EFFECTS OF HYDROXYAPATITE COATING WITH OXIDE INTERLAYER ON BIOACTIVITY PERFORMANCES IN CoCrMo ALLOY

MAS AYU BINTI HASSAN

UNIVERSITI TEKNOLOGI MALAYSIA
EFFECTS OF HYDROXYAPATITE COATING WITH OXIDE INTERLAYER ON BIOACTIVITY PERFORMANCES IN CoCrMo ALLOY

MAS AYU BINTI HASSAN

A thesis submitted in fulfilment of the requirements for the award of the degree of Doctor of Philosophy (Mechanical Engineering)

Faculty of Mechanical Engineering
Universiti Teknologi Malaysia

JUNE 2015
ESPECIALLY DEDICATED TO

My beloved and supportive husband

Rosdi bin Hj. Daud

My encouraging parents

Hj. Hassan Bin Hj. Sharif
Hjh. Juwita Binti Hj. Omar

My wonderful children

Muhammad Nur Aqemi
Muhammad Nur Ayman
Muhammad Nur Ammar

And last but not least to all my siblings, my relatives and my close friends

Thank you so much for all the prayers, encouragement, confident and trust that you all have gave to me throughout completing this thesis. May Allah bless all people that I love and it is my honor to share this happiness with my love ones.
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ABSTRACT

Cobalt-chromium-molybdenum (Co-Cr-Mo) alloys have been reported difficult to bond directly on hard tissues owing to encapsulation by fibrous tissues. Several attempts have been made to improve the situation including coating with a bioactive layer which is mainly hydroxyapatite (HA). Various HA coating methods have been introduced but massive micro crack surface, delamination and low adhesion strength of HA coating are still the major concerns that cause the harmful release of metal ions. In this study, an oxide interlayer on Co-Cr-Mo alloys was developed through thermal oxidation prior to HA coating with the objective to provide better anchorage of HA coatings on the substrate surface, reduce metal ions release and at the same time enhancing the cell attachment. The thermal oxidation process was conducted in a muffle furnace at different temperatures (850°C, 1050°C and 1250°C) for 3 hours to create an oxide interlayer on the substrate surface. It was followed by coating HA on the bare material and on the oxidized substrates using sol gel dip coating technique. Scratch test results showed that the bonding strength of the HA on the oxide interlayer is markedly higher than the HA coated substrates without oxide interlayer. It seems that rough surface of oxide interlayer provides better mechanical interlocking of HA particles to the substrate surface. Inductively coupled plasma-mass spectrometry (ICP-MS) test illustrated that the release of Co and Cr ions from the HA coated oxidized substrates reduced significantly after 28 days immersion as compared to bare material and HA coated substrates without oxide interlayer. This indicates that oxide interlayer is able to act as an additional barrier to suppress the metal ions release. Similarly, the HA coated substrates with oxide interlayer demonstrate strong attachment and proliferation of cells than the HA coated substrates without oxide interlayer. It is concluded that the introduction of an intermediate oxide layer on Co-Cr-Mo substrate prior to HA coating has shown a positive effect in terms of increment of the adhesion strength of HA coating as well as cell bioactivity performance.
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LIST OF ABBREVIATIONS

µg/l - Microgram per litre
µm - Micrometer
AAS - Atomic Absorption Spectrometry
AFM - Atomic Force Microscopy
AlN - Aluminum Nitride
CaP - Calcium Phosphate
CO₂ - Carbon dioxide
Co-Cr-Mo - Cobalt-Chromium-Molybdenum
Cr₂O₃ - Chromium Oxide
CrN - Chromium Nitride
CTE - Coefficient Thermal Expansion
DNA - Deoxyribonucleic Acid
EDX - Energy Dispersive X-ray Spectroscopy
FCC - Face Centered Cubic
FDA - Food and Drug Administration
FESEM - Field Emission Scanning Electron Microscopy
FHA - Fluoridated Hydroxyapatite
g - Gram
g/cm³ - Gram per cubic centimetre
GPa - Giga Pascal
HA - Hydroxyapatite
HCP - Hexagonal Close Packed
HDMEC - Human Dermal Microvascular Endothelial Cells
HPMEC - Human Pulmonary Microvascular Endothelial Cells
HUVEC - Human Umbilical Vein Endothelial Cells
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<tr>
<td>ICP-MS</td>
<td>Inductively Coupled Plasma-Mass Spectroscopy</td>
</tr>
<tr>
<td>ISO</td>
<td>International Organization for Standardization</td>
</tr>
<tr>
<td>min</td>
<td>Minutes</td>
</tr>
<tr>
<td>ml</td>
<td>Millilitre</td>
</tr>
<tr>
<td>mm</td>
<td>Millimetre</td>
</tr>
<tr>
<td>mm/min</td>
<td>Millimetre per minute</td>
</tr>
<tr>
<td>MOM</td>
<td>Metal-On-Metal</td>
</tr>
<tr>
<td>MPa</td>
<td>Mega Pascal</td>
</tr>
<tr>
<td>MSCs</td>
<td>Multipotent Stromal Cells</td>
</tr>
<tr>
<td>N</td>
<td>Newton</td>
</tr>
<tr>
<td>N/min</td>
<td>Newton per minute</td>
</tr>
<tr>
<td>ng/ml</td>
<td>Nanogram per millilitre</td>
</tr>
<tr>
<td>nm</td>
<td>Nanometer</td>
</tr>
<tr>
<td>nmol/l</td>
<td>Nanomole per litre</td>
</tr>
<tr>
<td>PCL</td>
<td>Poly ε-caprolactone</td>
</tr>
<tr>
<td>PIII</td>
<td>Plasma Immersion Ion Implantation</td>
</tr>
<tr>
<td>PLD</td>
<td>Pulse Laser Deposition</td>
</tr>
<tr>
<td>ppb</td>
<td>Part per billion</td>
</tr>
<tr>
<td>ppm</td>
<td>Part per million</td>
</tr>
<tr>
<td>PVD</td>
<td>Physical Vapour deposition</td>
</tr>
<tr>
<td>Ra</td>
<td>Surface roughness</td>
</tr>
<tr>
<td>rpm</td>
<td>Rotation per minute</td>
</tr>
<tr>
<td>RPMI 1640</td>
<td>Roswell Park Memorial Institute 1640 medium</td>
</tr>
<tr>
<td>SBF</td>
<td>Simulated Body Fluid</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning Electron Microscopy</td>
</tr>
<tr>
<td>TCP</td>
<td>Tricalcium Phosphate</td>
</tr>
<tr>
<td>TiN</td>
<td>Titanium Nitride</td>
</tr>
<tr>
<td>TJR</td>
<td>Total Joint Replacement</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumor Necrosis Factor</td>
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<tbody>
<tr>
<td>°C</td>
<td>Degree celcius</td>
</tr>
<tr>
<td>°C/min</td>
<td>Degree celcius per minute</td>
</tr>
<tr>
<td>Å</td>
<td>Armstrong (1 x 10⁻⁸ cm)</td>
</tr>
<tr>
<td>µ</td>
<td>Micro</td>
</tr>
<tr>
<td>~</td>
<td>Approximately</td>
</tr>
<tr>
<td>&gt;</td>
<td>More than</td>
</tr>
<tr>
<td>&lt;</td>
<td>Less than</td>
</tr>
<tr>
<td>wt.%</td>
<td>Weight percentage</td>
</tr>
<tr>
<td>E</td>
<td>Young’s Modulus</td>
</tr>
<tr>
<td>σ₀ₚ</td>
<td>Yield Strength</td>
</tr>
<tr>
<td>σₚₑₚₚ</td>
<td>Tensile Strength</td>
</tr>
<tr>
<td>σₚₑₚₑ</td>
<td>Fatigue Limit</td>
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CHAPTER 1

INTRODUCTION

1.1 Introduction

This chapter describes the general overview on the current issues such as corrosion resistance, release of toxic metal ions and bone bonding ability which is commonly encountered in biomaterial implants, followed by the recent work done to overcome these problems. Based on the literature study, the major problems were selected to be solved and become the problem statement for this research works. The determination of the problem statements, objectives, scopes, significance of the research and hypothesis of the results are also discussed in the following sections.

1.2 Research Background

Nowadays, the field of biomaterials is not new and has immense importance of the mankind as the existence and longevity for some of less fortunate human beings, for the aged population to increase their life span and also for patient who
experienced severe injury due to traumatic events. Apart from these reasons, young and dynamic people like athletes also need replacements due to fracture and excessive strain to help them continue normal life activities. The demands on biomaterial implants such as artificial joints, dental implants, bone plates, wires, and stents are continually increased especially after the world war and when global terrorism frequently strikes in today’s challenging world environment (Afolaranmi et al., 2011; Okur, 2009).

Currently, the uses of metallic biomaterials have widespread in replacing the damaged structural components of the human body due to their excellent mechanical properties such as high corrosion resistance, wear resistance, fatigue strengths and toughness. Some of their uses in medical devices are artificial joints, dental implants, bone plates, wires and stents. There are various types of biomaterials that have been used as implants such as steels, cobalt and titanium based alloys. Comparing these three alloys as metallic biomaterial in terms of biomedical properties and availability, cobalt based alloy which also known as Cobalt-Chromium-Molybdenum (Co-Cr-Mo) alloy are advantageous compared to stainless steel and titanium alloys. The biocompatibility of Co-Cr-Mo alloy is closely related to its excellent corrosion resistance, mainly due to the high chromium content (~ 30 wt.%) which is higher than 316L austenitic stainless steel (~ 18-20 wt.%) (Okur, 2009; Qingliang et al., 2010; Santavirta et al., 1998). Though stainless steels may have advantage in low cost, but because of its high toxicity of metal ions release and susceptibility in stress, they still have limited use in clinical practice.

Besides that, Co-Cr-Mo alloy also possess high fatigue strength in the porous-coated condition compared to titanium alloy, which is better potential use in extensively porous-coated hip systems, especially in the smaller size of implants that may be subjected to higher stresses (Disegi et al., 1999; Giacchi et al., 2011). Their favourable in tribological behaviour also make this alloy particularly suitable for metal-on-metal bearing surface such as the acetabular cup of total hip replacements (Igual Muñoz and Mischler, 2011). Furthermore, restrictions used of titanium alloy due to its inferior tribological properties and high costs when compared to stainless
steel and Co-Cr-Mo alloy, caused surgeon to shift for better biomaterial (Okur, 2009). In short, Co-Cr-Mo alloy is much preferable to be used as biomedical implant due to its combination of excellent mechanical properties, its workability to be forged into complex shape with compromise surface finish and low cost compared to other biomaterials alloy (Giacchi et al., 2011; Lutz et al., 2011). However, much effort is devoted to the design, synthesis and fabrication of Co-Cr-Mo orthopaedic implants in order to obtain long term stability and better anchoring between the metal implant and the bone. Essentially, this means the ability of the implant to sustain the dynamic and static loads when implanted in the human body. At the same time, the implant should also able to accelerate bone healing at the early implantation, with a very small failure rate and minimal discomfort for the patient. These factors are definitely influence the rate of implantation cost and restricting the widespread application of Co-Cr-Mo as biomedical implants. Since the formation of a living bone with direct contact to the implant surface is a critical issue and has received most attention from researchers, the common question arises as how to attain a better integration of the implant surface morphology.

Many attempts have been made to investigate and modify the implants surface in order to improve bone bonding ability. In recent years, there has been increasing interest to apply hydroxyapatite (HA) as coating layer since it is able to promote osseointegration on biomaterials implant (Sepehr et al., 2013; Shaylin and George, 2012). HA coating can be applied by a number of methods such as pulsed laser deposition (PLD) (Rajesh et al., 2011), plasma spray deposition (Cao and Liu, 2013; Coyle et al., 2007), biomimetic precipitation (Shaylin and George, 2012), sol-gel dip coating (Hongjian and Jaebeom, 2011) and electrochemical deposition (Lu Ning and Jing Li, 2011).

Among these methods, sol–gel dip coating technique has been attracted much attentions, due to its many advantages, which include high product purity, homogeneous composition and low synthesis temperature and low processing cost (Mathews et al., 2009). The low processing temperature and fusion of the apatite crystals have been the main attraction of the sol–gel dip coating process, in
comparison with high temperature process such as thermal spray. High temperature used in thermal spray has caused researchers to shift for alternative method that uses low processing temperature. In addition, sol–gel dip coating process also can produce mixture of fine grain microstructure from nano-to-submicron crystals. These crystals are reported to have good biocompatibility with host tissue (Hongjian and Jaebeom, 2011; Kim et al., 2004) and able to enhance the cell adhesion, proliferation and growth at the interface of implant materials.

Therefore, the aim of this research was to evaluate the effects of oxide interlayer created through thermal oxidation process prior to HA coating on Co-Cr-Mo alloy. The purpose to create oxide interlayer on Co-Cr-Mo alloy is to provide better adhesion of HA coating due to many researchers claimed that direct coating can caused delamination and cracks (Mohd Faiz et al., 2014; Purna et al., 2012). Thermal oxidation process was chosen as surface treatment techniques on Co-Cr-Mo alloy due to no reports were found in elsewhere to explain the effects of HA coating with incorporation of oxide interlayer when tested in metal ions release test and cells behaviour. Meanwhile, this study also seeks to address the following questions:

i. How thermal oxidation temperature influences the formation of oxide interlayer properties?

ii. Can oxide interlayer help to reduce metal ions release when immersed in simulated body fluid?

iii. What the maximum adhesion strength of HA can be achieved on the oxidized Co-Cr-Mo alloy?

iv. How well is the cell grow on the HA coated with oxide interlayer samples as compared to without oxide interlayer samples?

v. What is the feasible slurry concentration for obtaining a good HA coating on Co-Cr-Mo alloy?

vi. What is the best sintering temperature on HA coated samples in order to avoid massive cracks?
1.3 Problem Statement

Co-Cr-Mo alloy implant is known to corrode over the time and start releasing harmful metal ions (Co, Cr, Mo) into body fluids (serum, urine and blood) once implanted and exposed to the aggressive body environment. The level of metal ions release and accumulation of wear particles can cause adverse clinical responses which affecting the stability of the implant (Roberto and Anna, 2011; Sun et al., 2011). In order to overcome these problems, hydroxyapatite (HA) coatings can be introduced onto the metal surface to act as a barrier in reducing the release of excessive metal ions and also helps for bone to growth rapidly on the surface implants (Krishnamurithy, 2013; Liu et al., 2004a; Ramaswamy et al., 2009). It has also been reported that the survival rate of HA coated implants is high as compared to implants without HA coating (Kim et al., 2004; Surmenev et al., 2014).

There are various methods to coat HA on metal implants and one of them is sol-gel dip coating technique. This method have attracts most of researchers’ attentions due to its ability to coat sample at room temperature and the thickness of coating can be controlled much easily as compared to plasma spray (Ben Naceur et al., 2012; Fathi and Hanifi, 2007; Kim et al., 2004; Zhang et al., 2011). Despite many advantages of the sol-gel dip coating technique, there are several limitations exist arise. It has been reported that HA coating on metal implants often results in severe cracks and delamination which eventually lead to coating failure (Kirk and Pilliar, 1999; Roest et al., 2011; Yang and Chou, 2007; Zhen-lin and Rong-chang, 2010). This phenomenon occurs due to poor adhesion strength between HA and the underlying substrate (Case et al., 2005; Yang and Chou, 2007; Zhen-lin and Rong-chang, 2010) and the low cohesive strength of the coated material itself (Roest et al., 2011; Zhen-lin and Rong-chang, 2010). It is also noted that the poor mechanical properties of HA such as brittleness and toughness have restrict its used in load bearing applications (Diangang et al., 2008).
One of the solutions to overcome these issues is by introducing an intermediate layer in between the brittle HA coating and the metal substrate. It has been reported that the intermediate layer able to enhance the adhesive metal-ceramic (HA) bonding and coating integrity (García et al., 2004; Kirk and Pilliar, 1999; Purna et al., 2012; Rajesh et al., 2011; Yang and Chou, 2007). Although the results showed promising in improving adhesion strength on other metallic biomaterials (Cao and Liu, 2013; Man et al., 2009; Mohd Faiz et al., 2014; Shaylin and George, 2012), research on Co-Cr-Mo alloy is somehow still limited especially involving the application of intermediate layer prior to HA coating. There is also lack of research studies in evaluating the performances of the oxide interlayer on Co-Cr-Mo alloy in reducing the release of metal ions and their responses to the cell growth.

In summary, much attention has been paid on HA coating as a solution to reduce metal ions release but not much research on the application of HA coating with intermediate layer on Co-Cr-Mo alloy. There is possibility that oxide interlayer (intermediate layer) created on Co-Cr-Mo alloy able to helps in reducing metal ions release. However, the performances of oxide interlayer in promoting better cell growth are still limited. Therefore, this research works is required in order to assess the effectiveness of oxide interlayer in the body fluid and so the evaluation has been justified.

### 1.4 Objectives of the Research

The principal objective of this research is to establish the methodology to improve HA coating on Cobalt-Chromium-Molybdenum (Co-Cr-Mo) alloy for biomedical implant application. The objectives of the research are as follows:
i. To evaluate the effects of thermal oxidation temperature on Co-Cr-Mo alloy surface morphology, adhesion strength and metal ions release.

ii. To evaluate the effects of HA slurry concentrations and sintering temperature on Co-Cr-Mo alloy surface morphology, adhesion strength and metal ions release.

iii. To evaluate the cell growth and cell attachment performances on HA coated with oxide interlayer on Co-Cr-Mo alloy.

### 1.5 Scopes of the Research

The research was conducted in the following limits:

i. Cobalt-Chromium-Molybdenum (Co-Cr-Mo) alloy was used as the substrate material.

ii. Thermal oxidation process was used to create oxide interlayer on Co-Cr-Mo alloy within temperatures range of 850°C to 1250°C at fixed time duration in atmospheric condition.

iii. HA coating was deposited on the samples using HTWL-01 Desktop Dip Coater (MTI Cooperation, USA) at room temperature.

iv. The adhesion strength of oxide interlayer and HA coating were measured using Revetest Scratch test in order to determine the critical load.

v. RPMI 1640 medium solution was used as the simulated body fluid for metal ions release tests up to 28 days.

vi. In-vitro biocompatibility of the HA coated samples was tested using Multipotent Stromal Cells (MSCs) up to 14 days for cell attachment study.
1.6 Significance of the Research

In this study, a development of a simple and effective surface treatment technique is applied on Co-Cr-Mo alloy to improve the bonding and quality of HA coating using sol-gel dip coating method. Preliminary results from other biomedical materials showed that HA coating via sol-gel dip coating method have been successfully improved biocompatibility, increase corrosion resistance and reduce metal ions release when tested in in-vitro tests. By achieving this purpose, hopefully future implants are much cheaper and affordable for anyone who are in needs. It is also hope that the outcomes of this research will help the country to reduce power consumption and lessen the expensive costs in producing medical implants. The development of systematic understanding on exploring this material might also help engineers and scientists to come up with better and efficient implants for future used.

1.7 Hypothesis of the Results

It is expected that good adhesion strength of oxide interlayer formed on Co-Cr-Mo alloy through thermal oxidation process is able to act as a barrier to reduce excessive metal ions release into simulated body fluid. It is also expected that HA coated samples with oxide interlayer exhibits better performances in cell attachment and cell proliferation as compared to HA coated samples without oxide interlayer. Theoretically, it is believed that with the combination of oxide interlayer and HA coating on Co-Cr-Mo alloy, it will be able to accelerate the spreading of cell growth thus, resulting in shortening the healing time after implantation.
1.8 Organization of Thesis

This thesis consists of 5 main chapters. Chapter 1 gives an overview of the research background and problem statements in this study. Chapter 2 provides the literature review on the current biomaterial implants used and work done to solve problems with Cobalt-Chromium-Molybdenum (Co-Cr-Mo) alloy. Information regarding existing techniques to coat HA on metal implants and current method used to examine the coating performances are also covered in this chapter. Methodology for the whole experiments is included in Chapter 3 starting from sample preparation, method used to run the experiments until the existing equipment involved in executing this research work. Chapter 4 consists of results gathered from the experimental work and discussion on the findings obtained. Lastly, Chapter 5 provides conclusions of the research work and some suggestions for future work.
REFERENCES


