OPTIMIZATION AND BIOCOMPATIBILITY OF ALUMINA FOAM COATED SCAFFOLD FOR BONE TISSUE ENGINEERING APPLICATION

NOR SUHAIDA BINTI SHAHABUDIN

UNIVERSITI SAINS MALAYSIA

2016

OPTIMIZATION AND BIOCOMPATIBILITY OF ALUMINA FOAM COATED SCAFFOLD FOR BONE TISSUE ENGINEERING APPLICATION

by

NOR SUHAIDA BINTI SHAHABUDIN

Thesis submitted in fulfillment of the requirements
for the degree of
Master of Science

May 2016

ACKNOWLEDGMENT

Alhamdulillah, all praise to Allah S.W.T., the Most Beneficent and Most Merciful, for giving me to embark on my MSc. study and for completing this long and challenging journey successfully. I would like to express my sincere appreciation to the School of Materials and Mineral Resources Engineering, Universiti Sains Malaysis (USM), all academic staff, and technical staff for the well-equipped facilities, assistances and healthy working environment. I am very thankful to the Institute of Postgraduate Studies for providing me the financial support through the USM Graduate Assistant (GA) scheme.

I wish to express my deepest gratitude to Prof. Dr. Zainal Arifin Ahmad, who has served as my main supervisor. He gave me the resources and freedom to explore this research topic and guided the development of my experimental and theoretical of my thesis. His experience, insight, and guidance were critical components of my developments as an engineer. I truly admire his positive energy and admiration and dedication towards research. I would also like to thank Dr. Norazharuddin Shah Abdullah who served as the co-supervisor and constantly helped me during statistical analysis of this study. I really appreciate for helping me with his immense knowledge in field of tissue engineering.

I further thank to Assoc. Prof. Dr. Azman Seni from Toxico-Path Service Unit AMDI USM, for his valuable suggestions and considerations regarding on cytotoxicity study. I acknowledge as well the Ministry of Higher Education through the My Brain 15 sponsorship. I owe my special thanks to Dr. Abdul Rashid

Jamaludin, Dr. Wan Fahmin Faiz Wan Ali, Dr. Afifah Mohd Ali, Kho Chun Min, Mohamad Johari Abu, Nor Fatin Khairah Bahanurddin, Mohd Fariz Ab. Rahman, Hamdan Bin Yahya and members of Ceramics lab for their valuable friendly conversations, technical assistance and in valuable support throughout my master's thesis.

My special love and thanks go to my mother, Siti Aminah binti Wahab and my beloved family. Thanks for educating me and for the unconditional support and encouragement.

Suhaida Shahabudin
May 2016

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AF	Alumina Foam
ANOVA	Analysis Of Variance
BTE	Bone Tissue Engineering
CCD	Central Composite Design
DOE	Design of Experiment
DF	Degree of freedom
FCC	Face Centered Cube
HACAF	Hydroxyapatite coated Alumina Foam
HABCAF	Hydroxyapatite layer by Bentonite in between coated Alumina Foam
IC_{50}	50% Inhibitory Concentration
L929	Fibroblast mouse cell
MS	Mean square
MTS	Cell Proliferation Assay
PFR	Polymer Foam Replication
PU	Polyurethane Foam
ppi	Pores per inch
PVA	Polyvinyl Alcohol
RSM	Response Surface Method
SBF	Simulated Body Fluid
SS	Sum of square
TCP	Tri-calcium phosphate

LIST OF SYMBOLS

2^K	2- level
K	Number of factors
n_c, s	Center points
P-value	Probability value where $p < 0.05$

PENGOPTIMUMAN DAN BIOSERASI PERANCAH BUSA ALUMINA BERSALUT BAGI APLIKASI KEJURUTERAAN TISU TULANG

ABSTRAK

Perancah busa alumina (AF) bersalut adalah salah satu bio sintetik yang mendapat perhatian daripada ahli-ahli sains bahan bagi mengatasi sifat lengai supaya interaksi ikatan tisu tulang dipertingkatkan. Kajian ini bertujuan untuk menghasilkan AF bersalut dengan kekuatan mampatan yang lebih tinggi daripada 2 MPa kepada 12 MPa, keliangan yang lebih tinggi daripada 70% kepada 99% dan saiz liang yang besar daripada 100 µm hingga 1000 µm yang diperlukan dalam aplikasi kejuruteraan tisu tulang. Faktor- faktor penting dalam teknik replikasi busa polimer (PFR) adalah jumlah liang (ppi) busa polimer (PU), nisbah komposisi pepejal kepada air ternyah-ion, peratusan pengikat dan kekerapan proses mecelup. Analisis DOE menggunakan rekabentuk pecahan 2^k faktorial menunjukkan jumlah liang, nisbah komposisi dan bilangan mencelup adalah faktor terpenting mempengaruhi kekuatan mampatan perancah AF. Merujuk kepada rekabentuk kiub berpusat muka (FCC), nisbah komposisi 60/40, 20 ppi bilangan liang dan tiga kali proses mencelup adalah syarat yang memenuhi untuk perancah tulang manusia. Perancah AF dipertingkatkan dengan salutan hidroksiapatit (HACAF) dan hidroksiapatit-bentonit (HABCAF). Sampel-sample dinilai oleh analisis in-vitro iaitu rendaman di dalam simulasi cecair badan (SBF) dan ujian sitotosik (MTS assay). Analisis in-vitro menunjukkan permukaan perancah HACAF dan HABCAF mempunyai lapisan mendakan apatit dan kesan pertumbuhan sel yang positif.

OPTIMIZATION AND BIOCOMPATIBILITY OF ALUMINA FOAM COATED SCAFFOLD FOR BONE TISSUE ENGINEERING APPLICATION

ABSTRACT

Alumina foam (AF) coated scaffold is one of synthetics biomaterials that has received much attention to overcome inertness properties for enhanced bone tissue bonding interaction. This research aims to produce AF coated scaffold with compressive strength higher than 2 MPa to 12 MPa, porosity higher than 70% to 99% and pores size are larger than 100 µm to 1000 µm which required in bone tissue engineering application. The significant factors in polymer foam replication (PFR) techniques are number of pores (ppi) of polyurethane (PU) foam, the composition ratio of solid loading to deionized water, percentage of binder and number of dipping process. The DOE analysis using 2^k fractional factorial designs shows that the number of pore, composition ratio and number of dipping are most significant factors effect on the compressive strength of AF scaffold. According to the face-centered cube (FCC) design, the condition satisfied for human bone scaffold is prepared at 60/40 composition ratio, 20 ppi numbers of pores and three times of dipping process. The AF scaffold was further improved by coated with hydroxyapatite (HACAF) and hydroxyapatite-bentonite (HABCAF). The samples were evaluated by in-vitro analysis which is immersed in simulated body fluid (SBF) solution and cytotoxicity by MTS assay. The in-vitro analysis showed the surface of HACAF and HABCAF scaffold have precipitations of apatite layer and positive effect on cell growth.

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Human body is a perfect creation. It is a combination of many structural, cellular and functional levels in one-well organized unit. The structural elements of the human body are bones. Bones can be defined as a vascularised structure of living cells (Hench, 1996; Lickorish et al., 2007). Besides providing an internal structural framework for the body, bones also protect the internal organs, and are a reservoir for minerals that serve a metabolic function.

Being hard and strong does not mean that bones do not get injured. Bone injuries such as fractures and slips, are now more prevalent in numbers. Alarmingly, over 70,000 hip fractures occur in the UK and estimated 7.9 million patients suffering fractures in the USA annually (Victoria et al., 2009; Patel et al., 2013). The increasing numbers are due to several reasons. For example, an increase in life expectancy, i.e., age increase will result in reduced the bone density, causing decrease in bone strength, allowing easier fracture. Increased amounts of trauma cases, largely due to motor vehicle accidents and other mishaps is arguably another major contributor. Other than couple, diseases (such as Paget's disease and even cancer) and failures to heal (non-unions) also contributed to the increasing numbers of bone injuries. For example, approximately 10% of fracture cases in the USA resulted in non-unions and/ or delayed unions; the number tends to be higher in developing countries. Thus, widespread attention was focused on bone repair, up to a point that WHO has declared that year 2000 to 2010 as the Bone and Joint Decade (Lidgren, 2003). Current clinical bone repair strategies (i.e., autografts, allografts and

insertion of man-made materials) are associated with various bone problems. Autografts, although being the most reliable technique, however it is exhibit significant limitations, such as lack of sources (limited transplantable option), donor site morbidity and cellular reactions such as inflammation. Allografts meanwhile, are accountable for poor osteoconductivity and immune responses. As for man-made materials, leaving a foreign material in the body is perhaps not the best solution. This is where bone tissue engineering (BTE) comes into the scene (Gomes and Reis, 2004; Jones and Hench, 2003).

Since mid-80s, BTE becomes exciting field with extreme potential in the future. Shalak and Fox (1988), and Sachlos and Czernuszka (2003), describes TE as multidisciplinary research, combining multiple areas of research such as mathematics, engineering and biology to restore of repair tissue function. Not only for cellular level repairs, tissue engineering (TE) is also hoped to find and develop biological substitutes and cellular aids (e.g., scaffolds) to improve the quality of human life (Jones and Hench, 2003; Gomes and Reis, 2004).

Scaffold act as a template and as an artificial extracellular matrix. A template should have ability to support the human weight with appropriate mechanical strength. The range of mechanical strength for cancellous types is between 2-12MPa to mimic the original bone (Takaoka et al., 1996; Ramay and Zhang, 2003; Vitale-Brovarone et al., 2009). Besides, the scaffolds induced the formation of bone by guiding new tissue growth in three dimensionality (3D) structure. The 3D structure with porosity in a range of 50 to 90% is suggested to simulate an extracellular function closely (i.e., nutrient transportation, waste removal and gas diffusion) (Hutmacher, 2000; Vitale-Brovarone et al., 2007; Bellucci et al., 2011). In addition, scaffold should exhibit pore diameter size higher than 100μm as requirement for

osteoinduction process (Jun et al., 2003). The osteoinduction stimulates osteogenesis dependent on the surface materials response. Thus, the materials of scaffold must be biocompatible with the host tissue for cell-materials interaction. The findings were proved that ceramic materials have biocompatible chemical composition for implantable in human body (Hench, 1996).

Some of ceramic materials are classified as biomaterials. Biomaterials can be categorized into natural or synthetic materials that are suitable for implantable in human body (Chevalier and Gremillard, 2009). Ceramic is exhibiting good response to human body according to their various properties such as chemical reactivity, biocompatibility and resorbable. For example, alumina (Al_2O_3) and zirconia (ZrO_2) have been widely used in BTE field (Miao et al., 2007; Yang et al., 2011). Both having high mechanical strength, high density and high wear resistance. It also promotes excellent result from load bearing application. Alumina is suggested as the most widely used in orthopaedic applications due to minimum tissue rejection after implantation. Unfortunately, alumina has inherently an inertness property which does not support cell proliferation. Therefore, alumina coated with HAp has been working since 1995 to enhance bone bonding interaction (Takaoka et al., 1996). The bioactive silica glasses, hydroxyapatite (HAp) and tri-calcium phosphate (TCP) shows good sign of biological response at the interface of material and encourage the growth of new bone (Yang et al., 2011). Other than that, bentonite also chosen as coating materials based on its influence on bioavailability, non-toxicity and various proof that it acts as good binding agent for excellent coating (Carretero, 2012).

There are various fabrication techniques to produce porous biomaterials such as foaming consolidation, gel-casting, salt leaching, polymeric foam replication and rapid prototyping (Lyckfeldt and Ferreira,1998; Hou et al., 2003; Ryan et al., 2008;

Sopyan and Kaur, 2009). Control over fabrication route could help to obtain good implantable properties. Yet, it is still arguable which techniques are most excellent and pave an interesting route for design bone scaffold. Thus, polymer foam replication (PFR) was proposed as an effective technique to fabricate porous structure has been proven with controllable pore size, controllable interconnectivity and uniform pore size distribution (Lyckfeldt and Ferreira, 1998; Sopyan and Kaur, 2009). Moreover, due to the low cost of polymer foam, the PFR is also known as profitable technique and economical (Colombo and Modesti, 1999). PFR is dependent on several factors which, properties of foam, the preparation of slurry and the fabrication parameters influenced the properties of scaffold (i.e., mechanical strength, porosity, pore diameter size, interconnectivity and biocompatibility). These properties play very important roles in nutrient transportation to encourage cell growth in 3D.

The transportation of nutrient prediction performed through modelling approaches. Current modelling approaches for transportation involved various bioreactors design models for bone tissue growth for example hollow fibre membrane, confined perfusion bioreactor, and suspended tube bioreactor (Abdullah et al., 2009). The transport can be enhanced dependent on the types of bioreactor. For example, the transport restriction on batch system can be improved by addition of perfusion or by continuously refreshing the surrounding medium (Sengers et al., 2005). Designing of bioreactor for tissue engineered is important to allow tissue formation in 3D by good support for cell attachment, proliferation and vascularisation as well as enabling sufficient nutrient supply to cells. Modelling is requires for optimization process by identifying the main governing processes for practical TE efforts. In order to meet the goal of improving nutrient transportation,