CHARACTERISATION AND IN VITRO BIOACTIVITY OF ANODISED TITANIUM AND HYDROXYAPATITE AS IMPLANTS FOR BIOMEDICAL APPLICATION

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"Special dedicated with much love and affection to **my beloved family,**
SELIMIN BIN MUSTAPA, PATIMAH BINTI LATUWO, ISUHAINA BINTI SELIMIN, SAPNI BIN SAPENA, SURIATI BINTI SELIMIN, SAYNUDDIN BIN SELIMIN, SABLI BIN SELIMIN, ANY BINTI SELIMIN, ASMA BINTI SELIMIN, SITI NUR AISHA BINTI SELIMIN, and also to my family members
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ABSTRACT

Naturally, titanium (Ti) has a bioinert surface which is a non-preferred nucleation site for bone-like apatite. Therefore, this study aimed to modify bioinert to bioactive Ti using anodic oxidation in different electrolytes and pair Ti with HAP in SBF (new approach SBF). Besides that, there are also other novelties including ultraviolet (UV) light treatment, UV irradiation during SBF (UV-SBF) and prediction of anodised titanium properties. The anodisation parameters such as type of electrolyte and it concentration, applied voltage, and current density were varied. Meanwhile, HAP was mixed with binders, polyvinyl alcohol (PVA) and polyethylene glycol (PEG), and sintered at 1000-1200°C. The anodised Ti films were characterised based on it colour, morphology, mineralogy, cross-section view, and wettability. Various SBF approaches were used such as traditional SBF, UV-SBF and new approach SBF (pairing). The selected anodised Ti has undergone other testing including UV treatment and bioactivity test. The conceptual models that explain the changes in the morphology as a function of the experimental parameters were developed using the data as well as the prediction of anodised Ti properties. For the anodisation in $C_2H_4O_2$, there were three stages of oxide growth based on films properties. The data showed that the SP solution could produce better bioactive Ti, followed by SBC and SN. For HAP, the binders did not give any significant effect on HAP properties due to PVA and PEG were burnt off at high sintering temperature (> 700°C). Increasing the mechanical strength of HAP has led to low porosity. Anodised Ti with high crystalline of anatase and/or rutile, porous, and superhydrophilic enable better deposition of thick apatite on the Ti surface. Furthermore, the smooth Ti surface lacked nucleation sites for bone-like apatite growth. The new approach of pairing in SBF has produced weak bonding strength that bonded anodised Ti and HAP without fixation device. After 7 days in SBF, apatite bridge (thin layer) is formed between anodised Ti and HAP surfaces. The results are important to predict *in vivo* mechanism after completing the implantation process.

ABSTRAK

Secara semulajadinya, titanium (Ti) permukaannya bersifat biolengai, dimana ianya tidak membenarkan pertumbuhan apatit yang menyerupai tulang. Oleh itu, kajian ini dijalankan untuk mengubahsuai sifat permukaan Ti dari biolengai kepada bioaktif dengan menggunakan teknik pengoksidaan anod menggunakan pelbagai jenis elektrolit. Selain itu, terdapat beberapa pendekatan baru dalam kajian ini seperti penyinaran UV, UV-SBF dan jangkaan ciri-ciri Ti teroksida. Parameter pengoksidaan seperti jenis dan kepekatan elektrolit, voltan gunaan, dan ketumpatan arus turut dimanipulasikan. Bagi HAP, ianya dicampurkan dengan dua jenis pengikat iaitu PVA dan PEG, dan kemudiannya dibakar pada suhu 1000-1200°C. Ti teroksida dicirikan mengikut warna, morfologi, mineralogi, keratan rentas permukaan, dan keupayaan basah. Pelbagai pendekatan terhadap SBF diperkenalkan didalam kajian ini seperti SBF tradisional, UV-SBF, dan pendekatan baru SBF. Hanya Ti terpilih sahaja akan melalui ujian penyinaran UV dan bioaktiviti. Model konseptual mengenai perubahan morfologi dibangunkan menggunakan data termasuk ramalan terhadap ciri-ciri Ti teroksida. Bagi pengoksidaan menggunakan $C_2H_4O_2$, terdapat tiga peringkat pertumbuhan oksida dikenalpasti berdasarkan ciri-ciri filem. Data menunjukkan elektrolit SP mampu menghasilkan permukaan bioaktif yang lebih baik berbanding SBC dan SN. Bagi HAP pula, pengikat tidak mampu memberikan kesan yang signifikan terhadap HAP. Ini kerana ianya terbakar sepenuhnya pada suhu yang tinggi (> 700°C). Dengan meningkatkan kekuatan mekanikal HAP, ianya akan merendahkan keliangannya. Pembentukan apatit yang tebal mampu dicapai sekiranya Ti mempunyai kristal anatas dan/atau rutil yang tinggi, sangat berliang dan hidrofilik. Manakala, kawasan penukleusan apatit adalah sangat terhad pada permukaan Ti yang licin. Pendekatan baru SBF menghasilkan lekatan yang lemah untuk melekatkan Ti dan HAP tanpa alat penetapan. Selepas 7 hari didalam SBF, lapisan jambatan apatit (nipis) terhasil diantara permukaan Ti teroksida dan HAP. Keputusan daripada ujikaji ini sangat penting untuk meramal mekanisma *in vivo*.

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LIST OF SYMBOLS AND ABBREVIATIONS

%	-	Percentage
$(NH_4)_2SO_4$	-	Ammonium sulphate
$[-CH_2CHOH-]_n$	-	Polyvinyl alcohol
µm	-	Micrometre
•OH	-	Hydroxyl radical
AFM	-	Atomic force microscopy
ANO-C	-	Anodic oxidation on acetic acid
ANO-P	-	Anodic oxidation in phosphoric acid
ANO-S	-	Anodic oxidation in sulphuric acid
b	-	Breadth of plate
BG	-	Bioglass
$C_2H_4O_2$	-	Acetic acid
CA	-	Calcium acetate
Ca/P	-	Calcium to phosphorus
$Ca_{10}(PO_4)_6(OH)_2$	-	Hydroxyapatite
Ca^{2+}	-	Calcium ion
$Ca_3(PO_4)_2$	-	Tricalcium phosphate
$CaCl_2$	-	Calcium chloride
CB	-	Conduction band
Cl^-	-	Chloride ion
cm	-	centimetre
cm^{-1}	-	Per centimetre
Co-Cr	-	Cobalt-chromium
cpTi	-	Commercially pure titanium
D	-	Crystalline size
d	-	Depth of plate
D	-	Dry weight

d.c.	-	Direct current
E	-	Electrolyte
e^-	-	Electron
ETO	-	Ethylene oxide
eV	-	Electronvolt
F	-	Breaking load
FESEM	-	Field emission scanning electron microscopy
g/mol	-	Gram per mol
GAXRD	-	Glancing angle X-ray diffractometer
GIMS	-	Gas injection magnetron sputtering
H^+	-	Hydrogen ion
H_2O	-	Water
H_2SO_4	-	Sulphuric acid
H_3PO_4	-	Phosphoric acid
HALR	-	High anatase and low rutile
HAP	-	Hydroxyapatite
HCO_3^-	-	Bicarbonate
HCl	-	Hydrochloric acid
$HO(C_2H_4O)_nH$	-	Polyethylene glycol 6000
$HO_2\bullet$	-	Hydroperoxyl radical
HPO_4^{2-}	-	Phosphate ion
hr	-	Hour
I	-	Current
JCPDS	-	Joint Committee on Powder Diffraction Standards
K	-	Scherrer's constant
K^+	-	Potassium ion
$K_2HPO_4.3H_2O$	-	Dipotassium hydrogen phosphate trihydrate
KCl	-	Potassium chloride
L	-	Distance between supports span
L*	-	Brightness
L_1	-	Length after sintering
LAHR	-	Low anatase and high rutile
LANR	-	Low anatase and no rutile

L_o	-	Length before sintering
$mA.cm^{-2}$	-	Milliamp per centimetre square
MAO	-	Micro-arc oxidation
Mg^{2+}	-	Magnesium ion
$MgCl_2.6H_20$	-	Magnesium chloride hexahydrate
mL	-	Millilitre
mm	-	Millimetre
MOR	-	Modulus of rupture
Na^+	-	Sodium ion
Na_2SO_4	-	Sodium sulphate
NaCl	-	Sodium chloride
$NaHCO_3$	-	Sodium hydrogen carbonate
NaOH	-	Sodium hydroxide
Nb-OH	-	Niobium hydroxide
nm	-	Nanometer
°	-	Degree
O_2	-	Oxygen gas
O_2•	-	Oxygen radical
°C	-	Degree Celsius
°C/min	-	Degree Celsius per minute
OH^-	-	Hydroxyl ion
PEG	-	Polyethylene glycol
PO_4H_2	-	Phosphate
PSA	-	Particle size analyser
Pt	-	Platinum
PVA	-	Polyvinyl alcohol
rpm	-	Revolutions per minute
S	-	Suspended weight
SO_4^{2-}	-	Sulphate ion
S_a	-	Apparent surface area of specimen
SBF	-	Simulated body fluid
SEM	-	Scanning electron microscopy
SiC	-	Silicon carbide

Si-OH	-	Silicon hydroxide
STA	-	Simultaneous thermal analysis
Ta-OH	-	Tantalum hydroxide
TCP	-	Tricalcium phosphate
TFXRD	-	Thin film X-ray diffractometer
T_g	-	Glass transition temperature
TG	-	Thermogravimetric
Ti	-	Titanium
TiO_2	-	Titanium dioxide or titania
Ti-OH	-	Titanium hydroxide
t_o	-	Time deposition
Tris	-	Tris-hydroxymethyl aminomethane
TTCP	-	Tetracalcium phosphate
UV	-	Ultraviolet
UVA	-	Ultraviolet light type A
UVC	-	Ultraviolet light type C
UV-SBF	-	Ultraviolet irradiation during simulated body fluid
V	-	Voltage
VB	-	Valence band
V_s	-	Volume of simulated body fluid
W	-	Saturated weight
XRD	-	X-ray diffraction
XRF	-	X-ray fluorescence
Zr-OH	-	Zirconia hydroxide
α-TCP	-	Alpha tricalcium phosphate
$\beta_{1/2}$	-	Full width at maximum (FWHM) in radian
β-GP	-	β-Glycerophosphate
β-TCP	-	Beta tricalcium phosphate
γ_{LV}	-	Surface tension of liquid vapour interfacial
γ_{SL}	-	Surface tension of solid liquid interfacial
γ_{SV}	-	Surface tension of solid vapour interfacial
θ	-	Theta
λ	-	Wavelength of Cu Kα radiation

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CHAPTER 1

INTRODUCTION

1.1 Introduction

With the advance of modern medicine and the increase in human life expectancy, there is now a greater need for biomaterials to remedy problems related to the reconstruction of tissues and organs, as well as problems related to injury or diseases attribute to ageing or trauma from accidents (Zavaglia & Silva, 2016). Biomaterials are used for the purpose of biomedical or clinical applications to treat diseases or injuries by replacing or reconstructing broken parts, tissues and organs, or by functioning in close contact with the tissue of live organisms especially humans (Park & Lakes, 2007).

Many companies invest large sums of money towards the development of new products in the quest to find better and safer biomaterials. Biomaterials science encompasses elements of medicine, biology, chemistry, tissue engineering and materials science (Zavaglia & Silva, 2016). Figure 1.1 shows the examples of the broken parts of the human body (tooth and hip).

Biomaterials can be derived from natural resources or synthesised in the laboratory by using various techniques. They can be used to replace individual parts of living organisms or to function in close contact with tissues of living organisms for the purpose of performing examinations, treatments, improvements or replacements of individual tissues, entire organs, or some other functions (Williams, 1999). There are four main types of biomaterial implants in the industry, namely polymer, metal, ceramic, and composite. Figure 1.2 shows the types and examples of biomaterials.

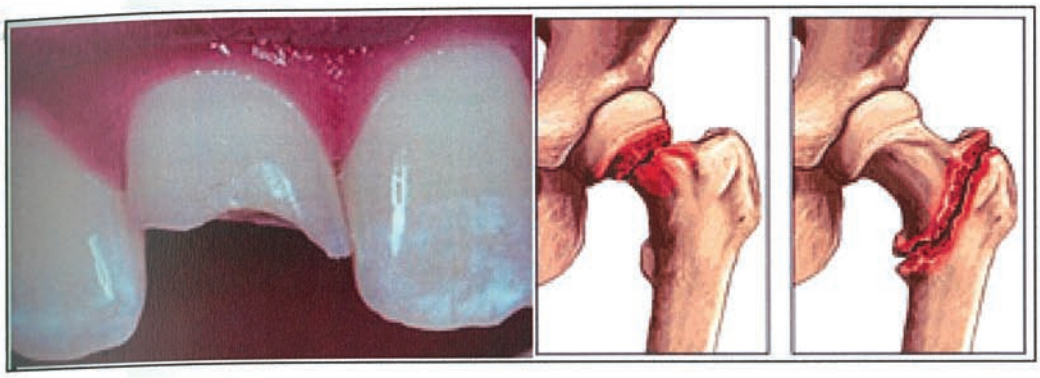

Figure 1.1: Chipped tooth and fractured hip joint (Scheinfeld, 2013 & Ma, 2016).

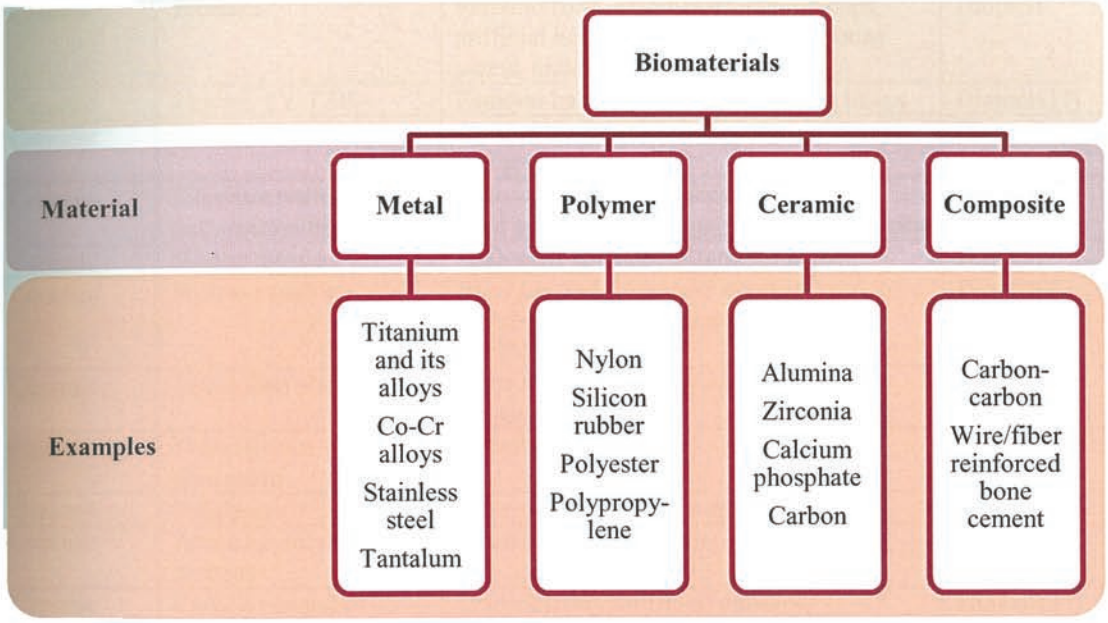

Figure 1.2: Types and examples of biomaterials (Wong & Bronzino, 2007).

Various materials have been utilised as biomaterials in order to meet the specific requirements of different parts or functions of the human body. Based on the findings of previous research, there are also biomedical materials which are harmful to the human body and tissues. For example, materials with vanadium elements are classified as toxic and may cause a tissular reaction (Ghosh *et al.*, 2015). Biomaterials have been used as part of the treatment to heal various parts of the human body. Some examples include dental implants, bone cement, bone fixations and screws, pacemakers, and knee joints (Patel & Gohil, 2012). Table 1.1 sets some examples of biomaterials, the applications, and the biological behaviour of each type of biomaterial in biomedical science.

Table 1.1: Application of biomaterials (Heimann & Lehmann, 2013)

Material group	Biomaterial	Selected applications	Biological behaviour
Metal	cp-titanium	Acetabular cup shells	Bioinert
Metal	Titanium alloys Ti6Al4V, Ti6Al7Nb, Ti4)	Shaft for hip implants, knee implants, coronary stents	Bioinert
Metal	Tantalaum	Vascular clips, cranial defect repair, coronary stents	Bioactive (?)
Metal	CoCrMo alloy	Femoral balls, shafts, knee implants	Bioinert
Metal	Stainless steel (AISI 316L)	Osteosynthetic plates and bone screws	
Metal	Mg Alloy (AZ31, AZ91)	Biodegradable implants	
Ceramic	Alumina	Femoral balls, insert of acetabular cups, artificial heart valves, dental roots, bone screw, endoscope	Bioinert
Ceramic	Zirconia (Y-TZP)	Femoral balls, dental veneers, tooth inlays	Bioinert (?)
Ceramic	Titania	Anti-microbial surfaces, bioactive Ti surface oxide	Bioactive
Ceramic	Titanium nitride, zirconium nitride	Anti-wear coating of femoral balls and knee prostheses, coating for coronary stents	Bioinert
Ceramic	Silicon nitride	Anti-wear coatings of femoral balls	Bioinert
Ceramic	Hydroxyapatite	Bone cavity fillings, ear implants, vertebrae replacement, hip implant coatings, bone scaffolds	Bioactive
Ceramic	Tricalcium phosphate	Bone replacement, UV-absorbing sunscreens	Bioactive/ bioresorbable
Ceramic	Tetracalcium phosphate	Dental cement	Bioresorbable
Ceramic	Bioglasses	Bone replacement, ear implants	Bioactive
Ceramic	Arbon (pyrolytic carbon)	Heart valve components	Bioinert
Ceramic	Carbon nanotubes (CNT)	Drug delivery, artificial muscles, bioelectronics	Bioinert (?)
Ceramic	Carbon (DLC,diamond)	Anti-wear coating, bioMEMS	Bioinert
Composite	Hydroxyapatite/mPCL	Tissue engineering scaffold	Bioresorbable

Biomaterials can also be classified based on the characteristics, such as the type of material and the tissue response. Basically, there are four general classifications for biomaterials based on their tissue response, namely toxic, bioinert, bioactive, and bioresorbable. Table 1.2 demonstrates how biomaterials are used in various applications in the biomedical field.

Studies have been conducted on artificial biomaterial implants in order to find ways to increase the quality of the implant and to reduce the level of risk which the implant poses to humans. The increasing demand in the global market for medical implants has triggered the growth of opportunities and challenges for researchers and manufacturers to produce biomaterials which are of high quality and safe for human

usage. The high demand for biomedical implants has also led to improvements in the quality of implant parts over time.

Table 1.2: Classification of biomaterials (Park & Lakes, 2007)

Classification	Explanation	Tissue response	Example
Toxic	Material is toxic	The surrounding tissues die	Copper and metals with Vanadium (V) element
Bioinert	Material is non-toxic and biologically inactive	A fibrous tissue of variable thickness forms	Alumina, zirconia, titania, titanium and their alloys
Bioactive	Material is non-toxic and biologically active	An interfacial bond forms, between the tissue and the material	Hydroxyapatite, bioactive glasses
Bioresorbable	Material is non-toxic and dissolves in surrounding environment	The surrounding tissue replaces it	Tri-calcium phosphate

Titanium (Ti) and its alloys are biocompatible, lightweight, corrosion-resistant and have superior mechanical properties. Therefore, they have been widely used in biomedical implants and dental applications. These characteristics make them very suitable elements compared to other biomaterials (Liu *et al.*, 2004).

Before titanium can be used as a material for implant manufacturing, it needs to first undergo a surface modification, namely to modify the oxide of the material from bioinert to bioactive. This material still requires more in-depth research before it will be able to control and enhance formation, growth, and biocompatibility of the apatite (bone-like mineral). Anodic oxidation is a promising technique that has the ability to enhance the physical and chemical properties of titanium without changing its microstructure. This method is able to produce anatase and/or rutile phases on the titanium surface (Liu *et al.*, 2004).

Meanwhile, hydroxyapatite (HAP) is a bioceramic material which is chemically and mineralogically similar to the non-organic component of natural bone and teeth (highly biocompatible and osteoconductive). It is a well-known material for treating bone defects. Current biomedical applications of bioceramics are commonly carried out in orthopaedic and dental implant coatings, dental implant materials, bone-graft substitute materials, and bone cement (Borkowska & Ginalska, 2010).

HAP has no carcinogenic properties and does not trigger allergic reactions. Nevertheless, despite its advantages, the application of this bioceramic material is limited due to its other less desirable characteristics such as poor resorption, a

substantially high Young modulus, and low fracture toughness (Porter *et al.*, 2004 & Best *et al.*, 2008). Table 1.3 below sets out a summary of the advantages and drawbacks of titanium and hydroxyapatite implants.

Table 1.3: Advantages and drawbacks of titanium and hydroxyapatite implants

	Type of implants	
	Titanium	**Hydroxyapatite**
Advantages	Excellent mechanical properties Lightweight Low modulus of elasticity High corrosion resistance Biocompatible	Bioactive Promote osseointegration Excellent interlocking with natural bone Similar properties with natural bone Ca/P ratio is 1.67
Drawbacks	Bioinert Do not promote osseointegration Poor mechanical interlocking with natural bone	Brittle Poor mechanical properties Mostly used for non-loading application

Materials that are used as implants need to undergo the proper handling procedures from the point of manufacturing up to the point when they are safely implanted inside the human body. In order not to be hazardous to health, there are two types of testing which can be done to analyse the materials: *in vitro* and *in vivo*. Meanwhile, there are four types of testing for *in vitro*: cytoxicity, genotoxicity, hemocompatibility, and the evaluation of bioactivities of the implant by using simulated body fluid (SBF) to predict its bone-like apatite precipitation (Peters *et al.*, 2009).

1.2 Problem statements

To date, it appears that no study has been published on the prediction of an adhesion of artificial titanium implant on the artificial bone by using SBF (*in vitro* test). Most of the studies conducted using SBF are done mainly to evaluate the ability of bone-like apatite precipitation on an artificial implant (single specimen in SBF) without observing the adhesion between the implant and the artificial bone (traditional SBF technique) (Kokubo *et al.*, 2006). Due to this, the SBF is unable to provide a more accurate prediction of adhesion of the artificial implant with natural bone during *in vivo* testing. The environment of traditional SBF without the existence of bone might be one of the reasons for this (Xin *et al.*, 2005 and Sun *et al.*, 2011). Therefore, to

better predict the adhesion of artificial implant on natural bone, a new approach on simulated body fluid test will be taken in this study to investigate the effectiveness of SBF.

Currently, the machined titanium implant in the market has bioinert characteristics due to its smooth surface, which restrict the implant to have better interlocking (anchoragement) with natural bone when implanted inside the human body (poor osseointegration) (Liu *et al.*, 2004). Due to poor osseointegration, the titanium implant needs a coating of bone cement on its surface in order to fix the implant on the natural bone.

Thus, to overcome the drawbacks of the titanium implant, anodic oxidation in various types of electrolyte will be conducted to modify the surface of the titanium from a bioinert to a bioactive oxide layer. The roughness or thickness of the titanium surface (oxide layer) can be controlled by varying the parameters of the anodic oxidation such as the applied voltage, the current density, and the concentration of electrolyte.

Apart from these parameters, a new approach on anodic oxidation using a mixture of sulphuric acid with other chemical solutions will be tested, given that there has not been any research findings reported on the effects of sulphuric acid mixtures upon the properties of the oxide layer of anodised titanium. Even though there are many studies were reported regarding the anodic oxidation of titanium in sulphuric acid, there still no article report on the prediction of anodised titanium properties especially based on the anodic oxidation reaction and the colour of anodic film obtained.

It is widely known that titanium, being a non-toxic and chemically stable material, is a good photocatalyst. By applying ultraviolet (UV) light treatment of the titanium, the bone-like apatite precipitation on the implant can be accelerated (Uetsuki *et al.*, 2010). UV treatment on anodised titanium is still a new development in biomedical application, as most UV treatment studies on titanium are for application in other field such as solar cell and the semi-conductor industry (Brunella *et al.*, 2007; Wang *et al.*, 2007 & Ohtsu *et al.*, 2013). Therefore, this study seeks to explore the effects of UV treatment on anodised titanium specifically for application in the biomedical field.

1.3 Objectives

This study aims to achieve the following objectives:

(1) To determine the effects of anodic oxidation on the oxide layer of titanium substrate in acetic acid ($C_2H_4O_2$) and sulphuric acid (H_2SO_4).

(2) To investigate the effects of post-treatment of UV irradiation on the wettability properties of anodised titanium.

(3) To investigate the effects of different types of sulphuric acid mixtures used as electrolyte on the properties of the anodised titanium.

(4) To evaluate the effects of binder and sintering temperatures on the properties of HAP.

(5) To evaluate the *in vitro* bioactivity of anodised titanium and HAP in SBF (traditional approach SBF).

(6) To evaluate the bone-like apatite growth and adhesion on pairing specimens (anodised titanium and HAP) in SBF using new approach SBF.

1.4 Scope of the study

In order to achieve the set objectives, experiments and observations under this study must be made in accordance with the selected scopes:

(i) Titanium:

(1) Titanium specimens were prepared using anodic oxidation method.

(2) The titanium foils with 0.05 mm thickness are cut into a dimension of 10 mm x 25 mm.

(3) The surfaces of the titanium films are hand wet-polished, cleaned, and dried using compressed air before undergoing anodic oxidation.

(4) The acetic acid and sulphuric acid are used as electrolytes for anodic oxidation of titanium, where these parameters are accordingly varied:

Concentration of electrolyte	:	0.3 – 1.8 M
Applied voltage:		50 – 350 V

Current density:	25 – 75 $mA.cm^{-2}$
Anodising time:	10 minutes
Temperature:	~ 25°C

(5) After characterisation of all the anodised titanium specimens, only selected specimen was undergoing the next stage (UV treatment) as stated below:

UV wavelength:	256 and 354 nm
UV treatment duration:	1 – 12 hours
pH of distilled water:	2 – 11
Mineralogy of titanium:	Ti, LANR, HALR, LAHR

(6) The characterisation of anodised titanium samples will be performed using glancing angle X-ray diffractometer (GAXRD), field emission scanning electron microscopy (FESEM), atomic force microscopy (AFM), goniometer contact angle, and colorimeter.

(7) Additional study of anodic oxidation using mixture of sulphuric acid with other chemical aqueous as electrolyte, as follows:

Electrolyte:	0.3 M H_2SO_4 + 0.3 M H_3PO_4
	0.3 M H_2SO_4 + 0.3 M NaOH
	0.3 M H_2SO_4 + 0.04 M β-GP + 0.3 M CA
Applied voltage:	75 – 200 V
Current density:	75 $mA.cm^{-2}$
Anodising time:	10 minutes
Temperature:	~ 25°C

(ii) Hydroxyapatite:

(1) Preparation of the hydroxyapatite sample using designated mixing ratio. The parameters of HAP preparation are as follows:

Binder ratio:	1 – 4 % of HAP weight
Mixing speed:	160 rpm

Mixing duration:	24 hours
Granule size:	250 μm
Pressing pressure:	51 MPa
Specimen dimension:	65 mm x 12 mm
Sintering temperature:	1000 – 1200 °C
Soaking duration:	2 hours
Heating rate:	2 °C/min

(2) Only 4% of the binder of the HAP specimen material will be used in this study under various combination ratios between the binders (PVA and PEG).

(3) The characterisation of hydroxyapatite samples was determined using X-ray diffraction (XRD), X-ray fluorescence (XRF), particle size analyser (PSA), field emission scanning electron microscopy (FESEM), three points bending (flexural test), shrinkage, porosity, and bulk density test.

(4) Only selected HAP specimens was undergoing the *in vitro* test.

(iii) Simulated body fluid:

(1) Similarly with Kokubo method, conventional simulated body fluid (SBF) was prepared for biological testing.

(2) Selected anodised titanium sample will be soaked in SBF-assisted ultraviolet (UV) irradiation (with or without UV irradiation) for bioactivity test for a certain period ranging from 1 to 3 weeks.

(3) As for a new approach of SBF, only selected anodised titanium and hydroxyapatite specimens were used in this study. The anodised titanium was act as an artificial titanium implant and the hydroxyapatite as an artificial bone. The characterisation of pairing adhesion in SBF will be observed by using FESEM.

(4) The single specimen in SBF (traditional method) was characterised using FESEM and GAXRD.

1.5 Novelty of study

In this study, some new techniques and approaches has been introduced in order to provide more knowledge and discover new findings in biomaterials studies. The following are several novelties of this study:

(1) A new approach in using simulated body fluid (*in vitro* test) to predict the adhesion of artificial implant on artificial bone was introduced to investigate the effectiveness of SBF in producing adhesion between the two materials.

(2) Ultraviolet (UV) treatment (post-treatment) is applied on anodised titanium specimens to study the effectiveness of this treatment in producing improved anodised titanium (prepared in sulphuric or acetic acid) implant with better osseointegration properties that are suitable for biomedical application.

(3) The combination of UV irradiation assisted SBF (UV-SBF) on anodised titanium is introduced to study the effectiveness of this technique on accelerating the bone-like apatite precipitation on the anodised titanium films.

(4) Performing anodic oxidation using electrolytes of sulphuric acid as the main mixture and other chemical solutions to modify the oxide layer of the anodised titanium in the said process.

(5) The prediction of anodised titanium properties based on colour of anodic films and the observation made during anodic oxidation was introduced in this study.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Biomaterials have been used to produce devices or parts to replace broken parts in a safe, reliable, economic, and physiologically acceptable manner (Park & Lakes, 2007). They consist of basic biology, medicine, engineering, and material science that intended to interface with biological systems to evaluate, treat, augment, or replace any tissue, organ or function to treat the disease or injury. In order to be implanted inside the human body, the materials must meet or exceed certain standardised as the following:

- Nontoxic
- Non-carcinogenic
- Non-allergic
- Biocompatible
- Non-inflammatory
- Not easy to fail during the implantation procedure
- Biofunctional for its lifetime and cannot affect the host

Some of the earliest biomaterial applications were dated back during ancient Phoenicia, when loose teeth were bound together with gold wires by tying artificial ones to the neighbouring teeth. An archaeologist has discovered artificial eyes, teeth, ears, and noses of Egyptian mummies which dated 4000 years ago (Manivasagam *et al.*, 2010). In the early 1900s, bone plates were successfully implemented to stabilise

bone fractures and to accelerate their healing. During the 1950s until 1960s, blood vessel replacement was still in clinical trials, where artificial heart valves and hip joints were in still in their early development (William & Cunningham, 1979).

For the application of blood contacting, the biomaterials are inserted into blood vessels or devices to be permanently implanted to remove and return the blood from the body. For soft tissue application, biomaterials are implanted to augment or redefine the damaged tissue. Besides that, biomaterials are implanted to repair the bone defect of the human body for orthopaedic and dental application (Nascimento *et al.*, 2007). Figure 2.1 shows the human anatomy, various implants, and devices that are used to replace or enhance the function of the tissues and organs.

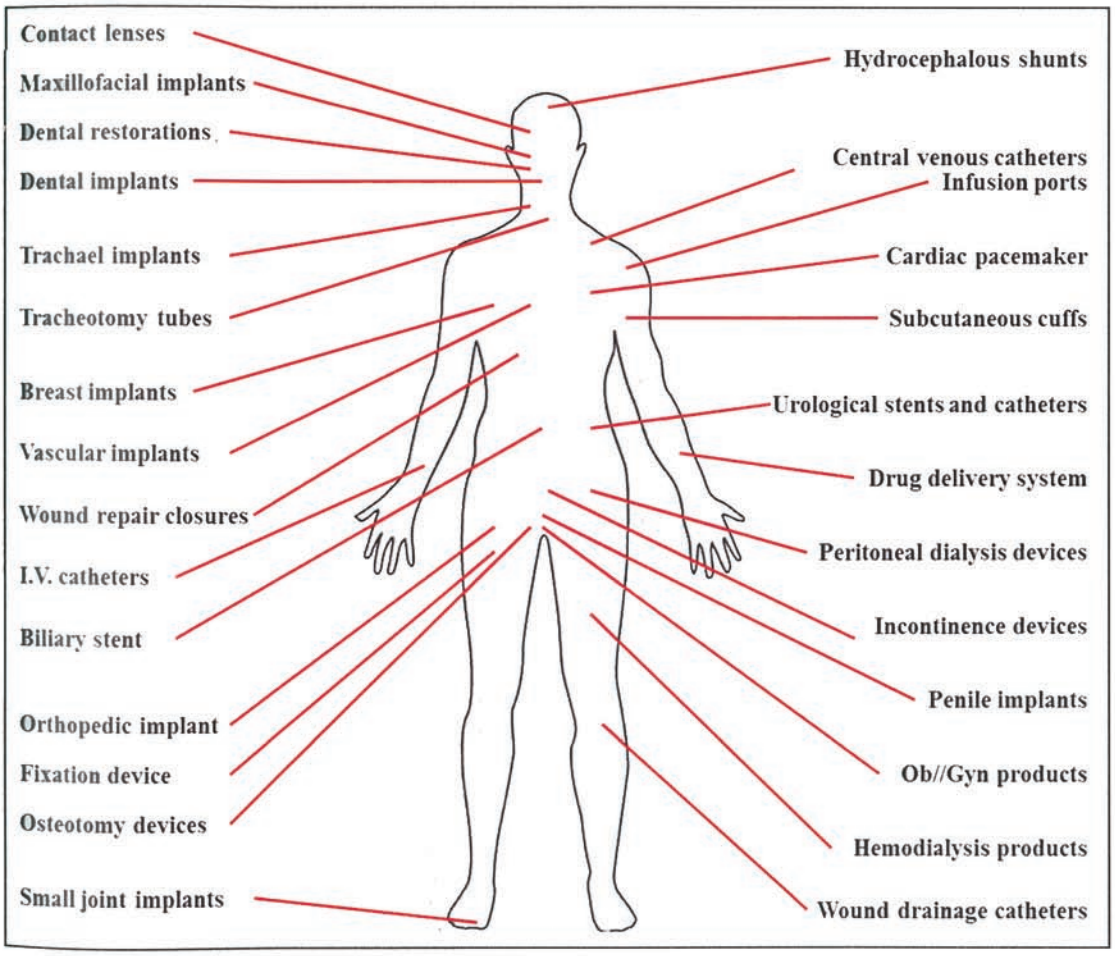

Figure 2.1: Various implants and devices used in the human body (Park & Lakes, 2007; Madihally, 2010).

2.1.1 Characteristics of biomaterials

The biomaterials that are implanted inside the human body are known as implants, and the reaction between the replaced implant with the host (tissues) need to be considered to unharmed host health. When a synthetic material is placed in the human body, the human tissue reacts with the implant in various ways depending on the type of material. The mechanism of tissue interaction (if any) depends on the tissue response to the implant surface (Boretos & Eden, 1984).

The biomaterials are very important for the mankind as it helps the less fortune such as people with diseases at birth, people who lost their abilities due to accident or illness, and aged population who requires biomedical implants to prolong their life span (Manivasagam *et al.*, 2010). Generally, biomaterials can be classified into four characteristics: toxic, bioinert, bioactive, and bioresorbable.

2.1.1.1 Toxic

A biomaterial should not be toxic, unless it is specifically engineered for such requirements (for example a "smart bomb" drug delivery system that targeted cancer cells and destroy them). The toxicology of biomaterials deals with substances that migrate out of the biomaterials. A biomaterial should not give off anything from its mass, unless it is specifically designed to do so. Therefore, it is important to determine the biocompatibility of materials before being used in biomedical application so that they would not cause any acceptable degree of harm to the host (Patel & Gohil, 2012).

The U.S. Department of Health and Human Services (2012) found that materials with vanadium elements are classified as toxic material as they are harmful to the human body and tissues. Vanadium is also linked to several disturbances, including infertility, disturbed iron metabolism, anemia, and impaired metabolism of bone, teeth and cartilage (Gruzewska *et al.*, 2014)

2.1.1.2 Bioinert

The term bioinert refers to any material that was once placed in the human body and it has minimal interaction with its surrounding tissue. Some of the examples are stainless steel, titanium, alumina, partially stabilised zirconia, and ultra-high molecular weight polyethene. Generally, bioinert materials consist of materials such as metals, alloys, polymer, composite and ceramic (Knepper *et al.*, 1997). However, there is currently no bioinert material as there is very low chemical reaction between the implant and host (Ravaglioli & Krajewski, 1992).

2.1.1.3 Bioactive

Bioactive biomaterials have specific chemical functional groups that are designed to develop specific interactions when placed in the human body, and they interact with the surrounding bone and in some cases, even soft tissue (Tathe *et al.*, 2010). The reaction occurs through time, dependent kinetic modification of the surface, and triggered by their implantation within the living bone. An ion exchange reaction between the bioactive implant and surrounding body fluids could result in the formation of a biologically active apatite layer on the implant that is chemically and crystallographically equivalent to the mineral phase in bone.

2.1.1.4 Bioresorbable

Degradable or resorbable materials are incorporated into the surrounding tissue, or may even dissolve completely over a period of time (Patel & Gohil, 2012). Some of the common examples for bioresorbable materials are tricalcium phosphate [$Ca_3(PO_4)_2$] and polylactic–polyglycolic acid copolymers. Calcium oxide, calcium carbonate and gypsum are other common materials that have been utilised during the last three decades.

2.1.2 Application of biomaterials

Biomaterials have been used for a wide range of medical applications. It has been used as earliest as ~ 1000 B.C. such as the use of gold strands as soft tissue sutures for hernia repair, silver and gold as artificial crowns, and gemstones as tooth replacements (inserted into the bone and extending into the oral cavity) (Burke *et al.*, 1996). Furthermore, the earliest application of titanium was in medical, surgical, and dental devices that were based on post-World War II advances for the manufacturing processes (Liu *et al.*, 2004).

Table 2.1 shows some examples of the materials used in biomedical. Biomaterials have been used in many functionalities including to replace a body part that has lost its function due to disease or trauma, to assist healing, to improve function, and to correct abnormalities (Patel & Gohil, 2012). The role of biomaterials has been considerably influenced by the advancements in many areas of biotechnology and science. For example, the advent of antibiotics has caused infectious disease to be less threatening compared to the previous time. Table 2.2 shows the examples of artificial devices or parts for the human body.

Table 2.1: Types of biomaterials (Wong & Bronzino, 2007)

Material	Examples
Metals	Titanium and its alloys, Co-Cr alloys, stainless steels, gold, silver
Ceramics	Aluminium oxide, calcium phosphates including hydroxyapatite, carbon,
Polymers	Nylon, silicone rubber, polyester, polytetrafluoroethylene
Composites	Carbon-carbon, wire or fibre reinforced bone cement

Table 2.2: Biomaterials in organs (Wong & Bronzino, 2007)

Organ	Examples
Heart	Cardiac pacemaker, artificial heart valve, total artificial heart
Lung	Oxygenator machine
Eye	Contact lens, intraocular lens
Ear	Artificial steps, cochlear implant
Bone	Bone plate, intramedullary rod
Kidney	Kidney dialysis machine
Bladder	Catheter and stent

2.1.3 Type of biomaterials

Basically, there are four major groups of material that have been used as biomedical materials, which are metals, ceramics, polymers, and composites. Recently, there has been the discovery of a new material and it is being used as biomedical material, which is classified under a new biomaterial group called biological materials. This material includes natural skin, arteries, veins, cord blood vessel, excised diseased, and defective tissues, and the most commonly used animal sources are porcine (pigs) and bovine (cow) (Madihally, 2010).

Another material which is grouped under biological materials is plant-derived substances such as cotton that is used as a wound dressing material (Dorozhkin, 2013). Table 2.3 shows the basic four types of biomaterial classification. Each biomaterial has its own unique functions that could be used for hard tissue implants or soft tissue implants. The selection of biomaterials is important to provide true biological and mechanical match for the living tissue.

Table 2.3. Comparison between the types of biomaterials and their applications (Suzuki & Ikada, 2011)

Material	Advantages	Disadvantages	Applications
Metals	Strong Tough Ductile	May corrode Dense Difficult to make	Joint replacement, bone plates and screws, dental root implant, pacer and suture wires
Ceramics	Very biocompatible Inert Strong in compression	Brittle Not resilient Difficult to make	Dental: femoral head of hip replacement, coating of dental and orthopaedic implants
Polymers	Resilient Easy to fabricate	Not strong Deforms with time May degrade	Sutures, blood vessels, hip socket, ear, nose, other soft tissues
Composite	Strong Tailor-made	Difficult to make	Joint implants, heart valves

2.1.3.1 Metal

Metals are used as biomaterials due to their excellent electrical and thermal conductivity, as well as their mechanical properties. Some metals are used as passive substitutes for hard tissue replacement such as hip and knee joints, fracture healing aids as bone plates and screws, spinal fixation devices, and dental implants due to their excellent mechanical properties and corrosion resistance. Some metallic alloys

are used for more active roles in devices such as vascular stents, catheter guide wire, orthodontic arch wires, and cochlear implants (Park & Kim, 2007). Table 2.4 shows some example of metals and their applications.

Table 2.4: Metals and their biomedical applications (Temenoff & Mikos, 2008)

Metal	Applications
Cobalt-chromium alloys	Artificial heart valves, dental prostheses, orthopaedic fixation plates, artificial joint components
Gold and platinum	Dental fillings, electrodes for cochlear implants
Silver-tin-copper alloys	Dental amalgams
Stainless steel	Dental prostheses, orthopaedic fixation plates, vascular stents
Titanium and its alloys	Artificial heart valves, dental implants, artificial joint components, orthopaedic screws, pacemaker cases, vascular stents

2.1.3.2 Polymer

Synthetic polymeric materials have been widely used in medical disposal supply, prosthetic materials, dental materials, implants, dressing, extracorporeal devices, encapsulants, polymeric drug delivery systems, tissue engineered products, and orthodoses as metal and ceramics substituents (Lee, 1989). Table 2.5 shows the types of biopolymer and their applications in biomedical.

Table 2.5: Types of polymer and their biomedical applications (Temenoff & Mikos, 2008; Dumitriu, 2001)

Type of polymer	Applications
Synthetic	
Poly (2-hydroxyethyl methacrylate)	Contact lenses
Poly (dimethyl siloxane)	Breast implants, contact lenses, knuckle replacements
Poly (ethylene)	Orthopaedic joint implants
Poly (ethylene glycol)	Pharmaceutical fillers, wound dressings
Poly (ethylene terephthalate)	Vascular grafts, sutures
Poly (ε-caprolactone)	Drug delivery devices, sutures
Poly (lactic-co-glycolic acid)	Resorbable meshes and sutures
Poly (methyl methacrylate)	Bone cements, diagnostic contact lenses
Poly (tetrafluoroethylene)	Vascular grafts, sutures
Poly (isoprene)	Gloves
Poly (propylene)	Sutures
Naturally derived	
Alginate	Wound dressings
Chitosan	Wound dressings
Collagen	Orthopaedic repair matrices, nerve repair matrices, tissue engineering matrices
Elastin	Skin repair matrices
Fibrin	Hemostatic products, tissue sealants
Glycosaminoglycan	Orthopaedic repair matrices
Hyaluronic acid	Orthopaedic repair matrices

The main advantages of polymeric biomaterials compared to metal or ceramic materials are ease of manufacturability to produce various shapes (latex, film, sheet, fibres), ease of secondary processability, reasonable cost, and availability with desired mechanical and physical properties. The required properties of polymeric biomaterials are similar to other biomaterials such as biocompatibility, stabilisability, adequate mechanical and physical properties, and manufacturability (Lee *et al.*, 2007).

2.1.3.3 Ceramic

Ceramic in the form of pottery have been used by humanity for thousands of years. Recently, their uses are limited due to their inherent brittleness, susceptibility to notches or micro-cracks, low tensile strength, and low impact strength. However, for the past century, the innovative techniques in fabricating ceramic have led to their use as high-tech materials (Chevalier & Gremillard, 2009). In recent years, someone realised that ceramic and their composites can be used to augment or replace various parts of the human body, particularly bone (Al-Sanabani *et al.*, 2013). Their relative inertness to the body fluids, high compressive strength, and aesthetically pleasing appearance have led to the use of ceramics in dentistry as dental crowns. Bioceramic can also be used as implants and tensile loading application such as artificial tendon and ligaments (Park & Lakes, 1992).

Unlike metal and polymer, ceramic are difficult to plastically shear due to their ionic nature of bonding and minimum number of slip systems. These characteristics have made the ceramics to become non-ductile and they are responsible for almost zero creep at room temperature (Park & Lakes, 1992). Ceramic are used in fabricating implants that are non-absorbable (relatively inert), bioactive or surface reactive (semi-inert) (Hench, 1998), and biodegradable or resorbable (non-inert) (Hentrich *et al.*, 1971; Graves *et al.*, 1972). Alumina, zirconia, silicon nitrides, and carbons are inert bioceramic. Certain glass ceramic and dense hydroxyapatites are semi-inert (bioactive), and calcium phosphates and calcium aluminates are resorbable ceramic (Park & Lakes, 1992; Billotte, 2007). Table 2.6 shows some examples of ceramic and their applications in biomedical.

Table 2.6: Ceramics and their biomedical applications (LeGeros & LeGeros, 1998; Temenoff & Mikos, 2008; Barinov & Komlev, 2008)

Ceramic	Applications
Alumina oxides	Orthopaedic joint replacement components, orthopaedic load-bearing implants, implant coatings, dental implants
Bioactive glasses	Orthopaedic and dental implant coatings, dental implants, facial reconstruction components, bone graft substitute materials
Calcium phosphates	Orthopaedic and dental implant coatings, dental implant materials, bone graft substitute materials, bone cements

2.1.3.4 Composite

Composite materials are solids that contain two or more distinct constituent materials or phases with a larger scale than the atomic. The term, composite, is usually reserved for materials that are in the distinct phases and they separated on a larger scale than the atomic, in which the properties of elastic modulus are significantly altered when compared to a homogeneous material. Accordingly, reinforced plastics such as fibreglass and natural materials such as bone are viewed as composite materials, but alloys such as brass are not composite materials (Davim, 2014).

Some of the applications for composites in biomaterial applications are dental filling composites, reinforced methyl methacrylate bone cement and ultra-high molecular weight polyethene, and orthopaedic implants with porous surfaces (Lakes, 2007). Table 2.7 shows the example of composite material and their applications.

Table 2.7: Composite and their biomedical applications (Davim, 2014)

Composite	Applications
Polyethylene based composites	Bone replacement, load bearing material in endoprostheses, bone graft substitute materials
Polymethymethacrylate based composites	Acetabular cups for hip prostheses, bone reconstructions, fixation of prostheses, bone cements
Polyester based composites	Bone repairs, treating skeletal defects, bone cements, treating osteonecrosis of the femoral head
Chitosan based composites	Tissue regenerating
HAP-metal based composites	Dental and orthopaedic implants
Hydrogels based on poly (vinylalcohol)	Cartilage replacement, tissue repairing matrices, tissue scaffolds
Polymer composites	Total hip replacement
Bioresorbable composites	Bone repair
Bioactive glasses and glass-ceramic	Dental prostheses, orthopaedic fixation plates, artificial joint components

2.1.4 Properties of biomaterials

Some of the properties for biomaterials include biocompatibility, good mechanical properties, non-toxicity, and much more that are very important in biomedical. In order to be implanted inside the human body, all biomaterials should meet or exceed certain properties that have been standardised for the acceptability of the human body (host) for long term usage without any negative effects such as nontoxic, noncarcinogenic, nonallergic, noninflammatory, biocompatible, biofunctionally for its lifetime and it cannot affect the host (Dujovny *et al.*, 1997; Horowitz *et al.*, 2009; Hung *et al.*, 2012). Biocompatibility is the prime requisite in orthopaedic and other implants. Biocompatibility is defined as the implant that does not interact adversely with the physiological environment or vice versa (Simon & Fabry, 1991).

There are three major factors that show how successful the material or implant: (i) the properties and biocompatibility of the implant; (ii) the health condition of the recipient, and (iii) the competency of the surgeon, who both performs the surgery and follow-up with the patient's progress (Manivasagam *et al.*, 2010). There are a lot of requirements that have to be fullfilled to ensure that the biomaterial would be accepted by the body. Besides biocompatibility, the material should possess other properties before being used as biomaterials such as nontoxic, nonallergenic, nonimmunogenic, and sometimes no time-dependent degradation. Other requirements include adequate mechanical strength and fatigue life, proper weight and density. The material should be relatively inexpensive, reproducible and easy to fabricate and process for a large-scale production (Davis, 2003).

An implant is inserted into the body and it will react differently according to the type of material (Park & Lakes, 2007). Fundamentally, there should be similar reaction if the body gets a simple splint. If the material is harmless to the body, the implant will be left in the body and the healing process will begin. An artificial implant that is accepted by the surrounding tissue and the body as a whole the material is known as a biocompatible implant.

The material that is inserted can be designed to degrade over the time. However, the products of degradation should be harmless to the tissue and organs. Overall the implant should be compatible with tissues in terms of mechanical, chemical, pharmacological and surface properties. Figure 2.2 presents some of the ideal property requirements of the materials for total joint replacement.

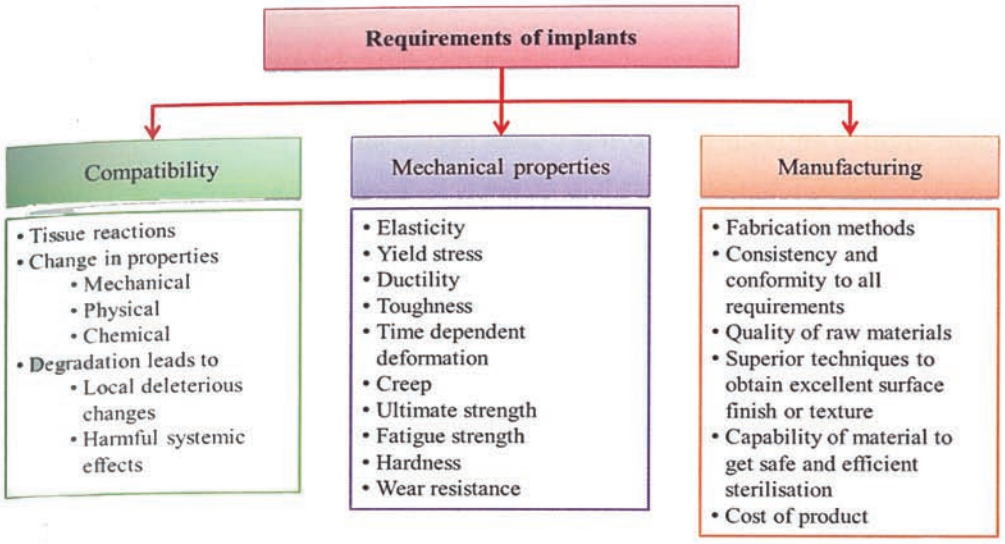

Figure 2.2: Implant material requirements in orthopaedic applications (Davis, 2003).

Besides that, the material should have other properties before being used in biomedical application (Teoh, 2004) as the following:

i. Biocompatibility: The material must not disturb or induce un-welcoming response from the host, but rather promote harmony and good tissue implant integration. An initial burst of inflammatory response is expected and it is sometimes considered as essential in the healing process. However, prolonged inflammation is not desirable as it may indicate tissue incompatibility.

ii. Sterilisability: The material must be able to undergo sterilisation. Sterilisation includes gamma, gas (ethylene oxide (ETO)), and steam autoclaving. Some polymers such as polyacetal will depolymerise and give off toxic gas formadehyde when subjected under high energy radiation by gamma. These polymers are best sterilised by ETO.

iii. Functionability: It depends on the ability of the material to be shaped to suit a particular function. The material must able to be economically shaped using engineering fabrication process.

iv. Manufacturability: There are many candidate materials that are biocompatible. However, it is often the last step, as the manufacturability of the material might hinder the actual production of the medical device.

2.2 Titanium

As biomaterials, titanium and its alloys have been widely used in various industrial applications for the last few decades and they are superior than other materials such as stainless steel, pyrolytic carbon, and many more (Tengvall *et al.*, 1989; Ishizawa & Ogino, 1995; Lee *et al.*, 2016a). Most of the applications of titanium are based on the special surface properties and catalytic properties of the titanium (Hupka *et al.*, 2006; Lee *et al.*, 2015a; Scuderi *et al.*, 2016). Titanium has become the focus of various investigations such as its application in the field of optical, electrical and microelectronic, photonic, chemical, aerospace, automotive, and medical. Titanium was once considered a rare metal, but nowadays it is one of the most important metals in the industry for instance, it is used as hard tissue replacements as well as in cardiac and cardiovascular biomedical application.

2.2.1 Application of titanium

Earlier applications of titanium in medical, surgical, and dental devices were due to post-World War II advancements in manufacturing processes, which was the result of the stringent requirements demanded by the aerospace and military industry (Liu *et al.*, 2004; Manivasagam *et al.*, 2010). There was an increasing trend in the use of titanium and its alloys as biomaterials stems due to their lower modulus, superior biocompatibility, and better corrosion resistance when compared to more conventional stainless and cobalt-based alloys. The attractive properties were the driving force for the early introduction of α (cpTi) and $\alpha + \beta$ (Ti-6Al-4V) alloys. The applications of titanium and its alloys can be classified according to their biomedical functionalities. Table 2.8 shows the applications of titanium in the medical field.

In term of its chemical property, titanium is one of the transition elements in group IV and period 4 of Mendeleef's periodic table. It has an atomic number of 22 and an atomic weight of 47.9. Titanium is more resistant to corrosion than other metal implants such as stainless steel, cobalt-chromium-molybdenum alloys and cobalt-nickel-chromium alloys, although these latter materials are also corrosion resistant to body fluids (Iman & Fraker, 1996). The corrosion resistance nature of

titanium is due to the formation of a protective oxide film. The composition of oxide film ranges from Ti_2O, Ti_2O_3, TiO, and TiO_2 (Iman & Fraker, 1996).

It is important that metallic biomaterials are corrosion resistant. Unlike other types of materials, titanium would either corrode very quickly or extremely slow, depending on the environmental conditions. When come into contact with body fluids that are close to neutral pH, the materials would exhibit corrosion rates that are extremely low and experimentally difficult to be measured. Hence, titanium is widely used in biomedical and dental applications. The stability and corrosion resistance of titanium are due to its native titanium dioxide film that protects the metal from further oxidation (Liu *et al.*, 2004).

Table 2.8: The applications of titanium and its alloys in biomedical field

Field	Applications	References
Orthopaedic	Bone fracture fixation (wires, nails, screws and plates) External bone fracture fixation	Liu *et al.*, 2004 Breme *et al.*, 1997
Hip Joint	Femoral head Cup Stem Fracture plates Screws	Liu *et al.*, 2004 Breme *et al.*, 1997
Knee Joint	Femoral component Tibia component Patella Intramedullary nails Plates	Liu *et al.*, 2004 Breme *et al.*, 1997
Substitute Parts	Shoulder Spine Elbow Hand	Breme *et al.*, 1997
Dental	Screw-sHAPed artificial tooth	Liu *et al.*, 2004
Orthodontics	Removable appliance and expansion screw Brackets and buccal tubes	Lindigkeit, 2003
Prosthetics	Crowns Bridges Combined denture Implant retained suprastructure	Lindigkeit, 2003
Implantology	Endosteal implants	Lindigkeit, 2003
Cardiac and Cardiovascular	Prosthetic heart valves Circulatory devices	Liu *et al.*, 2004
Pacemaker	Pacemaker case Valves	Liu *et al.*, 2004 Breme *et al.*, 1997

Orthodontics: Correction of tooth misorientation with mechanical or functional aids and tools.
Prosthetics: Reconstruction of mastication, aesthetics and phonetics by replacing the missing teeth or parts of them with prostheses.
Implantology: Reconstruction of mastication, aesthetics and phonetics by subperiosteal or endosteal implantation of artificial post into the jaw for the fixation of the prosthesis.

2.2.2 Titanium and its alloys

Table 2.9 shows the four grades of unalloyed commercially pure (cp) titanium for surgical implant applications. The impurity contents that separate them, which are oxygen, iron, and nitrogen should be carefully controlled. Oxygen, in particular, has a great influence on ductility and strength.

Table 2.9: Chemical composition of pure titanium and its alloy (Park & Kim, 2007)

Element	Grade 1	Grade 2	Grade 3	Grade 4	Ti6Al4V[a]
Nitrogen	0.03	0.03	0.05	0.05	0.05
Carbon	0.10	0.10	0.10	0.10	0.08
Hydrogen	0.015	0.015	0.015	0.015	0.0125
Iron	0.20	0.30	0.30	0.50	0.25
Oxygen	0.18	0.25	0.35	0.40	0.13
Titanium	Balance				

[a] Aluminium 6.00% (5.0), vanadium 4.00% (3.50-4.50), and other elements 0.1% maximum or 0.4% total.

All maximum allowable weight percent.

2.2.3 The properties of titanium

In term of its chemical property, titanium is one of the transition elements in group IV and period 4 of Mendeleef's periodic table. Table 2.10 shows its mechanical properties according to ASTM F136 of titanium and its alloy. Besides that, Table 2.11 describes some basic physical properties of unalloyed titanium.

Table 2.10: Mechanical properties of titanium and its alloy according to ASTM F136 (Park & Kim, 2007)

Properties	Grade 1	Grade 2	Grade 3	Grade 4	Ti6Al4V	Ti13Nb13Zr
Tensile strength (MPa)	240	345	450	550	860	1030
Yield strength (0.2% offset) (MPa)	170	275	380	485	795	900
Elongation (%)	24	20	18	15	10	15
Reduction of area (%)	30	30	30	25	25	45

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