BIOLOGICAL ASSESSMENT OF UNCOATED AND HYDROXYAPATITE-COATED FERUM AS BIODEGRADABLE SCAFFOLD FOR HARD TISSUE REGENERATION

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A thesis submitted in fulfillment of the
requirements for the award of the degree
of Master of Engineering (Biomedical)

Faculty of Biosciences and Medical Engineering
Universiti Teknologi Malaysia

MAC 2014

TO AYAH, MOHD DAUD OTHMAN
TO IBU, NGAMINI MARDIMIN
AND TO ALL MY BROTHERS;
MUHAMMAD ALIF
SULAIMAN
AHMAD DANIAL

WITH ALL MY LOVE

ACKNOWLEDGEMENT

In The Name of Allah, The Most Gracious, The Most Merciful

"Say: If the sea were ink for the words of my Lord, the sea would surely be consumed before the words of my lord are exhausted, though we were to bring the like of that (sea) to add"
(Surah Al Kahf: 109)

First and foremost, I would like to express my deepest gratitude to Allah S.W.T for His guidance and blessings throughout my life and the gift of strength in completing my thesis.

In particular, I wish to express my highest gratitude to my Master Degree Supervisor, Dr Hendra Hermawan for his critics, encouragement, consultations and funding throughout completing my experiment and thesis. Not forgetting my co-supervisor, Dr Asmah Hamid (UKM) and Prof Ir Dr Rafiq for their time and cooperation.

Special thanks goes to TCERG and MEDITEG group members especially kak Samsiah, for their support and informative discussion. My sincere appreciation extends to those who have provided assistance at various occasions. Their opinion and views are useful indeed.

Last but not least, I am also indebted to all my lovely family members especially my father, Mohd Daud Othman, my mother, Ngamini Mardimin and all my brothers for giving me such a valuable support and motivation.

May Allah gives His blessings to all of us and only Him could repay my debt to all of you. Amin.

And say, Lord increase my knowledge
(Surah Taha: 114)

ABSTRACT

Pure iron (Fe) and its alloys have been recently emphasized as potential biodegradable metals due to their good mechanical properties that are close to those of stainless steel 316L. This research was focused more on the study of cell-material interaction and to analyze the effect of corrosion product on cell behavior by performing degradation study. In this study, samples were prepared by coating hydroxyapatite (HA) and hydroxyapatite/poly (ε-Caprolactone) (HA/PCL) onto porous iron using dip coating method. Biosafety and biofunctionality of the sample were evaluated by using human skin fibroblast (HSF) and mesenchymal stem (MSC) cells. Analysis by Inductively Coupled Plasma- Mass Spectrometry (ICP-MS) revealed that concentration of ion Fe was decreased in the medium containing HA-coated Fe. However, the weight loss of the sample is high compared to pure porous iron and HA/PCL-coated Fe. A positive cell response to the Fe ions was revealed during the first 21 days of the cell toxicity study using indirect method. After 21 days the HSF cell viability decreased due to acidic eluates and the increase of Fe ions concentration that promoted the formation of the reactive oxygen intermediates (ROI). From the results obtained, it showed that the HSF and MSC cells exhibited higher viability when in contact with the Fe-HA and Fe-PCL/HA than with the Fe specimens. However, there is a significant decrease ($p<0.05$) of cells when cultured on three different samples after 3 days of incubation. HA-coated porous Fe also provides support for attachment of the cells. Observation under Scanning Electron Microscope (SEM) reveals that the filopodia of the mesenchymal stem cells preferred to develop onto irregular surface of HA-coated Fe. This study provided evidences of a good cell-material interaction on the porous Fe that may confirm the feasibility of using porous biodegradable ferum as hard tissue scaffolds.

ABSTRAK

Ferum tulen dan ferum berasaskan aloi telah dikenal pasti mempunyai potensi sebagai material terbiodegradasi kerana ciri mekanikalnya yang seakan-akan menyerupai *stainless steel 316L*. Namun begitu, kadar terdegradasi metal ini mengambil tempoh masa yang lama. Dalam kajian ini, sampel ferum yang poros dan disaluti dengan hidroksiapatit (HA) serta campuran hidroksiapatit/poly ε-kaprolakton (HA/PCL) menggunakan kaedah celup penyalutan (*dip coating)* telah disediakan. Hasil analisis menggunakan ICP-MS menunjukkan bahawa kepekatan ion Ferum yang diukur dalam medium yang direndam dengan ferum bersalut HA adalah rendah walaupun mempunyai pengurangan berat yang ketara berbanding sampel Ferum tulen yang poros dan sampel Ferum bersalut HA/PCL. Kajian ketoksisitan sel melalui kaedah tidak langsung ke atas ion ferum memberikan respon yang positif sehingga 21 hari. Selepas itu, viabiliti sel HSF menunjukkan penyerosotan disebabkan oleh elut medium yang berasid dan peningkatan kepekatan ion ferum yang menggalakkan pembentukan *reactive oxygen intermediate* (ROI). Seterusnya, ujian biokompatibiliti material dilaksanakan ke atas sel dengan menggunakan sel fibroblas manusia (HSF) dan sel stem mesenkimal manusia (MSC). Kedua-dua sel ini memberikan peningkatan dalam peratusan viabiliti apabila diuji menggunakan Fe-bersalut HA dan Fe-bersalut HA/PCL. Namun begitu, terdapat penurunan yang signifikan apabila sel dibiarkan selama tiga hari dengan semua sampel secara langsung. Pemerhatian di bawah SEM menunjukkan filopodia sel stem mesenkimal lebih mudah berkembang ke atas permukaan tidak rata pada ferum bersalut HA. Hal ini membuktikan bahawa sampel tersebut memberikan sokongan yang baik untuk pelekatan sel dan mengesahkan kebolehan material ferum terbiodegradasikan yang poros sebagai templat (scaffold) untuk tisu keras.

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LIST OF SYMBOLS AND ABBREVIATIONS

SEM	-	Scanning Electron Microscope
EDX	-	Energy Dispersive X-ray spectroscopy
μm	-	micrometer
DNA	-	Deoxyribo Nucleic acid
°C	-	Degree celcius
Mm	-	milimeter
w/v	-	Weight per volume
PBS	-	Phosphate Buffer Saline
Rpm	-	Rotate Per Minute
CO_2	-	Carbon Dioxide
μl	-	microliter
Nm	-	nanometer
Ppm	-	Part Per Million

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CHAPTER 1

INTRODUCTION

1.1 Background

In a case of organ function failure, biomedical implant made as a scaffold is needed to augment, repair and replace the function of affected tissue for reconstruction. The three-dimensional (3-D) scaffold should be porous, have a good mechanical strength and able to provide a necessary support for cells to perform its function [1]. Recently, biodegradable biomaterials are becoming interesting research topics due to its ability to support the healing process of a tissue and subsequently degrade on the site of implantation. Thus, degradable material represents a promising future in implant studies as they eliminate the risk of secondary surgeries. Furthermore, it will reduce the risk of refracture and stress shielding.

Studies on degradable materials such as polymer have shown excellent biocompatibility and an optimum degradation rate. However, polymer could not withstand a high strength application [2] which makes it less competence to use in the orthopaedic sector. Currently, metallic materials such as iron (Fe) and magnesium (Mg) have been introduced into the biomedical field and have received incredible interest. Iron has mechanical properties similar to bone and its degradation product is non-toxic to a human body [3].

Hydroxyapatite (HA) is a well known bioactive ceramics with nearly same chemical composition as a human bone. It has excellent biocompatibility and great bonding ability with bone structure [4]. However, shaping and implanting HA are difficult because of its weak and brittle mechanical properties. The use of HA coatings on metallic implants have been reported to stimulate bone healing. During the early stage of implantation, the coating shows improvement in the aspect of rate and strength of the metallic implant. It would speed up the rehabilitation of patients by decreasing the insertion time of implant to final reconstruction [1, 4-7]. To overcome the brittleness issue, double coating of HA with poly (ε-caprolactone) (PCL) were introduced by previous research [8]. Reportedly, the coating will become stable and flexible without experiencing crack or delamination compared to the hydroxyapatite single coating [8].

The relevant test is necessary to study the biosafety and biofunctionality of new devices by biomaterialists. Cell attachment is one of a vital part in determining the biocompatibility of a material upon implantation into the human body. This role will further gives information on the cell migration, differentiation and proliferation, thus making the iron based implant is suitable to be used in bone repair and regeneration.

So far, new interest of HA coatings on biodegradable metallic material was increasing in recent years. However, the rapid corrosion rate of Mg is the main restriction for biomedical application. HA has been shown to have the ability to decrease the corrosion rate and improve the bioactivity of Mg alloy [9-10]. Previous research demonstrates that coating of iron foam with calcium phosphate/chitosan gives mechanically remarkable results as it mimics human bone, which can minimize the stress-shielding effects [11]. Therefore, this research is going to be focused more on the study of cell-material interaction in porous iron. The porous iron was also coated with HA to see its effect on the cell behavior and its degradation study.

1.2 Problem Statement

Iron has been proposed as the potential biodegradable implant due to its mechanical properties that are similar to stainless steel. However, even it is considered as biocompatible, the range of application is limited by its toxicity at maximum concentration [3, 12]. Hydroxyapatite often stated as an osteoconductive material because of its ability to support bone tissue progression surrounding the implants and to induce fixation via chemical bonding [13]. At this point, this research explored the possibility of using iron for bone applications, whereas its bulk was minimized by forming porous structure. Hydroxyapatite (HA) and poly (ε-caprolactone)/hydroxyapatite (PCL/HA) coating was then applied to improve cell attachment and growth. HA-coated degradable implant with interconnected pores is expected to promote osseointegration without reducing its mechanical properties.

1.3 Objectives

In this study, porous Fe was coated with HA using dip coating, a simple and cost effective method. PCL was also used as polymeric binder to improve HA coating on the surface of the porous Fe. The cytocompatibility and degradation behaviour of those materials were investigated *in vitro*. Therefore, the objectives of this research are as follow:

1) To develop HA-coated porous Fe, HA/PCL-coated porous Fe and evaluate their degradation behaviour.
2) To analyse the cell-material interactions on the developed material by a series of cytocompatibility testing using two types of different cells; human skin fibroblast and human mesenchymal stem cells.

1.4 Significance of Study

Previous studies on iron underlining its degradation behaviour, mechanical properties and its biocompatibility have demonstrated its potential to be developed as degradable metallic biomaterials. Most of them were performed on solid Fe samples. This work was done on porous Fe samples with surface modification. The aims were to explore the biocompatibility of porous Fe, the way cells react and attach to the surface of the porous Fe structure. In addition, the effect of HA coating was investigated based on the behaviour of two different cells.

1.5 Scope of Study

Four scopes have been drawn to achieve the objectives of the research, that is:

1) Preparation of sample, where porous Fe coated with HA and PCL/HA by using dip coating method.
2) Characterization of material and coating by using SEM and EDX.
3) Determination of the degradation behaviour by measuring weight change and concentration of Fe release during immersion tests in cell culture medium.
4) Investigation of the effect of degradation towards cell viability, proliferation and morphology by using several assays and microscopic observation.

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