIR	Fourier Transfrom Infrared
XRD	X-Ray Diffraction
JPDS	Joint Committee on Powder Diffraction Standard

LIST OF SYMBOLS

μm	micro meter
ε	Porosity
ε’	Dielectric constant
ε”	Loss factor

Preliminari Analisis Menggunakan Ciri Ciri Dielektrik Untuk Mencirikan Kerangka nHA Berasaskan Kanji

ABSTRAK

Dalam kajian ini, kebarangkalian sifat dielektrik sebagai kaedah alternatif untuk mencirikan sifat-sifat fizikal perancah berasaskan nHA/kanji telah dijalankan. Hubungan antara sifat dielektrik dan sifat-sifat fizikal bahan dari kajian latar belakang yang berbeza telah disiasat, bagaimanapun, ia tidak lagi diaplikasikan untuk pencirian kerangka dalam bidang kejuruteraan tisu. Empat jenis sampel kerangka nHA yang berasaskan kanji dengan nisbah yang berbeza antara nHA: tepung jagung telah dihasilkan iaitu 20: 80, 30:70, 40:60 dan 50:50. Melalui pemerhatian yang dijalankan, apabila jumlah kanji meningkat, kerangka dapat menghasilkan corak liang yang lebih terususun dengan mikrostruktur yang mempunyai banyak liang. Kerangka dalam kajian ini mencatatkan peratus keliangan dalam lingkungan 68% hingga 80%. XRD dan FTIR mengesahkan bahawa, apabila jumlah kanji ditingkatkan, interaksi molekul antara nHA dan kanji jagung telah berlaku. Berdasarkan pengukuran dielektrik, dielektrik berterusan dan kehilangan faktor menurun disebabkan oleh susutan dalam polarisasi dielektrik. Ia telah disiasat polarisasi dielektrik menurun mungkin disebabkan oleh pembangunan mikrostruktur perancah dan interaksi molekul antara nHA dan kanji yang dipengaruhi oleh peningkatan jumlah kanji.

Preliminary Analysis Using Dielectric Properties to Characterize nHA/Starch Based Scaffold

ABSTRACT

In this study, the possibility of dielectric properties as an alternative method to characterize the physical properties of nHA/starch based scaffolds has been conducted. The relationship between dielectric properties and physical properties of materials from different background studies has been investigated, however, not yet applied for scaffold's characterization in tissue engineering field. Four types of samples of nHA/starch based scaffolds with different ratio between nHA:cornstarch were fabricated which is 20: 80, 30:70, 40:60 and 50:50. As amount of starch increased, nHA/starch based scaffold was able to create well-developed pore's pattern with more porous microstructure. nHA/starch based scaffolds recorded porosity value in the range of 68% to 80%. XRD and FTIR confirmed that, as the amount of starch increase, the molecular interactions between nHA and corn starch has occurred. Based on the dielectric measurement, the dielectric constant and loss factor were decreasing due to the decrement in the dielectric polarization. It was investigated that, the dielectric polarization was decreased possibly due to the development of scaffold's microstructure and molecular interaction between nHA and starch which is influenced by the amount of starch.

CHAPTER 1

INTRODUCTION

1.1 Introduction

The goal of tissue engineering is to develop special biological construction which is similar to real tissue and organ including skin (M.Alizadeh *et al*.,2013) , cartilages (Nasri-Nasrabadi *et al*., 2014), bones (An *et al*., 2012, Niccoletta *et al*., 2011 & Sadraie *et al*., 2014) , soft tissue (Hamid *et al*., 2010), cardiac tissue (Sin *et al*., 2010) and other organs of the human body. Hence, scaffold work as temporary synthetic structure on which they are able to proliferate. Without scaffold, the cells will be free to float, thus they are unable to connect to each other, communicate and later on, to form tissues. Thus, with an appropriate scaffold, they would have a structure to grow on which is needed for a period of time.

Scaffolds can be fabricated by using various types of materials including ceramics, synthetic polymer and natural polymer (Wiesmann & Meyer, 2009, Chen, 2013 & Lee, Kasper, & Mikos, 2014). Ceramics such as nano-hydroxyapatite (nHA) powder has been used widely as bone tissue substitute or scaffold because it has similar structure and chemical compound to bone since one of the major component in natural bone is nano-hydroxyapatite (nHA) itself (Azami *et al*., 2012, An *et al*., 2012, E. Engle *et al*., 2009 & Berzina Cimdina & Borodajenko, 2012).

Apart from that, natural polymer also has been used extensively as a material for scaffold production. Natural polymer such as alginate, gelatin, chitosan and collagen (Chen, 2013) have been used as primary materials for tissue engineering scaffold due to their bioactive properties which are able to stimulate good interaction with cells and then enhancing cell's performance during regeneration of tissues (Brahatheeswaran Dhandayuthapani *et al*., 2011). Starch, also, which is one of the natural polymer (Hsieh & Liau, 2013) is consists of amylose and amylopectin (Gomes, Salgado, & Reis, 2002 & Ochubiojo & Rodrigues, 2012).

Moreover, in order to fabricate the three dimensional scaffold, different methodology have been introduced. Here, nHA/starch based scaffolds were fabricated by using solvent casting/particulate leaching technique. Solvent casting/particulate leaching technique is an easier and straightforward approach to create porous scaffolds as this technique must use porogen such as sodium chloride (NaCl) and sodium bicarbonate ($NaHCO_3$) (V. Cannillo *et al*.,2010) to create pores. The usage of sodium chloride (NaCl) as porogen agent is a possible choice to fabricate nHA/starch based scaffold because it is cheap, easily available and able to dissolve in solvent (Ilyas *et al*., 2013).

Scaffold need to have these basic requirements such as porous structure, an adequate mechanical strength that match with implanted site, biocompatible and biodegradable (Bose & Roy, 2013) for tissue development. Generally, phase composition and porous structure are the major factors effecting the dielectric properties for porous composite (Feng *et al*., 2016). For this reason, a correlation between the dielectric properties of the scaffold and its physical characteristics may give additional information to the quality of tissue engineering scaffold fabricated. Here, ceramics such as hydroxyapatite (HA) powder has been selected to be combined with corn starch, which is expected to give further information to the physical-chemical properties and dielectric properties of the nHA/starch based scaffold.

1.2 Problem Statement

The relationship between dielectric properties with physical properties of materials from different background studies have been investigated, however, this has not yet applied for scaffold's characterization in tissue engineering field. Therefore, in this study, the dielectric properties are presumed to be used as an alternative method to characterize the scaffold's properties. Currently, the characterization of scaffold such as its porosity is assessed using scanning electron microscope. Another method which is inexpensive and easy to be applied is by analyzing the porosity through liquid displacement method. However, the major disadvantage of this method lies in its dependency on naked eyes reading which can be easily influenced by human errors.

Hence, the characterization of scaffold through dielectric measurement could be a reasonable alternative since dielectric properties measurement is dependent on the physical properties (composition and microstructure) of the medium. If the relation between dielectric properties and properties of the materials is known, the characteristic of the scaffold especially its porosity could be determined by measuring the energy propagation of the scaffold. Furthermore, it is expected that the dielectric constant would change accordingly to the microstructure of the biomaterials (X. Li *et al*., 2009). For example, BN/Si_3N_4 composite material has lower dielectric constant and loss factor due to its porous structure and phase composition and this had contributed to better dielectric properties (Feng *et al*., 2016). Hence, due to this issue, the study about the relationship of the physical properties and the dielectric characteristics of nHA/starch based scaffold is a new application for dielectric properties measurement and the findings can be applied as a basis of reference for measuring the porosity of biomaterials.

1.3 Objectives

a) To investigate the physical properties nHA/starch based scaffolds by using Field Emission Scanning Electron Microscope (FESEM) and Archimedes principle.

b) To identify the chemical composition and phase properties of nHA/starch based scaffold through X-ray Diffraction (XRD) and Fourier Transform Spectroscopy (FTIR).

c) To analyze the dielectric properties of nHA/starch based scaffold by using Performance Network Analyzer (PNA) with the frequency ranges from 8.4 – 12.8 GHz.

1.4 Scopes

The scaffold's morphology was determined through macro- and microstructures characterization which is identified by using Field Emission Scanning Electron Microscope (FESEM) and the porosity percentage (the percentage of void content present in the scaffold's body) through Archimedes principle. The composition of the nHA/starch based scaffolds was examined by through Fourier Transform Infrared Spectroscopy (FTIR) and X-ray Diffraction (XRD). Dielectric Properties of nHA/starch based scaffold was measured by using Performance Network Analyzer (PNA) with the frequency ranges from 8.4 – 12.8 GHz.

CHAPTER 2

LITERATURE REVIEW

2.1 Bone Tissue Engineering Scaffold

The body has the ability to heal itself through recovering and regeneration when it is injured or diseased (E. J. Lee et al., 2014). However, in order to heal, the cells in the body need a support system so that they would able to regenerate. Scaffold is a three dimensional structure and it is designed to have porous architecture (J. Igwe *et al*., 2011 & Livne & Srouji, 2012). Generally, scaffold is important to facilitate cells proliferation, differentiation, orientation and migration as well as mineralization of the cells in order to become tissues. Its architecture will determine the shape and the functions of newly generated tissue because it provides basic structure for cells to form three dimensional tissue (J. Igwe *et al*., 2011, Livne & Srouji, 2012, Wiesmann & Lammers, 2009).

Therefore, scaffolds for tissue engineering must follow stringent conditions in order to induce the regeneration of new functional tissues. Scaffolds should be biocompatible, biodegradable (the ability of scaffold to decompose), possessed high mechanical strength that can match the implanted site and have interconnected porous network. Pore sizes and porous environment in scaffolds are crucial for regeneration of cells because highly interconnected porous environment can support the cellular infiltration and this may allow proper exchange of nutrient and waste product in every part of the scaffold (R.Tran *et al*.,2011). Scaffold for osteogenesis (bone formation) should mimic bone morphology, structure and function in order to optimize integration into surrounding tissue. Thus, higher porosity around 50% - 90% is anticipated to promote osteogenesis (V.Karageorgiou & D. Kaplan 2005) in order to provide large surface area that allow cell to adhere and increase the amount of ion exchange and bone-inducing factor adsorption (Hannink & Arts, 2011 & O'Brien, 2011).

There are many suggestions regarding to the optimum pores size that scaffold should achieve in order to assist in tissue regeneration. Table 2.0 shows the summary of pore size according to previous studies.

Table 2.0: The summary of pores size based on previous study

Description of pore size	References
Scaffold should have macropores and micropores	(Mehrabanian & Nasr-Esfahani, 2011 & Bose & Roy, 2013)
Minimum pores sizes for scaffold is 100µm	(Karageorgiou *et al.*, 2005, Cannillo *et al.*, 2010) & Hannick & Arts, 2011)
Optimum pores size should be 100µm – 135µm	(O'Brien *et al.*, 2007)
Pores size >300µm are acceptable for bone development and vascularization within the scaffold	(O'Brien *et al.*, 2007)

A study about the porosity of a scaffold showed that, it should has macropores exceeding more than 150 µm and micropores lesser than 50µm (Mehrabanian & Nasr-

Esfahani, 2011). Current researches have showed that porous scaffolds including both microporous and macroporous are able to perform better than only having macro pores alone (Bose & Roy, 2013). Furthermore, Hannink & Arts (2011) had reported that, the minimum required pore size for scaffold in bone tissue engineering is 100 µm. A different study demonstrated by O'Brien *et al.* (2007) recognized that, the optimum recommended pore size for bone scaffold should range from 100µm - 135µm for sufficient diffusion of nutrient and oxygen to the bone within the scaffold in order to ensure survivability of the cell (Bose & Roy, 2013). Moreover, scaffolds with minimal pore sizes about 100µm in diameter is said to be suitable material to regenerate tissues (Cannillo *et al*., 2010). This study also has been supported by another research by Karageorgiou *et al*. (2005), that 100µm is the least sizes of the pores required for cell migration, vascularization and nutrient transportation.

However, subsequent investigation has shown well-developed bone tissue with scaffolds that had bigger pores than 300µm. Besides, it is suggested that, pore sizes bigger than 300 µm are acceptable for bone development and vascularization (the formation of blood vessels) within the scaffold and suitable for non-load bearing application (O'Brien *et al.,* 2007). In addition, Mantila Roosa *et al*. (2010) mentioned that larger pore sizes would yield immoderate void space and could affect the mechanical strength of the scaffold. Hence, it can be concluded that, both micro- and macropores give significant values for bone regeneration where, larger pores can direct bone formation, since they enable the vascularization and high oxygenation essential for bone regeneration, while smaller pores propagate osteochondral ossification (the formation of bone naturally). In addition, the pores size of the scaffolds need to be balanced in order to maintain mechanical properties of the bone scaffold and to ensure the diffusion of nutrient and waste product to every part of the scaffold.

Biocompatible materials should not induce toxic or injury effects at the implanted site that able to give corresponding effect on the biological system, in fact, it should generate appropriate beneficial tissue response and can optimize the clinical performance of the therapy (O'Brien, 2011 & Q.Chen, 2013). In bone tissue application perspective, the scaffold as bone substitute is said to have biocompatibility when it allows bone cells to adhere to itself and indicates that the cells has ability to function properly. In addition, the scaffold should permit the bone cells to migrate to the surface and hence through the scaffold and they will begin to reproduce. Besides, the scaffold will not respond towards immune reaction after implantation in order to prevent inflammatory reaction that might reduce healing process and thereafter, might be rejected by the body (O'Brien, 2011).

Since the scaffold is temporarily implanted within the body, it must be biodegradable which means, the scaffold has capability to decompose by biological action of the body and will not cause harm to the implanted site as well as body. Specifically, the biomaterials is said to be biodegradable when it has faster rate of degradation since the scaffold is only used as temporarily template for bone growth and the product of the degradation process will not cause any harm around the implanted site (Karageorgiou *et al.,* 2005). In term of mechanical strength, ideally, the scaffold must exhibit similar mechanical properties with the implanted site, practically, the scaffold should be strong enough to allow surgical handling during implantation (O'Brien, 2011). The research in tissue engineering is still ongoing in discovering appropriate materials for an ideal bone scaffold which have similar properties like the real bone. Scaffolds usually fabricated by using different types of materials including ceramics, synthetic polymer and natural polymer (Wiesmann & Meyer, 2009, Chen, 2013 & Lee, Kasper, & Mikos, 2014). These materials normally will be selected, combined and matched in order to produce scaffold based on the intended application (R. Dorati *et al*., 2010). Besides, these materials have their own advantages and disadvantage for example, synthetic polymers are widely used in clinical application especially for surgical suture. However, this material does not have many advantages since it does not exhibit similar chemical properties which familiar to cells. In summary, Table 2.1 shows the advantages and disadvantages of each biomaterials involve in scaffolds fabrication.

Table 2.1: The advantages and the disadvantages of biomaterials for tissue scaffolds (O' Brien, 2011)

Biomaterial	Advantages	Disadvantages
Synthetic polymer	-Easily fabricated -Degradation rate can be controlled	-Poor biocompatibility -Degradation process of PLLA and PGA may cause tissue necrosis
Ceramics	-High mechanical stiffness -Excellent biocompatibility	-Difficult to be shaped for implantation due to its brittleness -Have limited clinical application -Cannot sustain mechanical loading needed for remodeling the tissue
Natural polymer	-Biologically active -Can promote excellent cell adhesion and growth	-Poor mechanical properties

nano-Hydroxyapatite (nHA) is categorized as ceramic that are commonly studied, tested and used for clinical application (Berzina-Cimdina & Borodajenko, 2012). It is a favorite selected material for bone tissue scaffold due to its chemical and structural resemblance of real bone (Elisabett *et al*., 2009). nHA belongs to the calcium phosphate group (Chen, 2013) and it has a molecular formula of $Ca_{10}(PO_4)_6(OH)_2$ (Swetha *et al*., 2010). It is has been generally accepted as a bioactive material to guide the formation of bone tissue (Ghomi, Fathi, & Edris, 2011). This can be manifested by the development of bone-like apatite on the surface of nHA scaffold with apatite formation filled inside its pore, which showed that nHA scaffold has an excellent bioactivity after been immersed in simulated body fluid (SBF) within 28 days. This has proved that nHA is biocompatible and could exhibit osteoconductive properties which is favored by real tissue (Ghomi *et al*., 2011). However, because of its high brittleness and low mechanical strength (Francesca, Alessandro, & Giuseppe, 2013), if it is used solely, HA as a bone scaffold material solely is a failure as shown by S. Sadraie *et al*. (2014).

S. Sadraie *et al*. (2014) showed that, nHA scaffold which has been prepared by using gel casting and sponge replicating method was not suitable to be used as a bone scaffold but can only be applied as a filler in the defect area of the bone since the scaffold exhibited only low mechanical properties, where the compressive strength is 21.45±0.5 MPa and the Young's Modulus is 2.05 MPa whereas, the mechanical strength of a scaffold should be equivalent or better than the implanted site (Elisabett *et al*., 2009) in order to support the formation of the new tissue (Gerhardt & Boccaccini, 2010). Moreover, there was another proof which showed that nHA scaffolds do not display adequate mechanical support. Ghomi *et al*. (2011) fabricated porous nHA scaffold by using gel casting method and he found that the nHA scaffold has inadequate mechanical strength that was needed to support the regeneration of a new bone tissue. The nHA scaffold was poor in compressive strength and elastic modulus if compared to the actual strength of a real bone. In both case studies of nHA scaffold conducted by S.

Sadraie *et al*. (2014) and Ghomi *et al*. (2009) show that, the technique used to fabricate porous scaffold for their experiment had developed high porosity with larger pores sizes. However, poor mechanical properties are not only attributed solely by the fabrication technique, but also due to the properties of nHA too. The potential of nHA scaffold as bone scaffold had been evaluated and further improvement to the nHA scaffold's properties had been carried out. Other initiatives had been ventured by different researchers, where nHA has been combined with other potential materials ranging from natural polymer, synthetic polymer and ceramics itself (M.W Laschke *et al*., 2010, J.Liuyun *et al*., 2009, Peter *et al*., 2010, A. Mahmoud Azami *et al*., 2010, Azami et al., 2012, An *et al*., 2012).

One of the materials that have been combined with nHA is starch. Starch is classified as a biocompatible natural polymer (Rossella *et al*., 2010). The advantages of starch as potential material for bone tissue scaffold are still yet to be ventured. It can be found from different sources such as maize (corn), wheat, tapioca, rice and barley. Starch is composed of two types of polysaccharides; amylose and amylopectin. Amylose is a linear branched polymer consists of α-(1-4) linked anhydroglucose units while amylopectin is a highly branched polymer compromising of α-(1-4) anhydroglucose linked with α-(1-6) branch linkages as shown in Figure 2.0. The proportion of amylose and amylopectin is dissimilar based on the source of the starch (Robyt, 2008 & Shogren, 1998). For example corn starch has 25% of amylose 75% of amylopectin while rice starch has 19% amylose with 81% of amylopectin and tapioca has 17% amylose with 83% of amylopectin (Robyt, 2008). Besides biomacromolecules such as amylose and amylopectin, slight amounts of protein, lipids and phosphorus are also available in the starch composition (F.Xie *et al*., 2013).