Faculty of Manufacturing Engineering

THE PREPARATION AND CHARACTERIZATION OF POLYHYDROXYBUTYRATE-CO-VALERATE TRICALCIUM PHOSPHATE SCAFFOLDS FOR BONE TISSUE ENGINEERING

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DECLARATION

I declare that this thesis entitled "The Preparation and Characterization of Polyhydroxybutyrate-co-Valerate Tricalcium Phosphate Scaffolds for Bone Tissue Engineering" is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

Signature : ……

Name : Norfatiha binti Ishak

Date : ……

APPROVAL

I hereby declare that I have read this thesis and in my opinion this thesis is sufficient in terms of scope and quality for the award of Master of Science in Manufacturing Engineering.

Signature : ……

Supervisor Name : Dr. Zaleha binti Mustafa

Date : ……

DEDICATION

To my beloved mother and father.

ABSTRACT

Ceramic scaffolds have been widely used in biomedical application to treat bone defects. Enhancing the ceramic scaffold by using polymeric materials as coating may improve their strength however it will alter their pore characteristic as well as degradation behaviour which is crucial for the success of scaffold requirement. Polyhydroxybutyrate-co-valerate (PHBV) is degradable and has excellent compatibility properties for biomaterials. Thus the objectives of this study are (i) to determine the sintering temperature for the fabrication by using polymeric sponge method (ii) to analyse the effect of PHBV coated Tricalcium (TCP) scaffold after in-vitro immersion in simulated body fluid (SBF) solution for 16 weeks (iii) to evaluate the bioactivity and biocompatibility of the PHBV coated TCP scaffolds. Polyhydroxybutyrate-co-valerate (PHBV) coated TCP scaffolds were fabricated using polymeric sponge method. Two types of calcium phosphate (CaP) were used as matrix which are commercial (C-CP) and locally (L-TCP) supplied. The CaP scaffolds were sintered at optimised sintering temperature of 1450°C. The initial mechanical strength of the commercial and in-house TCP scaffolds are 36.64 KPa and 10.06 KPa respectively. The ceramic scaffold were then coated with PHBV using dip coating method, resulting in the increased of the compression strength to 140.00 KPa (PHBV coated C-CP) and 148.00 KPa (PHBV coated L-TCP) respectively. The scaffold contained interconnect pores with a range of size from 200 to 400µm, and porosity within 81% to 83%. Their mechanical strength in simulated body fluid (SBF) solution was retained up to 12 weeks with good pore integrity structure. The formation of bone-like apatite in the shape of globular and cauliflower-like cluster was observed after 4 weeks of immersion in SBF solution. The presence of apatite mineral was confirmed by FTIR, XRD and EDX analysis indicating bioactive ability of the scaffold. Biocompatibility analysis shows that the scaffolds were able to retain Saos-2 cells after 24 hours indicating their ability to allow cells proliferation to adhere to it. The increasing cell metabolic activity up to day 14 was also observed suggested that the scaffold is compatible with cells and non toxic. This finding has indicated that the PHBV coated TCP scaffold is a compatible and comparable material that is potential to be used in bone tissue engineering.

ABSTRAK

Perancah seramik telah digunakan secara meluas di dalam bidang perubatan bio untuk merawat kecacatan tulang. Polyhydroxybutyrate-co-valerate (PHBV) adalah bahan polimer yang boleh terurai dan memiliki sifat keserasian yang baik untuk digunakan sebagai bahan bio perubatan. Kekuatan perancah seramik boleh ditingkatkan dengan menyadurnya menggunakan bahan polimer, tetapi ini akan mengubah sifat liang serta sifat keserasian perancah yang mana ianya amat penting dalam memastikan kejayaannya sewaktu proses penggunaan. Oleh itu, objektif kajian ini adalah untuk menentukan suhu pembakaran perancah menggunakan kaedah replikasi span polimer, untuk menilai kesan tempoh perendaman in-vitro di dalam larutan cecair simulasi badan terhadap sifat mekamikal perancah trikalsium (TCP) bersalut PHBV dan untuk menilai sifat bioaktif dan sifat keserasian perancah TCP bersalut PHBV. Perancah TCP yang disaluti lapisan PHBV telah dihasilkan dengan menggunakan kaedah replikasi span polimer. Dua jenis bahan mentah kalsium fosfat (CaP) digunakan sebagai matrik iaitu bergred komersial (C-CP) dan tempatan (L-TCP). Perancah CaP dibakar pada suhu optimum iaitu 1450°C. Kekuatan mekanikal permulaan perancah TCP komersial dan tempatan adalah 36.64 KPa dan 10.06 KPa. Perancah seramik kemudiannya disalut dengan PHBV dengan menggunakan kaedah penyalutan celup. Penyalutan ini meningkatkan kekuatan mampatan perancah kepada 140.00 KPa dan 148.00 KPa bagi perancah C-TCP bersalut PHBV dan L-TCP bersalut PHBV. Perancah TCP bersalut PHBV memiliki liang saling bersambung yang bersaiz di dalam lingkungan 200 - 400μm, dan peratusan keliangan 81% hingga 83%. Kekuatan mekanikal dan struktur liang perancah sewaktu rendaman in-vitro di dalam SBF adalah bertahan dengan baik sehingga minggu ke- 12. Longokan apatit dalam bentuk global dan bunga kubis terhasil seawal 4 minggu semasa proses rendaman. Kehadiran mineral apatit ini dicirikan dengan menggunkaan FTIR, XRD dan EDX yang mana mengesahkan kebolehan sifat bioaktif perancah. Analisis sifat keserasian menunjukkan bilangan sel jenis Saos-2 kekal selepas 24 jam proses pengkulturan, membuktikan keupayaan perancah di dalam membantu percambahan sel. Peningkatan aktiviti metabolik sel sehingga hari ke-14 juga diperhatikan menunjukkan keserasian perancah di dalam menampung kehidupan sel dan tidak toksik kepadanya. Dapatan ini menunjukkan bahawa perancah TCP bersalut PHBV adalah serasi dan setanding dengan sifat tulang manusia dan memiliki potensi yang tinggi untuk digunakan di dalam bidang kejuruteraan tisu tulang.

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LIST OF SYMBOLS/ABBREVIATIONS

C-CP	-	Commercial Calcium Phosphate
C-CP	-	Commercial Calcium Phosphate
CP	-	Calcium Phosphate
CS	-	Chitosan
DBM	-	Dimeneralised Bone Matrix
ECM	-	Extracellular Matrix
HA	-	Hydroxyapatite
HV	-	Hydroxyvalerate
L-TCP	-	Local Tricalcium Phosphate
MSc	-	Mesenchymal Cells
MV	-	Matrix Vesicles
PCL	-	Poly-ε-Caprolactone
PEG	-	Poly(Ethylene Glycol)
PHB	-	Polyhydroxybutyrate
PHBV	-	Polyhydroxybutyrate-co-Valerate
PHV	-	Polyhydroxyvalerate
PLA	-	Polylactic Acid
PLGA	-	Poly(Lactide-co-Glycolic Acid)
PU	-	Polyrethane

PVA	-	Polyvinyl Alcohol
SA	-	Sodium Alginate
TCP	-	Tricalcium Phosphate
TE	-	Tissue Engineering
V	-	Total Volume of Scaffold
V1	-	Total Volume of Ethanol
V2	-	Total Volume of Ethanol and Scaffold
V3	-	Residual Ethanol Volume
α- TCP	-	Alpha Tricalcium Phosphate
β- TCP	-	Beta Tricalcium Phosphate

LIST OF PUBLICATIONS

Journals

1. Mustafa, Z., Ishak, N. F., Othman, R., Ahmad, N., Toibah, A. R., Fadzullah, S. S. M. and Tanner, K. E., 2018. In-Vitro Apatite growth on porous β-tricalcium Phosphate scaffolds coated with PHBV. *Journal of Advanced Manufacturing Technology*, 12(4), pp. 135-146.
2. Othman, R., Mustafa, Z., Ishak, N. F., Kien, P. T., Shamsudin, Z., Rosli, Z.M. and Mohd Noor, A. F., 2018. Intermediate Phases Formed during Synthesis of β-Tricalcium Phosphate via Wet Precipitation and Hydrothermal Methods. *Journal of Advanced Research in Fluid Mechanics and Thermal Sciences*, 48 (2), pp. 141-147.

Proceeding

1. Ishak, N. F., Mustafa, Z., Othman, R., Sheikh Md Fadzullah, S. H. and Mahamad Sahab, A. R., 2016. Effect of sintering on the physical properties of porous β-TCP scaffolds. *Proceedings of Mechanical Engineering Research Day 2016*, pp. 135-136.

CHAPTER 1

INTRODUCTION

The introduction presented in the thesis are classified into 6 sub-chapter including background, problem statement, objectives, research scope, significance of study and structure of thesis.

1.1 Background

In the field of bone tissue engineering, further research has been conducted for the past decades to achieve the best condition in term of materials as well as processing techniques to improve their application (Li et. al., 2013). As the results, materials used for porous scaffold which is suitable for bone healing and structural support with optimum osteogenesis abilities was accomplished. Throughout bone tissue regeneratqaion, bone scaffold act as a template. Nowadays, besides controlling the porosity of porous scaffold during fabrication process technologies being used also improve the properties of the scaffold. The uniqueness of natural human bone, they are associate of both mechanical properties and architectural design form macroscale to nano scale dimension as shown in Figure 1.1. However, the porous scaffold should imitate the natures of the bone to have the desired physical properties, mechanical strength and biological properties. It also must be biocompatible with the host tissue and able to support proliferation, adhesion and secretory activities of the cell (Yang et. al., 2001).

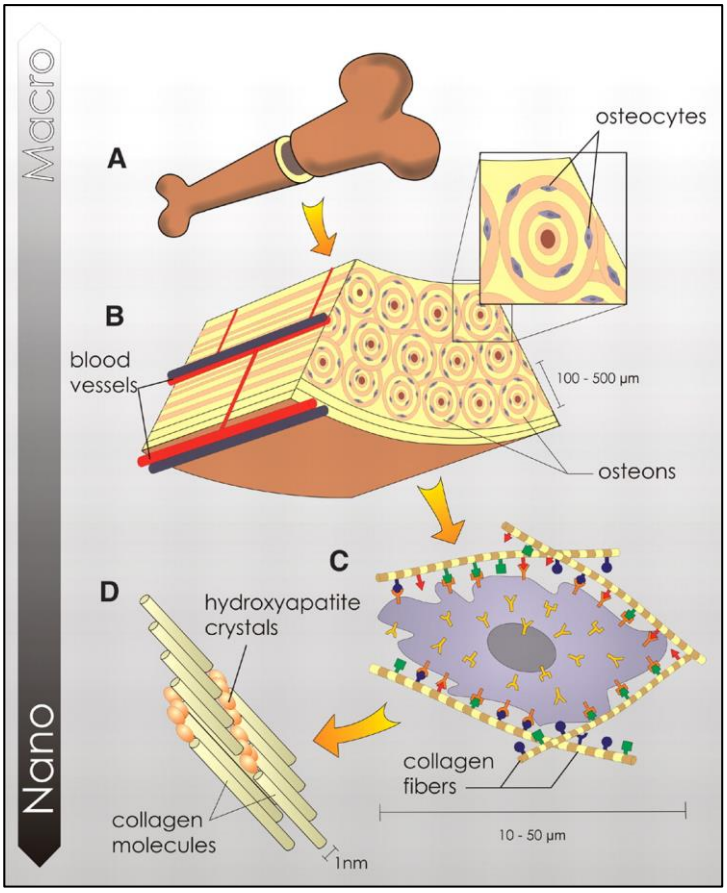

Figure 1.1: Macro to nano scale of bone cell (Li et. al., 2013)

The loss of severely damaged bone are replace through substitution either autologous or allogenic as an alternative method. This is the important aspects in regenerative medicine. The most notable studied, clinically tested and used synthetic materials based on calcium phosphate (CaP) are hydroxyapatite (HAp- $Ca_{10}(PO_4)_6(OH)_2$), β-tricalcium phosphate (β-TCP – $Ca_3(PO_4)_2$), and biphasic HAp/β-TCP mixture. CaP with high biocompatibility and bioactivity of CaP thus, make it the best candidate to fabricate porous scaffold. Otherwise, it also builds a connection between ceramic implant and bone tissue (Ge et. al., 2008). Besides that, in human healthcare, one of the most expensive cost involving tissue and organ failures which might be caused from infections, injuries and defects. And the core reason towards this problem is lack of organ and tissue donor.

Therefore, the solution that been discovered to encounter this issue is by introducing artificial substitutes and non-living processed tissues (Rai et. al., 2012). Though, the flaw of the bone or the outcome of the host tissue are not really solved by the substitutes such as lack of biocompatibility, non-bioactive material, vascularization of bone and bone remodeling (Roche et. al., 2015). Therefore, for a better result bioactive, biodegradable and biocompatible materials is a must being used in the field of tissue engineering (TE) (Puppi et. al., 2010).

There are gained in global interest of orthophosphate-based ceramics in orthopedics fixation because of the similar composition between mineral phase in human bone which promote organization of bone cell of the implants, hence they are the best alternative candidates over bio-inert materials such as chromium, cobalt, stainless steel and titanium. Within this TE field, β-TCP and HA are the most studied materials due to their greater biocompatibility and biodegradability compared to other biomaterials. However, when it comes to biodegradable composite, β-TCP are the most favored choice as in order to accommodate new bone formation because its resorption rate is better than other biomaterials.

Within regenerative medicine and tissue engineering, there are a lot of natural and synthetic polymer being used. However, according to Nair and Laurencin (2007) natural polymers has been considered to be among the first used for clinical application. According to Tang et. al. (2014), natural polymers include cellulose, chitin, silk, hyaluronic acid, chitosan, gelatin, albumin, collagen, keratin, elastin and actin. Nevertheless, because of their structural complexity, possible immunogenicity, and inferior biomechanical properties make they difficult to be used. In order to avoid the weaknesses of natural polymer, the potential candidates in tissue engineering which is synthetic polymer has been introduced. Synthetic polymer not only easy to tailor to any form for tissue engineering but

they also easy to get compared to natural polymers. In tissue engineering and regenerative medicine field, major synthetic polymers used are polycaprolactone (PCL), poly(ethylene glycol) (PEG), poly(lactic acid) (PLA), poly(glycolic acid) (PGA), poly(lactide-co-glycolic acid) (PLGA), polyhydroxybutyrate (PHB), poly(hydroxyvalerate) (PHV), poly(hydroxybutyrate- valerate) (PHBV), poly(dioxanone) (PDS), poly(propylene fumarate) (PPF), polyurethanes (PUs), polyphosphazenes, polyanhydrides, and polyacetals (Tang et. al., 2014).

1.2 Problem statement

A lot of efforts have been dedicated over the past decades in the development of biodegradable scaffolds which mimicking natural bone tissues in term of excellent biocompatibility as well as mechanical properties (Holland et. al., 2005; Wagoner-johnson et. al., 2011). As an example, according to Kang et. al. (2011), the widely studied of HA and β-TCP are to endeavor the strong biocompatibility, biodegradability, desire compressive strength of ceramics and flexibility of ductile polymers. Nevertheless, there are several methods involving in strengthening the mechanical strength of composites scaffold and one of them is polymer matrix approach as well as self-assembled mineralized collagen method (Fabbri et. al., 2010). Though by using these methods, the amount of ceramic matrix being used is limited hence, the composite's bioactivity will be affected (Bleach et. al., 2002). As instance, by increasing 10% to 40% volume of calcium phosphate which is HA, the materials become fragile so, to overcome the problem the HA content will be limited to 20% volume (Tan et. al., 2003). As a consequence, to improve the bioactivity properties, HA is being introduced with polymer to form a nanocomposite layers on the ceramic matrix (Roohani et. al., 2010) but, this method did not show in improving the strength of monolithic polymer also difficult to produce with high concentration of ceramic