Manufacturing of biomaterials with engineered porosity for the promotion of osseointegration : biocompatible duplex coating on titanium alloy for hard tissue replacement

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2005

Ng, B. S. (2005). Manufacturing of biomaterials with engineered porosity for the promotion of osseointegration : biocompatible duplex coating on titanium alloy for hard tissue replacement. Master's thesis, Nanyang Technological University, Singapore.

<https://hdl.handle.net/10356/6196>

<https://doi.org/10.32657/10356/6196>

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**Manufacturing of Biomaterials with Engineered Porosity
for the Promotion of Osseointegration:
Biocompatible Duplex Coating on Titanium
Alloy for Hard Tissue Replacement**

Ng Boon Sing

School of Mechanical & Production Engineering

A thesis submitted to the Nanyang Technological University
in fulfillment of the requirement for the degree of
Master of Engineering

2005

Abstract

Abstract

Potential nickel ion release from nickel-titanium alloy (NiTi) in physiological fluid environments has hindered the alloy's medical application thus far. Even Ti6Al4V, an established implant material, is known to release metallic ions *in-vitro* and *in-vivo*. To improve its biocompatibility, metal concentration at the surface was minimised by etching. A duplex titanium oxide, calcium phosphate (TiO/CaP) coating was then deposited by anodising NiTi; Ti6Al4V in a suitable electrolyte and subsequent immersion in simulated body fluid with three times the standard ion concentration (SBFx3).

X-ray photoelectron spectroscopy (XPS) study indicates that anodic titanium oxide has thicker top TiO₂ layer than those formed in air. Thickness of the oxide layer increases with anodising voltage. Mixtures of titanium sub-oxides exist between the TiO₂ and the metal substrate for all samples. The lack of typical titanium oxide (rutile, anatase) peaks during thin-film x-ray diffraction (TF-XRD) analysis shows that titanium oxide formed is amorphous in nature, or with poorly ordered crystal structures of size beyond the sensitivity of the instrument.

Electrochemical characterisation (electrochemical impedance spectroscopy and potentiodynamic polarisation) in simulated body fluid reveals that titanium oxide formed anodically on NiTi is compact, barrier-type layer. Anodised NiTi samples exhibit a three-fold improvement in their pitting resistance over orthopaedic-grade samples. Anodised Ti6Al4V samples have thicker titanium oxide, and exhibit four-fold improvement in their corrosion resistance over unanodised samples. Anodised NiTi has low pitting potential compared to Ti6Al4V (e.g. 1.0V verses 14.0V). However, within measured body potential (0.2-0.6V), NiTi exhibit passivity comparable to those of Ti6Al4V in SBF solution. The results show that enhancement in anodic oxide

Abstract

thickness, while maintaining the compactness, improves pitting resistance of NiTi and Ti6Al4V in simulated body fluid.

Effects of immersion bath temperature ($38.5\pm0.1^{\circ}\text{C}$ versus $39.5\pm0.1^{\circ}\text{C}$), different substrate surface treatment on successive steps of calcium phosphate nucleation and growth were monitored using Fourier transform infrared spectroscopy with an attenuated total reflection (ATR-FTIR) and scanning electron microscopy (SEM). Analyses of the results reveal that elevating the immersion bath temperature enhances the heterogeneous nucleation of amorphous calcium phosphate globules onto the titanium alloys. Modifying the titanium oxide layer can control the rate of crystallisation, from globules of amorphous calcium phosphate to plate-like crystalline apatite. Results from FTIR analysis indicate that the final coating at 96 hours immersion is a B-type carbonated calcium-deficient apatite, similar with those in natural bone.

Further analyses of this coating using XPS, TF-XRD reveal that the apatite exhibits structure (amorphous or poorly crystalline), chemical composition (with carbonate, hydrogen phosphate and magnesium substitutions) and calcium-to-phosphorous ratios close to those of natural bone (1.14-1.60) than hydroxyapatite (1.67).

Deposition of a duplex anodic titanium oxide, bone-like apatite coating seems to possess a potential in producing titanium alloy prostheses with enhanced corrosion resistance, and this can be an important step towards direct bonding of implants with bone. However, strict measures have to be taken to ensure aseptic conditions (autoclave, UV radiation, etc.) to prevent susceptibility to bacteria contamination during immersion in SBF at physiological temperature (around 37°C).

Acknowledgements

Acknowledgements

This work was carried out at the Division of Manufacturing Engineering, School of Mechanical and Production Engineering, Nanyang Technological University (NTU), in collaboration with Singapore Institute of Manufacturing Technology (SIMTech) during the years 2002-2004. The author is deeply grateful to NTU and SIMTech for giving her the opportunity to take up this collaborative project for her Master of Engineering studies.

Sincere thanks are extended to Associate Professor Khor Khiam Aik, Ph.D, NTU/MPE/ME, and Dr. Andrew Soutar, Ph.D., Senior Scientist, SIMTech, for their supervision and invaluable advice.

Special thanks goes to Dr. Ingegerd Annergran, Ph.D, Assistant Professor Butler Lee David, Ph.D., NTU/MPE/ME and Dr. Anders E. W. Jarfors, Ph.D., and for their support in many ways.

A word of thanks goes to Dr. Jason Paul Pickering, Dr. Gu Yan Wei, Ph.D., Dr. Andrew Spowage, SIMTech, and Madam Chow Shiau Kee, Senior Laboratory Executive, NTU/MPE for extending their instrumental expertise and knowledge.

The author would also like to express her appreciations to, Mr. Ronnie Tew, Miss Cassandra Ngoh, Dr. Montserrat Espanol Pons, Mr. Goi Lim Seng and Mr. Joshua Thong for their invaluable help and inputs in the writing of this report.

Finally, yet importantly, the author would like to thank her family who have been supportive in all manners.

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1. H. Li, B. S. Ng, K. A. Khor, P. Cheang, *Raman spectroscopy determination of phases within thermal sprayed hydroxyapatite splats and subsequent in-vitro dissolution examination*, Acta Materialia, Vol. 50, No. 2, pp. 445-453 (2004).
2. B.S. Ng, I. Annergren, A. M. Soutar, K.A. Khor and A.W.F Jarfors, *Characterisation of a duplex TiO₂/CaP coating on Ti6Al4V for hard tissue replacement*, (Paper accepted for publication in *Biomaterials*, work presented in 2nd International Conference on Materials for Advanced Technologies & IUMRS-International Conference in Asia 2003, Singapore).
3. B.S. Ng, K.A. Khor and A. M Soutar, *Spectroscopic and microscopic characterisation of apatite deposited onto anodised NiTi*, (paper submitted for publication).
4. B.S. Ng, K.A. Khor, A. M Soutar and I. Annergren, *Characterisation of Surface Oxideformed on Anodised NiTi for Hard Tissue Replacement*, (paper submitted for publication)

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55-Nitinol	Equiatomic nickel-titanium alloy with 55 atm.% nickel
AC	Alternating current
AFM	Atomic force microscopy
aq.	Aqueous
AISI	American Iron and Steel Institutes
atm.%	Atomic percentage
ATR	Attenuated total reflection
BE	Binding energy
BEs	Binding energies
c	Cation
CaP	Calcium phosphate
Ca:P	Calcium-to-phosphorous ratio in atomic percentage
CPE	Constant phase element
DC	Direct current
C _{dl}	Capacitor
DCPD	Dicalcium phosphate dihydrate (Brushite)
ECF	Extracellular fluid
EDX	Energy dispersive for X-ray analysis
E _f	Forming/anodising voltage
E _{corr}	Corrosion potential
E _f	Forming voltage
EIS	Electrochemical impedance spectroscopy
E _{pitt}	Pitting corrosion potential
ε	Dielectric constant
FEGESEM	Field emission gun environmental scanning electron microscopy

Lost of Symbols

FESEM	Field emission scanning electron microscopy
FTIR	Fourier transform infrared spectroscopy
g	Gaseous
HA	Hydroxyapatite
HVOF	High velocity oxygen-fuel
i	Current density
ICP-AES	Inductively coupled plasma-atomic emission spectroscopy
M	Molar
NiTi	Nickel-titanium shape memory alloy (also Nitinol)
OCP	Octacalcium phosphate
ox.	Oxidation
PMMA	Polymethyl methacrylate
PTFE	Polytetraflouroethylene
R_a	Roughness average
R^*	Polarisation resistance
R_q	Root mean square average
R_s	Resistor
s	Solid
S.A.	Surface area
SBF	Simulated body fluid
SCE	Saturated calomel electrode
SEM	Scanning electron microscopy
Ti6Al4V	Titanium-aluminium (6%)-vanadium (4%)
TF-XRD	Thin film- X-ray diffraction
TJR	Total joint replacement
Ti:O	Titanium to oxygen ratio in atomic percentage

Lost of Symbols

TRIS	Trishydroxymethylaminomethane
UHMWPE	Ultra-high molecular weight polyethylene
V	Volts
Vitallium	Cobalt-chrome-nickel-tungsten alloy
XPS	X-ray photoelectron spectroscopy
XRD	X-ray diffraction

Glossary

Glossary

Albumin	A class of simple, water-soluble proteins that can be coagulated by heat and are found in egg white, blood serum, milk, and many other animal and plant juices and tissues.
Bioactive	Having an effect on or eliciting a response from living tissue.
Cancellous	Having a spongy or porous structure; made up of cancelli; cancellated; as, the cancellous texture of parts of many bones.
Cytotoxic	Chemicals directly toxic to cells, preventing their reproduction or growth. Cytotoxic agents can damage healthy noncancerous tissues or organs, which have high proportion dividing cells e.g. bone marrow.
Endodontic	A branch of dentistry that deals with diseases of the tooth root, dental pulp, and surrounding tissue
Endosseous	Surgical insertion of an implant into the bone.
(wound healing)	Healing of tissues surrounding the inserted endosseous implant.
Glycosaminoglycans	A polymerised sugar (see polysaccharide) commonly found in carious connective tissue.
Glycoprotein	A compound consisting of carbohydrate protein. The carbohydrate is generally hexosamine, an amino sugar.
<i>In-vitro</i>	In glass, as in a test tube. An in vitro test is one done in laboratory, usually involving isolated tissue, organs, or cells.
<i>In-vivo</i>	In the living body or organism. A test performed in a living organism.
Lacuna	A depression; a small blank space; a gap or vacancy; a hiatus.
Maxillofacial	Pertaining to the jaws and face, particularly with reference to specialised surgery of this region.
Organometallic	Pertaining to, or denoting, any one of a series of compounds of certain metallic elements bound to organic radicals.

Glossary

Ossteoblasts	A cell that makes bone. It does so by producing a matrix that then becomes mineralized.
Ossteoconductive	Support of bone tissue growth.
Ossteoinductive	To induce growth of bone tissue.
Ossteointegration	Direct contact of bone tissues to an implant surface with fibrous membrane.
Orthopaedics	The branch of medicine that deals with the prevention or correction of injuries or disorders of the skeletal system and associated muscles, joints, and ligaments.
Polysaccharide	Polymerised sugar molecules found in tissues as lubricant (synovial fluid) or cement (between osteon, tooth root attachment) or complexed with proteins such as glycoproteins.
Prosthesis	An artificial substitute for a missing body part, such as an arm or leg, eye or tooth, used for functional/ cosmetic reasons.
Shake-up lines	Satellite XPS peak of emitted photoelectron from ion left in an excited state a few electron volts above the ground state. Such peaks are found at higher binding energy (eV) than the main peak.
Stent	A device used to main the bodily orifice or cavity
Stroma (cell)	The connective tissue framework of an organ, a gland, or other structure, as distinguished from the tissues performing the special function of the organ or part.
Traumatology	The study of wounds and injuries caused by accidents or violence to a person, and the surgical treatment and repair of the damage. It is often considered a subset of emergency medicine, also known as accident surgery.

Chapter 1: Introduction

Natural joints such as hip, knee, spinal joints, are complex but delicate structures capable of functioning under critical conditions (Long & Rack, 1998). Such capability is result of optimal combination of mechanical and biological properties of physiological materials. Unfortunately, human joints are prone to degenerative and inflammatory diseases that lead to pain and joint stiffness. Partial or even dramatic degradation of tissues and organs with age is becoming more widespread. In fact, 80% of the population over the age of 40 suffer a certain degree of degenerative joint disease (AAOS, 2001). Premature degeneration of joints may arise from deficiencies in joint biomaterial properties, from excessive loading conditions, or from failure of normal repair processes. However, the exact process of degeneration is not yet understood. The need to repair, or even to replace, worn out body parts, bones and organs has made biomedical engineering one of the most heavily studied areas in recent years. Weight bearing joint surfaces must be replaced by metal, plastic, or ceramic artificial materials through arthroplasty surgery, with total joint replacement (TJR) being a major advancement in orthopaedic surgery. Within this area, total hip replacement is the most common artificial joint in human beings. Approximately 300,000 hip and knee arthroplasties were performed in America alone during year 2000, and the rate is increasing (AAOS, 2000). Synthetic prostheses have therefore become increasingly important and new biomaterials; improved processes and techniques have been progressively discovered and developed. Longevity has also become one of the main criteria for permanent implants, especially when the implants are built for young patients (Hallab et al., 2000).

Titanium and its alloys, have been extensively used as load-bearing biomaterials due to their low modulus, superior biocompatibility and enhanced resistance compared

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to conventional stainless steels and cobalt-based alloys (Jacobs et al., 1998; Jones, 2001; Iman et al., 1979; Long & Rack, 1998). Ti6Al4V is currently the most commonly used titanium alloy. However, the differences in elastic modulus of these materials with bone lead insufficient load transfer from artificial implants to adjacent remodelling bone. The consequences are bone resorption (Wolff's Law) (Wolff, 1986), followed by prosthesis failure (Long & Rack, 1998; Park & Kim, 2000).

NiTi or Nitinol, is a promising new implant material that possesses unique superelasticity (pseudoelasticity), thermal shape memory, deformation compatibility, good damping properties and biocompatibility (Duerig et al., 1999; Park & Kim, 2000). An equiatomic alloy (55-NiTi) has superelasticity and flexibility, allowing up to 8% of strain to be fully recovered. This alloy also has a Young's modulus (28-50MPa for martensite and 83-120MPa for austenite phase) (Hodgson et al., 1999) and stress-strain hysteresis very similar to that of natural bone (7-40MPa) (Duerig et al., 1999). These unique properties of NiTi improve the physiological similarity between bone and implants, enhancing the healing process and causing less post-operational trauma by sharing the load with the surrounding tissues (Kapanen et al., 2001; Park & Kim, 2000). Contradictory reports on potential nickel ion release from NiTi and its effect on cytotoxicity and biocompatibility (Filip et al., 2001; Kapanen et al., 2002; Ryhanen et al., 1998; Wever et al., 1998) have been published. However, there is still much interest in this material's potential to yield a significantly more efficient tissue/implant interaction (Bogdanski et al., 2002; Castleman et al., 1976; El Medawar et al., 2002; Kujala et al., 2002; Thompson, 2000).

The biocompatibility of titanium and its alloys is probably due to the spontaneous formation of a dense oxide film on its surface. This titanium dioxide (TiO₂) film covering the surfaces of these materials constitutes an effective barrier to

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metal ion diffusion (Firstov et al., 2002; Jones, 2001; O'Brien et al., 2002; Shabalovskaya et al., 2003; Thierry et al., 2002; Wever et al., 1998). Anodisation is a simple technique that can be employed to modify, and enhanced pitting resistance of titanium alloy. Depending on the parameters such as applied voltages, process time, electrolyte type and concentration, titanium oxide with controlled thickness, structure and composition can be achieved (Asami et al., 1990; Badekas & Panagopoulos, 1987; Blondeau et al., 1977a; Blondeau et al., 1977b; Bojinov, 1996; Blackwood et al., 1989; Firstov et al., 2002; Sul et al., 2001). However, anodisation of NiTi is not common.

As the surface oxide is exposed to the tissue, it is the chemical properties of the titanium oxide, and not the bulk metal, that determines the tissue response after an implantation. Negatively charged titanium oxide, when exposed to physiological fluids, has an ion exchange property that binds positively charged calcium ions. This property is shared by other materials that integrate with bone (e.g. hydroxyapatite-HA and oxides of zirconium), which has been suggested as basis for biocompatibility of titanium. The increased in subsequent adsorption of proteins (e.g. serum) (Ellingsen, 1991) and acidic macromolecules (e.g. albumin and glycosaminoglycans) (Bernardi & Kawasaki, 1968; Bernardi et al., 1972) after adsorption of calcium ions is a property of titanium oxide has in common with HA. Topology, chemical composition, stoichiometry, crystal structure and defect density of this oxide determine the diffusion rate of calcium/phosphate ions into the oxide layer, influencing apatite nucleation (Barrere et al., 1999; Kokubo et al., 1999; Li & Ducheyne, 1998; Stoch et al., 2000; Tanahashi et al., 1996). The induced calcium phosphate layer could also act as a further barrier against titanium ion diffusion through titanium oxide layer into the electrolyte (Sousa & Barbosa, 1996; Wever et al., 1998).

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Hydroxyapatite (HA), a good example of bioactive ceramic, is a form of calcium phosphates (CaP) effective in promoting spontaneous bone growth and bone integration i.e. good bone fixation (Billotte, 2000; Hench & Wilson, 1993a). However, despite the excellent results shown in numerous *in-vivo* studies on these materials (Aoki, 1994; Ellingsen, 1999; Hench & Wilson, 1993a; Manjubala et al., 2002; Tanahashi et al., 1996; Yuan et al., 2001), they have limited load-bearing applications (e.g. hip and teeth implants) due to their brittle nature, low tensile strength and fracture toughness. de Groot resolved this problem in the 1980s by plasma spraying calcium phosphate onto metallic prosthesis. This technique is able to produce CaP coatings in tens to hundreds of microns (Ding et al., 2003; Khor et al., 2003b; Porter et al., 2003; Taddei et al.) and has been proven by many successful clinical reports (Hench & Wilson, 1993b). The high temperatures involved, however, make it impossible to coat heat sensitive material. The line-of-sight nature of this technique further limits its applications in coating implant with complex/porous geometry.

The biomimetic process is an alternative low temperature technique developed to cater for the above-mentioned applications. This process imitates or ‘mimics’ the natural biomineralisation occurring during bone formation in human and animals. Thin coatings (few microns) of metastable calcium phosphate phases with incorporated drugs and essential proteins to enhance prostheses survival can be deposited onto different substrates. It is an immersion process, which substrates are immersed into the Kokubo’s simulated body fluid (SBF) (Kokubo et al., 1990), at physiological pH (7.4) and temperature (37°C) (Furuzono et al., 2000; Kokubo, 1996; Li & Ducheyne, 1998; Takadama et al., 2001; Stigter et al., 2002). Kokubo et al. established that the essential criterion for successful biomimetic coating is the substrate’s ability to initiate calcium phosphate nucleation on its surface (Kokubo et al., 1999). Structure and composition of

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biomimetic calcium phosphates formed vary with the substrate's surface morphology, chemistry, and with the SBF solution's ion concentrations. Octacalcium phosphate, carbonated apatite or calcium deficient apatite are just some examples (Barrere et al., 2002b; Takadama et al., 2001; Tanahashi et al., 1996). These calcium phosphate phases actually have structures and compositions closer to that of natural bones, which are mainly calcium-deficient carbonated hydroxyapatite (HA) (Aoki, 1994). Calcium-to-phosphorous ratios in bones are different from HA, and are normally less than 1.67 (Turek, 1984). Bone was found to deposit directly onto commercially pure or commercially pure-titanium plate that had been biomimetically coated with calcium phosphate without any intervening soft tissue (Yan et al., 1997). The formation of biomimetic calcium phosphate coatings on titanium and its alloys could take seven to forty-eight days (Kokubo, 1996; Liu et al., 2001; Stigter et al., 2002; Rey et al., 1991). The kinetic of calcium phosphate nucleation can be enhanced by increasing SBF solution's concentration (Barrere et al., 2002b; Dorozhkina et al., 2003), controlling pH of SBF solutions (Chou et al., 2004), elevating immersion temperature (Lin et al., 2002) or modifying substrate's surface (Liu et al., 2002b; Kokubo, 1996; Oliveira et al., 2003b; Oyane et al., 2003; Yang et al., 2004).

The present study aims to change the surface chemical properties of NiTi and Ti6Al4V to improve their *in-vitro* corrosion resistance, providing henceforth favourable consequences for the biological properties of the implants. The approach adopted is to apply a duplex coating to 1) improve the *in-vitro* corrosion resistance of NiTi and Ti6Al4V in physiologically buffered SBF solution, by enhancing thickness and compactness of the titanium oxide layer, and 2) induce formation of a bone-like apatite coating on the titanium oxide.

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Numerous reports on coating biomimetic calcium phosphate (CaP) on Ti6Al4V and commercially pure titanium are available (Barrere et al., 1999; Kokubo, 1996; Liu et al., 2001; Stoch et al., 2000; Stigter et al., 2002; Takadama et al., 2001; Tanahashi et al., 1996; Yan et al., 1997). Effectiveness of anodising titanium alloys in improving their corrosion resistance in simulated physiological fluids, in comparison with alternative conventional surface treatments, has been evaluated and reported by many authors (Blackwood & Chooi, 2002; Cai et al., 2003; Cortada et al., 2000; Gonzalez & Mirza-Rosca, 1999; Hukovic et al., 2003; Seah et al., 1995; Pouilleau et al., 1997; Valereto et al., 2002; Velten et al., 2002). The *in-vitro* corrosion resistance of as polished, passivated, heat-treated etc. NiTi samples have also been heavily studied (Aziz-Kerrzo et al., 2001; Chen et al., 2004; El Medawar et al., 2002; Es-Souni et al., 2002; Firstov et al., 2002; Marino et al., 2001; Shabalovskaya et al., 2003; Starosvetsky & Gotman, 2001). However, there are few reports on anodisation of NiTi available within the literature (Asami et al., 1993; Asami et al., 1991). No report is available, within the literature search, on *in-vitro* corrosion resistance of anodised NiTi. Only a couple of reports are available on depositing biomimetic calcium phosphate on NiTi (Chen et al., 2004; Chen et al., 2003; Choi et al., 2003) and anodised Ti6Al4V (Yang et al., 2004; de Sena et al., 2002; Wang et al., 2003b), but none for anodised NiTi.

The present study therefore also attempts to investigate 1) the feasibility and effectiveness of employing an anodisation process to enhance the NiTi's surface titanium oxide; 2) the relationship between anodising parameters (electrolyte-type and applied voltage) and the resultant *in-vitro* corrosion resistance of anodised NiTi and Ti6Al4V; 3) calcium phosphate nucleation ability on anodised NiTi and Ti6Al4V; and finally 4) effects of surface treatments/substrates surface chemistry on the composition and morphology of the deposited calcium phosphate.

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This work is based on plate-form material and will be used as groundwork for the future development of process for porous Ti6Al4V and equiatomic NiTi, which, is not within the scope of this project. The work in this report is presented as follows:

Chapter 2 gives a survey of the fundamental aspects of the available biomaterials. It covers recent progress in surface modifications of titanium alloy substrates and deposition of biocompatible calcium phosphate coatings via the biomimetic route.

Chapter 3 describes the experimental procedures, techniques and machine parameters employed to modify the titanium alloy's surface to improve its corrosion resistance (electrochemical anodisation approach) and biocompatibility (biomimetic route). Sample preparation methods and techniques used for surface chemical composition studies, structure analysis, corrosion resistance assessment, surface roughness measurements and observation of surface morphology are also described in detail.

Chapter 4 compiles the experimental data and graphs.

Chapters 5 and 6 cover the discussion of anodisation process, *in-vitro* corrosion resistance assessment, chemical composition, structure and morphology studies of the duplex coatings produced. Structure and chemical composition of the coatings are discussed. Relative thickness of anodic versus air-formed titanium oxide on NiTi and Ti6Al4V are determined. The effects of different surface treatments employed on the materials' topography and *in-vitro* corrosion resistance in SBF are discussed. An attempt to relate variations in morphology, chemical composition and relative thickness of the final calcium phosphate coating, with immersion temperature in SBFx3 ($38.5 \pm 0.1^\circ\text{C}$ versus $39.5 \pm 0.1^\circ\text{C}$), substrate-type and surface treatment is made.

Chapter 7 concludes the findings of the present study.

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Chapter 8 lists the recommended future work necessary to fulfil the objectives of the project, and to allow a better understanding of the influence of surface properties on the subsequently applied coatings.

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2.1 Biomaterials for Hard Tissue Replacement

Biomaterials are used to direct, supplement, or replace the functions of living tissue in the human body in a safe, reliable and physiologically accepted manner (Hench & Ethridge, 1982). A variety of devices and materials are used in treatment of degenerative disease. Some examples are bone plates, total joint replacement, screws and teeth filling. The success of biomaterials depends on factors such as its properties, design and biocompatibility. However, subjective factors such as surgery techniques, health and condition of patient and post-operative activities of the patients also affect performance of these materials in the internal body environment (Park, 2000). For example the pH of the body fluids in various tissues, shown in Figure 1, can vary between 3.5 to 9 (Park & Kim, 2000). Loads on a hip joint can range from three to ten times body weight, stress cycles for finger joint or hip joint motion can be as high as 1×10^{16} per year (Ramakrishna et al., 2001). It is therefore important that biomaterials demonstrate both 'biofunctionality', and 'biocompatibility'. 'Biofunctionality' concerns the ability of the implant to adapt, in particular to the mechanical behaviour of the host tissue with minimum mismatch at the implant/tissue interface. 'Biocompatibility' is a descriptive term that indicates the ability of a material to perform with an appropriate host response while serving its specific application (Hench & Wilson, 1993a).

According to Wolffs law, bone remodels and adapts itself to the applied mechanical environment (Wolff, 1986). Density of living bone is influenced by the stress condition applied to the bone. Higher applied stress leads to denser bone, and conversely, applied stress below normal physiological loads leads to a decrease in bone mass and hence bone weakening (Wolff effect) (Cowin, 1981; Hayashi, 1996; Long &

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Rack, 1998; Wolff, 1986). Insufficient load transfer from an artificial implant to adjacent remodelled bone, due to substantial difference in elastic modulus between e.g. titanium alloy (110GPa) and bone (7-40GPa) (Long & Rack, 1998; Park & Lakes, 1992), can also result in bone resorption (Wolffs Law). These mismatches of the mechanical properties between bone and metallic/ceramic implants can lead to loosening or fracture and eventual prosthesis failure (Long & Rack, 1998) and should be avoided or minimised.

Figure 1 Pourbaix diagram for Chromium, showing regions associated with various body fluid (Park & Kim, 2000)

Implants are designed to be temporary or permanently in contact with living tissues (Park, 2000; Hench & Wilson, 1993a). The overall reaction of a body towards a foreign material implant is dependent on several factors, which determines whether the implant is biocompatible and biofunctional. Biocompatibility is a system property, which includes mechanical, chemical, biological, medical and design aspects (Wieland et al., 1999). Based on their effect on the surrounding bone tissue; implant materials can be classified as either

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1. Tolerant materials result in a distant osteogenesis with a fibrous tissue layer formed between implant and bone.
2. Bio-inert implants are encapsulated by a layer of thin fibrous tissue.
3. Bioactive implants enhance bonding with the bone by influencing and participating in bone nucleation, formation and growth (Aoki, 1994).

Biofunctionality defines an implant's ability to perform its designed functions, namely:

1. Mechanical properties such as tensile strength, fracture toughness, elongation at fracture, fatigue strength, Young's modulus;
2. Physical properties like density (orthopaedic implants), thermal expansion (bond cement), and
3. Surface chemistry like degradation resistance, oxidation and corrosion.

To fulfil both biofunctionality and biocompatibility requirements, various types of bone substitutes have been developed: metallic materials (titanium, titanium alloys, stainless steel, cobalt-chromium alloys); ceramics (aluminium oxide, carbon, calcium phosphate, Bioglass©) polymers (silicon rubber, PMMA – poly(methyl methacrylate), Polyactive©), and composites (hydroxyapatite coating on metal implants, hydroxyapatite-reinforced Polyactive©), the choice of material depends on the part's application (Shabalovskaya et al., 2003). Calcium phosphate ceramics have demonstrated unique bioactive properties for bone repair. Nevertheless, they are limited when a load-bearing application is required due to their brittle nature (Billotte, 2000) e.g. hydroxyapatite has modulus comparable to, but tensile strength much lower than those of titanium alloy (95 GPa verses 116 GPa and 50 MPa verses 965MPa respectively) (Long & Rack, 1998; Park & Lakes, 1992; Ramakrishna et al., 2001). Even though metals are able to meet the mechanical requirements for load-bearing

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applications, they generally need surface modification to improve their biocompatibility, and must be properly fixed onto bone.

2.1.1 Ceramic implants

Alumina, titania, zirconia, bioglass or bioactive glass and hydroxyapatite (HA) are widely considered as biocompatible ceramics. Bone bonds spontaneously to some glasses in the $\text{Na}_2\text{-CaO-SiO}_2\text{-P}_2\text{O}_5$ system (called BioglassO) with no fibrous encapsulation (Hench & Wilson, 1993a). In particular, calcium hydroxyapatite (HA), tri-calcium phosphate and other such materials promote bone-tissue formation at the interface and promotes chemical bonding with osseous tissues after being implanted (Liu et al., 2002a; Oonishi et al., 2000). General observations of calcium phosphate coatings accelerating early healing in endosseous sites could be due to an increase in affinity for, and absorption of proteins to, this material (HA is employed in protein separation columns). HA has also been shown to play a role in the osteoconduction stage of endosseous wound healing (Davies & Hosseini, 1999). Mechanical properties of some typical ceramic biomaterials are shown in Table 1.

Ceramics are well known for their good biocompatibility, corrosion resistance and high strength under compression. However, these materials have mechanical drawbacks, which include brittleness, low fracture toughness and low modulus (when compared to metal), limiting their load bearing applications (Billotte, 2000).

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Table 1 Mechanical properties of typical ceramic biomaterials (Ramakrishna et al., 2001).

Material	Modulus (GPa)	Tensile strength (MPa)
Alumina	380	300
Zirconia	220	820
Bioglass®	35	42
Hydroxyapatite	95	50

Apatite is the most common crystalline form of calcium phosphate and is largely used in biological applications. Hydroxyapatite (HA), $\text{Ca}_{10}(\text{PO}_4)_6\text{OH}_2$, is the most commonly used biceramic. The word ‘Hydroxyapatite’ is a combination of ‘hydroxy’, OH^- , and ‘apatite’, a mineral name (Aoki, 1994). Stoichiometric HA is represented by a general formula:

where Me is a divalent metal (Ca^{2+} , Sr^{2+} , Ba^{2+} , Pb^{2+} etc.), XO_4 a trivalent anion (PO_4^{3-} , AsO_4^{3-} , VO_4^{3-} etc.) and Y is a monovalent anion (F^- , OH^- , Cl^- , Br^-).

For stoichiometric HA, substitution of trivalent XO_4^{3-} by divalent ions (e.g. HPO_4^{2-}) induces lacunas in the lattice. The resultant apatite is known as calcium-deficient apatite, and possesses decreased crystallinity. Carbonate ion substitution can occur at different sites in the crystal, resulting in different types of deficient apatite: B-type carbonated apatite (CO_3^{2-} occupies the XO_4^{3-} site); A-type carbonated apatite (CO_3^{2-} occupies the Y site) and AB-type carbonated apatite (CO_3^{2-} occupies both XO_4^{3-} and Y₂ sites). These apatites have structure closer to that of natural bone (Aoki, 1994; Barrhe, 2002; Kim, 2003; Treboux et al., 2000b).

A convenient way to classify calcium phosphates is by their Ca:P ratios. Hydroxyapatite has the chemical formula $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, and its Ca:P ratio is therefore $10/6=1.67$. Ca:P ratios of currently known calcium phosphate compounds are shown in Table 2. Calcium phosphates of higher Ca:P ratio are precipitated in alkaline

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solution, which acidic condition favour lower Ca:P ration compounds. HA is precipitated in either neutral or alkaline solution (Aoki, 1994).

Octacalcium phosphate (OCP) has the chemical formula $\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$. Its crystalline form is triclinic, with alternating 'apatite layers' (with similar calcium phosphate groups arrangement to that of apatite) and 'hydrated layers'. OCP has Ca:P atomic ratio of 1.33, and amorphous OCP is believed to be the transient intermediate formed during precipitation of the more thermodynamically stable HA and biological apatite (Rey et al., 1991). This metastable amorphous phase transform to poorly crystalline apatite at rates dependent on the temperature and composition of solution.

Table 2 Various calcium phosphates with their respective Ca:P atomic ratios (Aoki, 1994)

Ca:P	Formula	Name	Abbreviation
2.0	$\text{Ca}_4\text{O}(\text{PO}_4)_2$	Tetracalcium phosphate	TTCP
1.67	$\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$	Hydroxyapatite	HAp
	$\text{Ca}_{10-x}\text{H}_{2x}(\text{PO}_4)_6(\text{OH})_2$	Amorphous calcium phosphate	ACP
1.5	$\text{Ca}_3(\text{PO}_4)_2$	Tricalcium phosphate (α , β , γ)	TCP
1.33	$\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$	Octacalcium phosphate	OCP
1.0	$\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$	Dicalcium phosphate dihydrate (Brushite)	DCPD
1.0	CaHPO_4	Dicalcium phosphate (Monetite)	DCP
1.0	$\text{Ca}_2\text{P}_2\text{O}_7$	Calcium pyrophosphate (α , β , γ)	CPP
1.0	$\text{Ca}_2\text{P}_2\text{O}_7 \cdot 2\text{H}_2\text{O}$	Calcium pyrophosphate dihydrate	CPPD
0.7	$\text{Ca}_7(\text{P}_5\text{O}_{16})_2$	Heptacalcium phosphate (Trömelite)	HCP
0.67	$\text{Ca}_4\text{H}_2\text{P}_6\text{O}_{20}$	Tetraclacium dihydrogen phosphate	TDHP
0.5	$\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$	Monocalcium phosphate monohydrate	MCPM
0.5	$\text{Ca}(\text{PO}_3)_2$	Calcium metaphosphate (α , β , γ)	CMP

Amorphous calcium phosphate (ACP) can vary widely in composition, with a Ca:P ratio ranging between that of HA and TCP ($1.67 < \text{ACP} \leq 1.50$), due to the possible insertion or substitution of several secondary ions (Aoki, 1994). These materials exhibit broad and featureless x-ray diffraction halos and infrared monocomponent PO_4^{3-} bands.

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Its basic structure is a cluster of ions ($\text{Ca}_9(\text{PO}_4)_6$) packed with interfacial water to form bigger entities (Betts & Posner, 1974; Treboux et al., 2000b).

Most calcium phosphate biomaterials are prepared by wet chemistry, using the conventional double decomposition method between a calcium salt and a phosphate salt at very high supersaturation levels (Rey et al., 1991).

2.1.2 Metal Implant - titanium and its alloys

The main issue with initial fixation of fractured bones by metallic wire was tissue reaction to the implant (Laing, 1978). Blame was put on the implant for problem with infection. Corrosion fatigue test in Hank's solution has shown that Ti6Al4V has the longest fatigue life over 316L stainless steel and Co-Cr-Mo (Iman et al., 1979). Implants with both adequate strength and corrosion resistance, in short with biocompatibility as per the current understanding, is recommended.

Various metallic biomaterials have been used as passive substitutes for hard tissue replacement in applications include total hip and knee joints replacement, bone plates and screws as fracture healing aids, spinal fixation devices and dental implants. The main advantages of these materials are their excellent mechanical properties and corrosion resistance. Alloys of some metals have been utilised for devices such as vascular stents, catheter guide wires, orthodontic archwires, and cochlea implants, where specific properties are required (Park & Kim, 2000). Titanium and its alloys, have been extensively used as load-bearing biomaterials due to their low modulus, superior biocompatibility and enhanced corrosion resistance compared to conventional stainless steels and cobalt-based alloys (Jones, 2001; Kohn, 1998; Long & Rack, 1998). The mechanical properties of some typical metallic biomaterials verses bones are shown in Table 3, where they are compared with bone.

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Table 3 Mechanical properties of typical metallic biomaterials (Long & Rack, 1998; Park & Lakes, 1992; Fung, 1993).

Material	Modulus (GPa)	Tensile strength (MPa)
Stainless steel	190	586
Co-Cr alloy	210	1086
Ti-alloy	116	965
Bone (cortical)	7-40	52-152
Bone (cancellous)	0.4	7.4

Titanium alloys

Titanium and associated alloys stand out for their combined mechanical strength and superior biocompatibility. Their favourable performance as an implants are believed to be a consequence of the combination of various surface properties:

- 1) The presence of a thin (1.5-10nm), passive titanium dioxide film that protects the underlying material, giving the material excellent corrosion resistance in natural environment (indicated electrochemically by a low level of electronic conductivity) (Aziz-Kerrzo et al., 2001 ; Blackwood & Chooi, 2002; Ellingsen, 1999; Ku et al., 2002; Park & Kim, 2000; Sul et al., 2001).
- 2) A hydrolysed oxide gel (containing hydroxyl group) has moderate negative charge under physiological pH conditions, which favours the nucleation of calcium phosphate (Barrke, 2002; Kokubo et al., 2003).
- 3) The oxides have low solubility and hence very low concentrations of ionic corrosion species ($\leq 10^{-6}$ M) are present at neutral PH.
- 4) TiO_2 is a semiconductor (n-type), having a dielectric constant ($\epsilon=40-110$) similar to that of water ($\epsilon=80$). Titanium and titanium alloy surfaces therefore do not lead to strong or excessive interaction with, and hence denaturalisation

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of, protein in the extracellular matrix. On the contrary, its' 'water-like' nature allow it to interact gently with the hydrophilic outer shell of the protein molecules (Wieland et al., 1999).

Ti6Al4V is currently the most common titanium alloy used for implants. It can be heat-treated to various strength levels, is readily weldable and is relatively easy to machine (Critchlow & Brewis, 1995). Most of the orthopaedic titanium alloys have acceptable strength levels and ductility. However, titanium and its alloys do not bond to bone in the early stage of implantation (<6 months). Metal ions released by metallic implants have brought about concerns of cytotoxicity, and their possible contributory role to the loosening of hip and knee prostheses (Ku et al., 2002; Thompson & Puleo, 1996). Insufficient load transfer from artificial implants to adjacent remodelling bone, due to the substantial difference in elastic modulus between titanium alloy (110GPa) and bone (7-40GPa) (Long & Rack, 1998; Park & Lakes, 1992), could also result in bone resorption (Wolff's Law). This could also lead to loosening or fracture and eventual prosthesis failure.

NiTi

Nitinol, or NiTi, is a new promising implant material that possesses unique superelasticity (pseudoelasticity), thermal shape memory, deformation compatibility, good damping and biocompatible properties (Kapanen et al., 2001; Park & Kim, 2000). The mechanical properties of Nitinol depend on its phase state and final shape-setting temperature (Hodgson et al., 1999). The shape memory effect and superelasticity properties of the equiatomic alloys (55NiTi), occur as a result of an austenite to martensite transition, induced by applied stress or change of temperature (Thompson, 2000). Diagrammatic representations of these properties are shown in Figure 2. Table 4 summarises some of the mechanical properties of martensitic and austenitic 55NiTi.

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Conventional metallic implants such as AISI 316L stainless steel and titanium are very stiff compared to biological materials (see Table 3). The 55NiTi exhibits extraordinary compliance with its superelasticity feature. It allows a deformation up to 8% strain to be fully recoverable as compared to, for example, 1% for stainless steel (Thompson, 2000). These alloys also have a Young's modulus and stress-strain hysteresis very similar to those of natural bone (7-40MPa) (Park & Kim, 2000). The superelasticity of Nitinol compared to stainless steel, and its mechanical similarity to biological materials are illustrated in Figure 3.

Figure 2 Diagrammatic representation of the shape memory and superelasticity effect of NiTi alloy (Thompson, 2000).

Table 4 Typical properties of 55-Nitinol (Thompson, 2000; Duerig et al., 1999; Ramakrishna et al., 2001).

Property	55-Nitinol austenite	55-Nitinol martensite	316 Stainless Steel	Ti6Al4V
Young's modulus (GPa)	83-120	28-50	190	116
Tensile Stress (MPa)	690-1380	-	586	965
Super-elastic recoverable strain	≤ 8%	-	NA	NA

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Such unique properties of Nitinol improved the physiological similarity between bone and implants, and make possible processes like spinal viscoelasticity, growth and bone remodelling. Osseointegration and proper healing by sharing the load with surrounding tissue is also likely to be promoted (Assad et al., 2003a; Duerig et al., 1999). The possibility of more efficient tissue/NiTi implants interaction makes this material of significant medical and scientific interest. Orthopaedic devices including hip implants, bone spacers, stents, skull plates (Duerig et al., 1999), and endodontic instruments such as a root canal (Thompson, 2000) have been manufactured with this material in recent years, taking advantage of its properties.

Figure 3 The stress–strain curve of NiTi is superimposed on a) stainless steel and b) those of biological materials (Duerig et al., 1999).

Porous titanium alloys, particularly porous NiTi, possess several mechanical and physiological advantages over their solid counterparts (Blackwood & Chooi, 2002; Duerig et al., 1999; Li et al., 2002b; Li et al., 2002a). Porous biomaterials with certain pore size (100 to 500 μ m) allow human tissue ingrowths and body fluid transmission. Porous materials with advantages mechanical properties have been applied successfully

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as biomedical implants (e.g. orthopaedics, traumatology, vertebrarium, maxillofacial surgery etc.) (Assad et al., 2003b; Li et al., 2002b).

Li et al. (Li et al., 2002b) have reported that the compressive strength of porous NiTi shape memory alloy (SMA) met the strength requirement for human cancellous bone, with corresponding porosity. Figure 4 shows some examples of NiTi porous structure produced at different preheating temperatures. Traditional powder sintering and novel combustion synthesis methods using blended or pure metal powders can be employed to prepare parts with porous structure.

Figure 4 a) Porous Ni–Ti synthesized at different preheating temperatures, b) SEM picture of the porous structure (Li et al., 2000).

Seah et al. (Seah et al., 1998; Seah et al., 1995), Blackwood et al. (Blackwood et al., 2000; Blackwood & Chooi, 2002) and Li et al. (Li et al., 2002a) reported that corrosion of the porous material in SBF is significantly higher than solid implants under simulated physiological conditions. Initial failure of the oxide film on porous titanium was found to occur within the pores. The need to remove the initial air-formed oxide layer via an acid etch prior to additional surface treatment, and the associated

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difficulty in removing residual traces from pore structures, may lead to increased corrosion rates.

NiTi is advantageous for specific medical applications. However, the high nickel content still raises concerns for its long-term usage. Potential nickel metal ion release due to localised corrosion could lead to toxicity or hypersensitivity (Hallab et al., 2000). Reported values for initial ions release from NiTi is about three times higher than those for Ti6Al4V and austenitic stainless steels during an *in-vitro* corrosion test in human body simulated fluid (Rondelli, 1996). However, despite the higher initial Ni dissolution rate, *in-vitro* study in human cell culture (Ryhanen et al., 1997), and *in-vivo* tests using rats (Ryhanen et al., 1998) carried out on NiTi have shown that no toxic effect was induced.

2.1.3 Polymer Implants

A large number of polymers are used for a wide area of applications due to their availability in a variety of compositions and forms with wide ranging properties. They can be readily fabricated into complex shapes and structures, but tend to be flexible and generally too weak to meet the mechanical demands of implants in orthopaedic surgery. Table 5 shows the mechanical properties of some typical polymeric biomaterials. These properties vary significantly depending on, for example, the molecular weight, and crystallinity of the polymer.

Polymeric biomaterials have advantages of manufacturability to various shapes, ease of secondary processes with comparatively low cost versus properties ratio. They possess a wide range of properties, and are most similar to soft tissues or non-mineralised tissues. A good example is the use of polypropylene (PP) as artificial vascular grafts due to its high flex-life, excellent environment stress-cracking resistance and its gas and water vapour permeability. Some of the applications of polymeric

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biomaterials are 1) load-bearing ultra-high molecular weight polyethylene (UHMWPE) as acetabular cup of total hip and the tibia plateau and patellar surface of knee joints; 2) polytetrafluoroethylene (PTFE) as catheter and artificial vascular grafts; and 3) polymethylmethacrylate (PMMA) as bone cement and implantable ocular lens etc. (Lee et al., 2000a). Nevertheless, a possible problem with polymeric biomaterials is that they may absorb liquids, swell and leach undesirable products (e.g. monomers, fillers, plasticisers etc.) (Ramakrishna et al., 2001).

Table 5 Mechanical properties of typical polymeric biomaterials (Ramakrishna et al., 2001).

Material	Modulus (GPa)	Tensile strength (MPa)
Polyethylene (PE)	0.88	35
Polyurethane (PU)	0.02	35
Polytetrafluoroethylene (PTFE)	0.5	27.5
Polyacetal (PA)	2.1	67
Polymethylmethacrylate (PMMA)	2.55	59
Polyethylene terephthalate (PET)	2.85	61
Polyethyl etherketone (PEEK)	8.3	139
Silicon rubber (SR)	0.008	7.6
Polysulfone (PS)	2.65	75

2.1.4 Composites implants

Composite biomaterials, in particularly polymer composites, offer the ability to overcome some of the shortcomings of homogenous materials, enabling considerable control over materials properties. Stiff, strong and lightweight materials as well as highly resilient and compliant materials can be produced. The mechanical properties of

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Table 6 Mechanical properties of typical composites biomaterials (Lakes, 2000).

Material	Modulus (GPa)	Tensile strength (MPa)
Graphite-epoxy (unidirectional fibrous, high modulus)	215	1240 (tension)
Graphite-epoxy (quasi-isotropic fibrous)	46	579 (tension)
Dental composite resins (particulate)	10-16	170-260 (compression)

Examples of applications are dental filling composites, reinforced methyl methacrylate bone cement and ultra-high molecular weight polyethylene, and orthopaedic implants with porous surfaces. Typically, the reinforcing materials are carbon fibres, polymer fibres, bioceramics and glasses. Each constituents of the composite must be biocompatible and their interfaces should not degrade when exposed to human physiology (Lakes, 2000). Preparation of HA-based ceramic composites utilizing the favourable bioactive properties of HA is also an interesting approach. However, it has been shown that with the advantage of increased toughness and strength, the introduction of ‘foreign’ materials into the HA matrix could affect its biocompatibility and promote decomposition of HA. It is also known that processes to achieve composites with homogeneously distributed reinforced particles or fibres with controlled orientation are very difficult to develop.

2.2 Ti Alloys Prostheses

In the orthopaedic and dental fields, an implants’ surface composition and topography significantly affect its osseointegration (direct contact without intervening soft tissue between implant and remodelled bone). The nature of an implant’s topology determines its initial interaction with proteins, glycoproteins and saccharides in the body fluid (Park & Kim, 2000). Surface characterisation of biomaterials is essential in

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understand the biocompatibility of implants and the design of biomaterials (Wieland et al., 1999; Fini et al., 1999).

The presence of a thick and porous titanium oxide is known to be more effective in encouraging healing and bone growth around implants, when compared to conventionally turned surfaces (Liang et al., 2003). This phenomenon is not fully understood and the ingrowth of tissue into pores is still debated (Goransson. et al., 2003). Nevertheless, factors mentioned indicate an increased topography (by incorporating a porous structure, particle blasting, oxide layer with increased roughness etc.) and reduction of metal ion release (by increasing oxide thickness and uniformity and/or coating with bioactive ceramics) could improve titanium alloys' biocompatibility (Critchlow & Brewis, 1995; Hench & Wilson, 1993a; Trepanier et al., 1998; Velten et al., 2002).

Several techniques to modify the implant surface mechanically (Cooper, 2000), chemically (Gu et al., 2003) or electrochemically (Shabalovskaya et al., 2003) have been studied. Surface modification presents the possibility to achieve both biocompatibility and biofunctionality within a single implant. Through the combination of the two concepts, i.e. bulk material properties (tensile strength, stiffness etc) with desired surface properties (biocompatibility, bioactive, controlled surface roughness etc), it was hoped that better control over the biological response of the body towards implants would be obtained (Wieland et al., 1999).

2.2.1 Surface of Ti6Al4V

A thin TiO₂ layer forms spontaneously on metallic titanium in the presence of oxygen or water at room temperature. Layer thickness and properties depend on metal composition, the surrounding medium, surface finish and the maximum temperature reached during manufacturing.

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Electron diffraction studies of anodic oxide film on titanium and Ti6Al4V revealed a “quasi-amorphous” layer. This film is composed of microcrystallites, of 15-25 atoms, having anatase symmetries that shaped like platelets or polyhedral aggregates, which convert to crystalline anatase at higher voltages (Blondeau et al., 1977a). These findings are supported by a Raman spectroscopy characterisation of anodic titanium oxide (Velten et al., 2002). The percentage of amorphous titanium oxide has been reported to reduce with increasing anodising voltage. Electrolyte's concentration apparently enhanced the crystallisation of amorphous titanium oxide, as anatase TiO_2 with small amount of rutile was detected on Ti6Al4V anodised at $E_f > 8\text{V}$ in 40% H_2SO_4 (Shirkhanzaddeh & Sims, 1997).

Figure 5 A schematic representation of a proposed three-layer model with surface oxide, a) TiO_2 , b) an intermediate sub-oxide, c) the TiO and d) the bulk metal Ti . Adapted from (Pouilleau et al., 1997; Blackwood et al., 1989).

A three-layer model, shown in Figure 5, with an intermediate sub-oxide TiO_{2-x} layer between a TiO and outermost TiO_2 layer, has been proposed by Pouilleau et al. and Blackwood et al. (Blackwood et al., 1989; Pouilleau et al., 1997) for the titanium oxide layer.

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The primary interaction between implant and the biological environment is at the implant-tissue interface i.e. titanium oxide-tissue interface. This interaction is dependent on the surface morphology of the titanium oxide layer and not on the bulk material itself. Combinations of the beneficial properties of the oxide layer could be the reason for the excellent biocompatibility of titanium alloy, leading to favourable tissue responses to these implants relative to other metals (Shirkhanzaddeh & Sims, 1997; Sul et al., 2001; Wieland et al., 1999). Titanium dioxide powder was demonstrated to be a much better nucleation promoter for calcium phosphate nucleation, compared to titanium powder, during a nucleation in a test system saturated with respect to both calcium and phosphate (Ellingsen, 1999). However, an in-vivo study revealed that there were no significant differences in tissue reaction to the implant (bone contact area and thickness) between commercially pure titanium, anodic oxidised titanium and Ti6Al4V implants retrieved from rat's tibia 168 days after implantation (Takeshita et al., 1997)

2.2.2 Surface of NiTi

A passivation layer covers the surface of NiTi implants, due to titanium's high affinity for oxygen. This surface oxide consist mainly TiO₂, with smaller amounts of NiO and Ni₂O₃ (Armitage & Grant, 2003; Firstov et al., 2002; Shabalovskaya et al., 2003), or in some cases even metallic Ni (Wever et al., 1998). Depending on its composition, structure, thickness and stability, this layer may act as a barrier to nickel diffusion (Firstov et al., 2002; O'Brien et al., 2002; Shabalovskaya et al., 2003; Thierry et al., 2002; Wever et al., 1998). Successful passivation requires complete coverage of the metallic implant surface before implantation and stability of the achieved passivation layer has to be considered during implant design. Destruction of this passivation layer during significant deformation or complex interaction involving wear, due to the difference in mechanical properties of TiO₂ and NiTi, could be the cause of

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reported negative tissue response to these alloys (Filip et al., 2001). Other possible factors include poor surface finish with manufacturing defects (Es-Souni et al., 2002) or non-uniform TiO₂ (Kuphasuk et al., 2001; Trepanier et al., 1998).

Calcium phosphate film is known to form on the surface of titanium alloys in biological or simulated biological environment (Kokubo et al., 1999; Liu et al., 2001; Li & Ducheyne, 1998; Stoch et al., 2000; Tanahashi et al., 1996). This film could possibly act as a physical barrier against titanium ion diffusion through titanium oxide layer into the electrolyte (Sousa & Barbosa, 1996; Wever et al., 1998).

2.2.3 Corrosion of Ti and Ti alloys

Although titanium alloy implants have stable oxide films, they still undergo electrochemical exchange and release metallic ions into the physiological environment, which is believed to be the cause of implant failure (Kohn, 1998).

EDS analysis of the fibrous tissue enveloping Ti6Al4V implants retrieved after 1-6 years implantation revealed titanium and aluminium ions in the proximity of metal parts (Zaffe et al., 2004). Titanium is known to cause a particulate tissue inflammatory response, and histological evidence of symptomatic titanium particulate tissue inflammation following joint replacement performed on two patients has been published (Khoo et al., 2004). Study on tissues adjacent to titanium implants, retrieved from rats tibia after 48 weeks of implantation, revealed the presence of titanium and its alloying elements (Okazaki et al.). Ions associated with Ti6Al4V were found to inhibit normal differentiation of bone marrow stroma cells to mature osteoblasts *in-vitro*, suggesting that ions released from implants *in-vivo* may cause implant failure (Thompson & Puleo, 1996). A gene expression analysis of osteoblasts cultured on Ti6Al4V revealed that once accumulated metal ions released exceed a threshold concentration, cell growth is diminished (Ku et al., 2002). Velten et al. assessed the corrosion properties of

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differently treated Ti6Al4V under physiological conditions (Velten et al., 2002). He reported that anodised samples have thicker TiO₂ than polished samples and exhibit lower current density compared to thermally oxidised and polished samples.

Potential nickel ion release from high nickel content NiTi alloy within the body could lead to metal hypersensitivity. Such reactions occurred when the body treats the organometallic complexes formed by the released metal ions and native proteins, as antigens or allergens (Hallab et al., 2000; Takeshita et al., 1997). Many contradicting reports on the role of nickel and its alloy's biocompatibility and toxicity have been produced, but with no conclusive statements (Filip et al., 2001; Kapanen et al., 2002; Takeshita et al., 1997). Aziz-Kerrzo et al. (Aziz-Kerrzo et al., 2001) studied and compared the corrosion susceptibility of Ti, Ti6Al4V and Ti45Ni in buffered saline solution using anodic polarisation and electrochemical impedance measurements. They reported that Ti45Ni exhibit pitting at relatively low potentials (200mV). Once initiated, these pits continue to propagate even when the applied potential was reversed. The propagation rate of the initiated pits remains the same regardless of type of surface modification. These results are conflicting with those of Wever et al. (Wever et al., 1998), who reported that the near equiatomic nickel-titanium demonstrated a passive behaviour when polarised in Hanks solution at 37°C. The latter study suggested that NiTi has good corrosion properties and promising biological response.

Assessment of the cytotoxicity effect of NiTi has been carried by *in-vitro* cell culture experiments on functionally graded Ni-NiTi-Ti materials by Bogdanski et al. (Bogdanski et al., 2002). No cytotoxicity effects on osteoblast and fibroblast cells are observed for Nitinol with up to 50 atm.% Ni content. They concluded that equiatomic NiTi has good biocompatibility under the conditions studied. Ryhanen et al. also reported good biocompatibility of NiTi during *in-vitro* study in human cell culture

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(Ryhanen et al., 1997), and *in-vivo* study using rats (Ryhanen et al., 1998), despite an initial high rate of Ni ions release. Differences in sample preparation, preexistence of surface defects or rupture of surface titanium oxide during experiment (Es-Souni et al., 2002; Filip et al., 2001; Kuphasuk et al., 2001; Trepanier et al., 1998).

2.2.4 Modifications of the oxide layer on titanium alloys

To improve titanium alloy implants' biocompatibility, many different approaches have been employed to reduce ion release and to enhance topography. Primary interaction of an implant with the physiological environment occurs at the oxide-tissue interface. By altering the composition, structure, thickness and stability of this surface oxide, metal ion leaching to surrounding electrolyte can be reduced/prevented (Solar et al., 1979; Thierry et al., 2002; Wever et al., 1998). Considerable effort has been directed at thickening and modification of this oxide layer (Critchlow & Brewis, 1995; Cui et al., 2003; Ellingsen, 1999; Firstov et al., 2002; Garcia-Alonso et al., 2003; Kilpadi et al., 1998; Leng et al., 2002; Lopez et al., 2003; Man et al., 2001; O'Brien et al., 2002; Schreckenbach & Marx, 1999).

Cooper reviewed the effect of topology on commercially pure titanium implants (Cooper, 2000). He concluded that an increase in the implant's surface topography improves bone-to-implant contact, enhancing the mechanical properties of the interface.

Khor et al. chemically modified Ti6Al4V surface by high velocity oxygen fuel (HVOF) spraying of fully crystalline bioactive hydroxyapatite (HA), up to the thickness of about one hundred microns. Reduction in process-induced decomposition of HA enhances the integrity (*in-vitro*) of the coating when immersed in SBF solution (Khor et al., 2003a).

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Surface treatments, such as laser melting, promote oxidation of NiTi, and improve its corrosion resistance. However, the process is very expensive and inappropriate for implants with complex geometries (e.g. stents, porous parts) (Trepanier et al., 1998).

Modification of NiTi surface oxide can be achieved by inexpensive conventional methods such as mechanical polishing, electropolishing, heat treatment, chemical treatment, and nitric acid passivation (Firstov et al., 2002; Trepanier et al., 1998; Zhao et al., 2001). The primary objective is to replace defective native oxide layer with one having greater uniformity.

Anodisation is one of the techniques employed (Schreckenbach & Marx, 1999; Liu et al., 2003a) to modify and enhance the *in-vitro* corrosion resistance of titanium and its alloys. *In-vivo* studies also revealed that bone to implant contact was greatly enhanced on anodised titanium (Sul, 2003; Sul et al., 2002b). Several studies have been reported on the galvanostatic anodisation of titanium. However, the number of research reports for potentiostatic anodisation of titanium is limited (Badekas & Panagopoulos, 1987; Blondeau et al., 1977a; Pouilleau et al., 1997; Sul et al., 2001). Anodisation of Nitinol for medical or other usage is uncommon. Few papers available on anodisation of NiTi are by Asami et al., who assessed passivity of crystalline and amorphous NiTi in sulphuric acid (Asami et al., 1990; Asami et al., 1991; Asami et al., 1993). They revealed that oxide layer thickness increased linearly, up to few nanometers with potential. Anodised NiTi exhibit passive current comparable to those of titanium until a potential where primary passivity of nickel breaks down and successful evolution of oxygen.

Sul et al. studied the effects of different electrolytes, current densities and anodising voltages on the anodic oxide formed (Sul et al., 2001). He concluded that

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regardless of the electrolyte, behaviour of the oxide grown is dependent on the electrochemical parameters in the following order:

current density > electrolyte concentration > electrolyte temperature? agitation speed > chemical composition of the titanium electrode in terms of the anodic forming voltage.

He showed that anodic oxidation of titanium in acid and alkaline electrolytes resulted in thickening of the oxide, interference colours giving an indication of oxide layer thickness.

Yang et al. employed spark-discharge anodic oxidation in H₂SO₄ to produce porous titania on biomedical titanium, which could induce apatite formation when immersed in SBF for three to six days (Yang et al., 2004).

Electrochemical anodisation is therefore a quick, simple, versatile technique that allows controlled thickness, structure and composition of titanium oxide by adjusting the electrochemical settings.

Anodic titanium oxide

The electrochemical titanium oxide growth resulted from a redox reaction at the oxide film/electrolyte interface:

Subscripts indicate: s- solid, aq- aqueous, g- gaseous

Titanium at the metal/oxide interface is oxidised to produce titanium cations, Ti⁴⁺ that in turn reacts with water that diffuses through the existing defect layer of titanium oxide. These reactions are presented schematically in Figure 5.

Pouilleau et al. (Pouilleau et al., 1997) report that only peaks for the metallic substrate were detected during TF-XRD analysis of commercially pure-titanium

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anodised with $E_f=20V$ in $0.5M H_2SO_4$. Even though the presence of TiO_2 was confirmed by XPS, XRD peaks characteristic of anatase and rutile were only detected after heat treatment at $600^\circ C$. They therefore concluded that the outer layer of oxide is composed of amorphous TiO_2 . Velten et al. (Velten et al., 2002) studied the structure of anodic TiO_2 formed on $Ti6Al4V$ anodised at E_f up to 100 volts in $0.5M H_2SO_4$. They report the absence of anatase or rutile XRD peaks, but confirmed the presence of TiO_2 using Raman spectroscopy. They also concluded that the anodic TiO_2 formed is amorphous, with a small amount transformed to crystalline anatase structure at higher voltages (approximately 30% at 90V).

Figure 6 Schematic structure of the electrical double layer at the titanium oxide/electrolyte interface. Adapted from (Sul et al., 2001)

Calciumphosphate (CaP) coating

Designing of biomaterials to elicit a specific biological response in, for example encouraging bone bonding and growth, has gained importance in view of its applications in promoting rapid wound healing (Jones, 2001). Calcium phosphates (particularly hydroxyapatite-HA) are bioactive ceramic materials that promote spontaneous bone

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growth and bone integration, henceforth good bone fixation (Hench & Wilson, 1993a; Billotte, 2000). Numerous studies have been done on this material showing excellent *in-vivo* results (Aoki, 1994; Ellingsen, 1999; Hench & Wilson, 1993a; Manjubala et al., 2002; Tanahashi et al., 1996; Yuan et al., 2001).

The limitation in load bearing applications for these ceramics was overcome by de Groot who chemically modified a metallic prosthesis by coating it with calcium phosphate using a plasma-spraying technique. Thick layer of calcium phosphate (tens to hundreds of microns) can be coated onto a metallic prosthesis. Osteoconductive calcium phosphate coatings for total joint arthroplasty have been in clinical use since the mid 1980s. Of all the calcium phosphate coatings, HA coatings have found the most widespread application in hip arthroplasty. Implants with plasma-sprayed coating have clinical records of accomplishment demonstrating its osseointegrative capabilities (Ding et al., 2003; Fini et al., 1999; Porter et al., 2003; Taddei et al.). Dong et al. (Dong et al., 2003) reported that *in-vitro* test on plasma sprayed Ti6Al4V/HA composites on sand-blasted Ti6Al4V substrate found interfaces inside the coating maintained good microstructural integrity. Their clinical advantages over more conventional implant surfaces are evident in primary and revised hip arthroplasties. In a group of young patients with revision arthroplasty using hydroxyapatite-coated femoral components, an 11-year survival rate of 93% was obtained. Histologic analysis of specimens retrieved at autopsy confirmed the excellent bone fixation of components (Geesink, 2002).

By contrast, resorption of HA coating on dental implants and other prostheses was reported to compromise long-term implant survival (Porter et al., 2004). These reports were also challenged by results of a meta-analytic review, which concluded that the survival rates reported for HA-coated implants were similar to those reported for uncoated titanium implants (Lee et al., 2000b). In addition, the high temperatures

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involved and line-of-sight nature of the process have limited its applications in coating heat sensitive materials or implants with complex geometry/pores (Rey et al., 1991; Kokubo, 1996). High Velocity Oxygen-fuel (HVOF) spray (Khor et al., 2003a; Khor et al., 2003b); electrochemical deposition (Kuo & Yen, 2002) and sol-gel coating (Liu et al., 2002a; Cavalli et al., 2001) are examples of alternative techniques studied to counter this limitation. Coatings produced using these techniques demonstrate interesting *in-vivo* and *in-vitro* results, however, insufficient adhesion has also been reported (Kumar & Wang, 1999). Most of the processes mentioned require post-treatment of coating in the form of heat treatment, which are normally high energy consuming (Kuo & Yen, 2002; Cavalli et al., 2001; Liu et al., 2002a; Huang et al., 2000).

2.3 Biomimetic Strategy

Demand for new bio-interactive materials with enhanced or novel physical (mechanical), chemical and biological functions has lead to innovative strategies to produce biomaterials that interact with targets within the body or mimic tissue architecture (Kim, 2003). Designing interactive biomaterials or ‘biomimetic’ materials is inspired by studying and copying the way nature works. Biomimetic strategies for design of functional, interactive biomaterials include biodegradable and ‘intelligent’ materials for targeted drug delivery applications and tissue (both soft and hard) engineering. These strategies also cover modification of biomaterials surfaces for implants or wound-healing applications (Dillow & Lowman, 2002).

Pertaining to hard tissue healing and/or replacement, bioactive ceramics (BAC) like (Na₂-CaO-SiO₂-P₂O₅) Bioglass®, sintered HA, sintered β-tricalcium phosphate and glass ceramic are the only material sources that provide biological tissue-integration in tissue defect in-situ (Aoki, 1994; Billotte, 2000; Hench & Wilson, 1984;

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Hench & Wilson, 1993a; Park & Lakes, 1992). Their brittle nature, however, renders them of limited use for load-bearing applications. Together with the increasing knowledge in bone biology (Puleo & Nanci, 1999; Turek, 1984) and bone mineralisation (Betts et al., 1981; Cuisinier, 1996; Thull, 2002; Legrand et al., 2002; Magne et al., 2001; Pak & Skinner, 1968), there raise a trend to produce implant closer to biological bones to stimulate bone-implant contact (Andrade et al., 2002; Burg et al., 2000; Kokubo et al., 1999; Kuo & Yen, 2002; Shin et al., 2003). One innovative, alternative strategy to produce biomaterials with enhanced novel physical, chemical and biological functions is protein-free biomimetic modelling. This approach learns from and imitates the biomineralisation of calcium phosphate nano-crystals on BAC during bone formation in human and animals (Kokubo et al., 1990).

2.3.1 In-vivo biomineralisation

A synthetic material is normally encapsulated with non-adherent fibrous connective tissue when implanted in a living host (D'Hermies et al., 1998; James et al., 1999; Porter et al., 2004; Vercaigne et al., 1998). Tissue integration onto BAC is due to biomineralisation of bone mineral phase at the BAC/tissue interface. The layer between the BAC and integrated bone tissue is an interfacial calcium phosphate phase, characterised by nano-crystallites of apatite slightly differing in composition and structure from that of synthetic HA (Gross et al., 1993; Hench & Anderson, 1993; Kokubo et al., 1999). This apatite has a defective structure with low crystallinity, and is calcium-deficient containing sodium, magnesium, chloride and carbonate. Its structure and composition bear close resemblance to the mineral phase in bone tissue (Aoki, 1994; Glimcher et al., 1981; Turek, 1984), which is not observed to form on non BACs, implying surface biomineralisation of bone mineral-like calcium phosphate is a prerequisite for bioactivity (Kim, 2003).

2.3.2 Biomimetic calciumphosphate coating

Blood plasma is the first phase BAC is exposed to when implanted in a living host. Plasma is believed to interact with, and drive BAC towards biomineralisation of calcium phosphate. The blood plasma contains various proteins that significantly affect the biomineralisation. However, the system is too complex to mimic *ex-vivo* for designing new bioactive materials. Blood plasma is also a solution supersaturated with respect to calcium and phosphate, which is finely controlled in a metastable stable condition to deposit the calcium phosphate solely by surface-induced heterogeneous nucleation and growth (Kim, 2003). Biomimetic calcium phosphate coatings can be formed on substrates by immersion in simulated physiological fluids e.g. simulated body fluid (SBF), at physiological pH and temperature (Furuzono et al., 2000; Kokubo, 1996; Li & Ducheyne, 1998; Takadama et al., 2001; Tanahashi et al., 1996). Protein-free simulated body fluid (SBF) was first used by Kokubo et al (Kokubo et al., 1990). It is prepared based upon the chemical analysis of human body fluid. SBF has ion concentrations and pH similar to human blood plasma (Table 7). These simulated body fluids are buffered to pH=7.4, physiological pH, at 37°C using tris-hydroxymethyl aminomethane ($(\text{CH}_2\text{OH})_3\text{CNH}_2$) and hydrochloric acid. Under such physiological temperature and pH conditions, the solution is supersaturated towards apatite. Immersion of a substrate provides sites for the nucleation of apatite onto the surface, which grows over time to homogeneously cover the substrate.

2.3.3 Biomineralisation studies

The exact mechanism of calcium and phosphate ion nucleation, accumulation and formation of an apatite layer is not known (Cuisinier, 1996). Possible nucleation precursors attributed to Posner's cluster (amorphous calcium phosphate-ACP, Ca_9 -

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(PO)₄) (Betts & Posner, 1974), have been detected by dynamic light scattering spectrometry *in-vitro* in simulated body fluids (Kanzaki et al., 2001). Clustering of ACP is considered as important in biomineralisation because clustering is involved in the formation of hydroxyapatite (Treboux et al., 2000a). Size and composition of the final crystalline phases evolved depends on pH, temperature and presence of crystal growth inhibitors (Barrere et al., 2002b; Barrere et al., 1999).

Table 7 Ion Concentrations (mM) of SBF and human blood plasma (Kim et al., 2001).

Ion	Concentrations (mM)	
	Stimulated Body Fluid	Blood Plasma
Na ⁺	142.0	142.0
K ⁺	5.0	5.0
Mg ²⁺	1.5	1.5
Ca ²⁺	2.5	2.5
Cl ⁻	147.8	103.0
HCO ₃ ⁻	4.2	27.0
HPO ₄ ²⁻	1.0	1.0
SO ₄ ²⁻	0.5	0.5

High-resolution electron microscopy (HREM) study of the bone crystal growth mechanism generated a four-stage development model shown in Figure 7. The first stage of crystal formation is held to be ionic adsorption onto a substrate (Figure 7a), leading to nucleation and formation of nanometre-sized particles (Figure 7b). Individual ionic groups that are destined to form the nucleation core diffuse randomly throughout the solution, forming stable clusters. Studies have shown that these clusters could be closely related to HA but are definitely not stoichiometric. The mechanism by which nanoparticles accumulate in localised/precise areas is not known. Stable nuclei then grow further by ionic deposition (Figure 7c) with the direction of growth being governed by the substrate and the presence of neighbouring particles. Space restriction

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eventually induces these particles or globules to fuse together (Figure 7d) (Cuisinier, 1996).

Figure 7 Bone crystal growth mechanisms, a) ionic adsorption, b) nucleation, c) crystal growth, d) crystal fusion (Cuisinier, 1996).

These two models/findings both speculate on apatite clusters as nucleation precursors. By adjusting the experimental parameters, the composition and size of the final crystallised phase evolved can be altered to catered to specific applications.

2.3.4 Formation of biomimetic calciumphosphate onforeign substrates

Nucleation induced by a foreign substrate is known as heterogeneous nucleation. The degree of supersaturation required to induce heterogeneous nucleation of calcium phosphate is higher than that for secondary nucleation (induced by seed of similar nature) (Barrere, 2002).

The essential criterion for successful biomimetic coating is the substrate's ability to initiate calcium phosphate nucleation on its surface (Kokubo et al., 1999). It

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have been shown that presence of surface functional groups, formed by hydrated silica or titania abundant in Si-OH or Ti-OH groups, play an important role in such nucleation on surgical titanium implants (Kokubo et al., 1999; Takadama et al., 2001; Liu et al., 2002b). TEM-EDX and XPS studies on these functional groups show that they induce calcium phosphate nucleation by forming amorphous calcium compounds e.g. calcium titanate and amorphous calcium phosphate, which eventually stabilises into a crystalline apatite (Takadama et al., 2001). FTIR and TFXRD analyses also revealed that composition and structure of the apatite formed are similar to those in bone with type-B carbonated apatite. The apatite formed has calcium-to-phosphorous (Ca:P) ratio lower than that of stoichiometric hydroxyapatite (1.67). From the morphological study, the apatite formed was found to exist as smaller crystallites in the nanometre size range. It was speculated that bone-producing cells like osteoblast could preferentially proliferate and differentiate on this bone-like apatite layer. In fact, it has been shown that alkaline phosphatase activity is considerably increased on the bone-like apatite layer that forms on both Cerabone A-W (glass-ceramic containing crystalline apatite and Wollastonite in a matrix of MgO-CaO-SiO₂), and NaOH and heat-treated bioactive titanium alloys in SBF (Kokubo et al., 1999). However, in the next pioneering study by the same authors, apatite formation was enhanced on sodium-free titania with specific structure of combine anatase and rutile (Uchida et al., 2002). This result suggests it may be beneficial to employ an additional sodium removal process, or to form titania via a different route.

Biomimetically formed calcium phosphates varied with the substrate's surface (morphology and chemistry), and SBF solutions composition (ion concentrations and pH). Some examples are octacalcium phosphate, carbonated apatite or calcium deficient apatite (Barrere et al., 2002a; Takadama et al., 2001; Tanahashi et al., 1996).

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These actually have structures and compositions close to that of natural bones, which are mainly carbonated hydroxyapatite $[\text{Ca}_5(\text{PO}_4, \text{CO}_3)_3(\text{OH})]$ (Takadama et al., 2001; Yan et al., 1997). There is also speculations that these are OCP $[\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}]$, since the Ca:P ratio in bones are rarely exactly 1.67 (calcium-deficient), as in the case of HA (Cuisinier, 1996; Turek, 1984). Biomimetically formed calcium phosphate-coated commercially pure titanium plates (Tanahashi et al., 1996) and NiTi rods (Chen et al., 2004) were tested *in-vivo* using rabbits. Bone was found to deposit directly onto the apatite coating of the retrieved implants without any intervening soft tissue.

Besides calcium and phosphate ions, SBF solutions contain magnesium and carbonate ions. These ions, in particularly carbonate ions, have been reported to enter the apatitic lattice (Barrere et al., 1999; de Sena et al., 2002; Li & Ducheyne, 1998; Li et al., 1997; Stigter et al., 2002; Stoch et al., 2000). Studies on the influence of magnesium ions on calcium phosphate nucleation show Mg^{2+} favours heterogeneous nucleation of tiny globules on the substrate. Magnesium ions are also thought to complex phosphate ions on the surface of the calcium phosphate nuclei. A consequence is defects generated in the crystal lattice stabilised the amorphous calcium phosphate phase. The carbonate groups enter the apatite lattice by substituting phosphate or hydroxyl groups. The formation of lacuna in the lattice further decreased crystallinity of apatite (Barrere et al., 2002b).

The formation of biomimetic calcium phosphate coatings normally required an average immersion period of seven to forty eight days (Gil et al., 2002; Oh et al., ; Stigter et al., 2002). The kinetic of calcium phosphate nucleation can be enhanced by increasing SBF solution's concentration (Barrere et al., 2002b; Dorozhkina et al., 2003), controlling pH of SBF solutions (Chou et al., 2004), elevating immersion temperature (Lin et al., 2002) or modifying substrate's surface (Kokubo, 1996; Liu et

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al., 2002b; Oliveira et al., 2003b; Oyane et al., 2003; Yang et al., 2004). A two-step biomimetic process involving immersion in two separate SBF solutions, of different concentrations, has also been studied and found to produce coatings *in-situ* with controlled grain size (Tanahashi et al., 1996; Turek, 1984). The calcium phosphate film formed was reported to have very similar structure to bone's apatite, and it remained on the substrate and continuously bonded to a rabbit's living tissue after eight weeks of implantation. Apatite layers have also been obtained on various substrates by soaking the materials in SBF solution (Kokubo et al., 1999; Takadama et al., 2001; Yan et al., 1997).

Takadama et al. (Takadama et al., 2001) enhanced calcium phosphate nucleation by modifying the surface of commercially pure-titanium using alkali (NaOH) and heat-treatment (600°C, 1hr). He reasoned that passive TiO₂ dissolves in alkaline solution to form negatively charged hydrated titania species according to equation 3:

These species in turn react with positively charged alkali ions to produce an alkali titanate hydrogel layer on the surface (Kokubo, 1996). The mechanism of apatite formation on the modified titanium (alloy) metal in SBF solution proceeds by ion exchange at the oxide-aqueous interface. Na⁺ ions are released from the surface into solution via exchange with H₃O⁺ ions. This causes an appreciable pH increase and raises the degree of supersaturation of the surrounding fluid with respect to apatite, thereby accelerating apatite nucleation. Water reacts with Ti-O-Ti bonds (hydrated) forming numerous Ti-OH groups on the surface (hydroxylated layer). This hydroxylated layer can show positive or negative charge, and acidic or basic properties depending on the environmental pH. At physiological pH (7.4), this layer is slightly

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negatively charged and gel-like (Kokubo et al., 1999; Park & Kim, 2000). The slight negative charge promotes incorporation of calcium ion to form calcium titanate. This in turn incorporates phosphate ions in the solution to form numerous amorphous calcium phosphate nuclei. A schematic representation of this process is shown in Figure 8. These nuclei grow spontaneously by consuming Ca^{2+} and PO_4^{3-} ions from the surrounding fluid.

Since the biomimetic calcium phosphate layer is formed in physiological conditions similar to that in a living body, it should have similar chemical and mechanical properties to natural bone (Yan et al., 1997). The Ti-OH species are believed to form nucleation sites for calcium phosphate. By increasing the concentration of Ti-OH, more calcium phosphate nuclei and therefore nano-sized calcium phosphate crystallites could be potentially formed on the implants' surface. The apatite layer formed is tightly bonded to the substrate by chemical bonding through the hydrated titania and titanium oxide layer (Kokubo, 1996).

Studies have shown that TiO_2 interacts with calcium and/or phosphate ions (Park & Kim, 2000; Yan et al., 1997). However, unexpectedly, considering the negative charge on the hydroxylated layer, some studies report that under physiological conditions phosphate groups are present first on TiO_2 surface, followed by calcium ions, (Barrkre, 2002; Frauchiger et al., 1999).

The good adhesion of the biomimetic calcium phosphate layer to the metallic substrate was demonstrated by a study of Yan et al. Specimens, freshly retrieved from test animals, fractured in the bone instead of at the bone-implant or coating-implant interfaces during detachment test (Yan et al., 1997).

Figure 8 Chemical change of titanium implant surface (Park & Kim, 2000).

2.4 *In-vitro* Assessment

The biocompatibility of an implant could be assessed by both *in-vitro* and *in-vivo* tests. It has been reported that the same materials behave differently during *in-vitro* and *in-vivo* testing (Ratner & Shi, 1999). However, *in-vitro* testing still provides a first indication of an implant's biocompatibility and bioactiveness (Kokubo et al., 1999).

2.4.1 *Material's bioactivity test*

A layer of apatite was found to form at bioactive artificial implant/bone interfaces during *in-vivo* study (Kokubo et al., 1999; Kokubo et al., 2003). Studies on the bonding to bone showed that the apatite layer formed on the implant's surface during the early stage of implantation. Characterisation of this *in-vivo* formed apatite revealed nano-crystals of carbonated, defective structure with low crystallinity. These characteristics of apatite are very similar to those of the mineral phase in bone.

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Henceforth, it is believed that bone-producing cells (osteoblast) could preferentially proliferate and differentiate on this layer. This results in direct contact of surrounding bone with the apatite layer (Chen et al., 2004; Kokubo et al., 2003). It has been shown, and confirmed, that the same materials also form an apatite layer on their surfaces when immersed in an SBF with ion concentrations and pH nearly equal to blood plasma (Kokubo et al., 1999).

Increased alkaline phosphatase activity and direct bone bonding (Tanahashi et al., 1996) to the bone-like apatite layer formed in SBF solution have been observed. The formation of apatite layer on the surface of a substrate immersed in SBF solutions is therefore assumed to be an essential requirement for it to exhibit bioactivity or bone-bonding (Kokubo et al., 2003; Liu et al., 2002b; Tirrell et al., 2002; Takadama et al., 2001). *In-vitro* testing by immersion in SBFx1 solution under physiological conditions (pH7.4, 36.9°C) has been used in numerous studies (Chan et al., 2003; Cleries et al., 2000; de Sena et al., 2002; Ding et al., 2003; Gil et al., 2002; Gu et al., 2003; Khor et al., 2003b; Kim et al., 2001; Kokubo et al., 1999; Oliveira et al., 2003a).

2.4.2 Corrosion Resistance Studies

Many corrosion resistance alloys exhibiting excellent passivity, when in contact with different environments, are imperfectly resistant to solutions containing dissolved chloride, which is abundantly found in human body fluid. The *in-vitro* anticorrosion ability of titanium alloy, with or without modified surfaces, can be evaluated by electrochemical corrosion tests in simulated physiological fluids. Hoar and Mears demonstrated that the corrosion results for titanium in Human blood, Hank's solution and chloride solutions are similar (Hoar & Mears, 1966). This led to a conclusion that inorganic solutions are satisfactory substitutes for blood, and other extracellular fluid, for *in-vitro* corrosion resistant assessment of titanium.

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Potentiodynamic polarisation (Hall & Hackerman, 1953; Hoar & Mears, 1966; Kao et al., 2002; Solar et al., 1979; Valereto et al., 2002) and electrochemical impedance spectroscopy (EIS) (Ibris & Rosca, 2002; Hukovic et al., 2003) are two common and useful testing methodologies employed.

Electrochemical impedance spectroscopy (EIS)

The electrochemical impedance of a system or cell can be measured by analysing the response of working electrodes (current through the cell) to an AC potential with small-amplitude alternating potential signals over a widely varying frequency (Jones, 1992). It is a non-destructive test capable of detecting defects or diffusion paths in the coating or surface oxide on test specimen (Ibris & Rosca, 2002).

Impedance measured by the EIS method contains real and imaginary parts. By plotting the negative-imaginary part (y-axis) against the real part (x-axis), a Nyquist plot, shown in Figure 9 can be obtained. In this plot, the y-axis is negative (-imag); with each point on the plot being the impedance at one frequency. Data for lower frequencies are on the right and higher frequencies on the left of the plot.

Equivalent circuit models are frequently used to interpret EIS data (Gamry, 2003; Ibris & Rosca, 2002; Jones, 1992; Trethewey & Chamberlain, 1995). The simplest cell model used is the Randles cell containing elements corresponding to solution resistance (R_s), a capacitor (C_{dl}) and a polarisation resistance (R_p), which is the resistance of the conducting path (e.g. surface oxide) (Figure 10). The semicircle in the Nyquist plot in Figure 9 is typical of a Randles cell; the intercept at high frequency gives the value of R_s , while the diameter of the semicircle gives R_p .

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Figure 9 A Nyquist plot for a typical Randles cell (Gamy, 2003).

Figure 10 Randles cell schematic diagram (Gamy, 2003).

Capacitors in EIS experiments normally behave like a constant phase element (CPE) instead of an ideal capacitor. Several theories have been proposed to account for the non-ideal behaviour of the CPE element, but none have been universally accepted. Ibris and Rosca (Ibris & Rosca, 2002) used this technique to study the influence of alloying elements (in titanium and its alloys) on oxide film resistance and growth rate in Hank's solution. They concluded that alloying elements probably enhance the diffusion of the titanium ions, leading to a thicker oxide film.

Potentiodynamic polarisation

When the potential of an electrode is forced away from its value at open circuit, the electrode becomes polarised, and current may flow by electrochemical reactions at the electrode surface. This current can be monitored as a function of time or varying potential, allowing different oxidation conditions at the electrode surface to be examined (Marino et al., 2001).

Potentiodynamic polarisation is a technique used to measure the polarisation behaviour of a metal in a solution. This technique is a powerful tool for the determination of the test metal behaviour in a wide range of oxidising conditions in a designated environment. In a polarisation curve, applied electrochemical potential is plotted against the measured electrode current density. Polarisation behaviour is determined by continuously scanning the potential, while monitoring the current response.

A cyclic pitting scan can be used to assess the passivation behaviour of a titanium dioxide film for example. Figure 11 is an example of a typical potentiodynamic polarisation curve for AISI 304 stainless steel. The starting point is normally the open circuit potential, from which potential is scanned in the cathodic (negative potential) direction, before proceeding in the anodic direction (positive potential) to a predetermined potential. After that, a reverse scan will bring the system to the free corrosion potential (E_{corr}), where the test terminates. The pitting susceptibility of various materials can be compared by their polarisation behaviour. Pitting corrosion occurs when a protective film breaks down, as indicated by a large increase in current density, and the corresponding potential is known as the pitting corrosion potential (E_{pitt}). Above this potential, the current density (i) increases rapidly even with small changes in potential as more pits propagate. Materials with high E_{pitt}

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would generally be less susceptible to pitting. The important parameters to be considered in this test are the sweep rate, selection of the starting potential and selection of the stopping potential (Trethewey & Chamberlain, 1995).

Figure 11 A cyclic polarisation curve for type 304 stainless steel in aerated seawater at 25deg C, scan rate:0.1mV/sec. The broken line shows a similar plot using aerated seawater + 4.5M sodium chloride and HCl, adjusted to pH1.2 (Trethewey & Chamberlain, 1995).

Hall and Hackerman were the first to observe pitting corrosion of commercially pure-titanium (at 10V) when anodically polarised in 0.5M NaCl solution (Hall & Hackerman, 1953). Solar et al. made a comprehensive study of corrosion of surgical implant materials in Ringer's solution. Varying the solution's pO₂, pH, amino acid additions, the test samples' surface finish and treatment (steam sterilisation), they report that titanium and its alloys possessed very high E_{pitt} (Solar et al., 1979).

Blackwood et al. (Blackwood et al., 2000; Blackwood & Chooi, 2002) and Seah et al. (Seah et al., 1995; Seah et al., 1998) employed the polarisation technique to assess the corrosion behaviour and the stability of protective oxide films on solid/porous titanium and titanium composites, in simulated physiological media/conditions. Solid

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and sintered porous titanium parts demonstrated distinct passive ranges, with porous structures being more susceptible to pitting due to their larger surface area.

Polarisation curves, performed in Hank's solution under physiological conditions, revealed a passive behaviour for NiTi alloy. It was also demonstrated that NiTi exhibits better corrosion resistance than AISI 316L stainless steel, a well-established implant material (Wever et al., 1998).

2.5 Summary

Biomaterials for hard tissue replacement have to demonstrate both 'biofunctionality' and 'biocompatibility'. 'Biofunctionality' concerns the ability of an implant to match the mechanical behaviour to that of the host tissue with minimum mismatch at the implant/tissue interface. 'Biocompatibility' refers to the ability of a material to induce an appropriate host response, while serving its specific function.

Titanium and its alloys have been extensively used as load-bearing biomaterials due to their low modulus, superior biocompatibility and enhanced corrosion resistance compared to conventional stainless steels and cobalt-based alloys (Long & Rack, 1998; Ramakrishna et al., 2001). Ti6Al4V is most commonly used for metallic implants fabrication. However, substantial difference in elastic modulus between titanium alloy (110GPa) and bone (7-40GPa) could result in bone resorption (Wolff's effect). This eventually leads to the loosening or fracture and ultimately prosthesis failure (Long & Rack, 1998; Park & Kim, 2000).

An equiatomic NiTi (55wt.% or 50 atm. % Ni) possesses unique superelasticity (pseudoelasticity), thermal shape memory, deformation compatibility and good damping properties. It has a Young's modulus (Hodgson et al., 1999) and stress-strain hysteresis very similar to that of natural bone (Duerig et al., 1999). The improved physiological similarity between bone and implants is likely to promote

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osseointegration and proper healing by sharing the load with the surrounding tissue (Kapanen et al., 2001; Park & Kim, 2000).

Contradicting reports on NiTi's cytotoxicity effect (due to potential nickel release) and biocompatibility (Filip et al., 2001; Kapanen et al., 2002; Wever et al., 1998) hindered greater expansion of its application in medicine. The potential nickel metal ion release due to localised corrosion could lead to toxicity or hypersensitivity (Hallab et al., 2000). However there is much interest in this material due to the possible yield in a significantly more efficient tissue/NiTi implant interaction (Assad et al., 2003b; Bogdanski et al., 2002; Castleman et al., 1976; El Medawar et al., 2002; Kapanen et al., 2002; Kujala et al., 2002).

A passivating titanium dioxide (TiO₂) layer covers the surface of both Ti6Al4V and NiTi due to titanium's strong affinity for oxygen. This layer may act as a barrier to metal ion e.g. Ni, diffusion. Anodisation is one of the techniques employed to modify and enhanced the corrosion resistance of this oxide layer. By adjusting the anodisation parameters, thickness, structure and composition of the anodic titanium oxide formed can be controlled to cater for particular applications (Sul et al., 2001). However, unlike Ti6Al4V, anodisation of NiTi is not common.

At the implant-tissue interface, it is the protective oxide and not the bulk material itself, which is in contact with the physiological environment. The topology, chemical composition, stoichiometry, crystal structure and defect density of the titanium dioxide may therefore determine the diffusion rate of calcium/phosphate ions into the oxide layer. This in turn influences the nucleation of apatite (bone-bonding). Calcium phosphate film is known to form on the surface of titanium alloys in biological or simulated biological environment (Kokubo et al., 1999; Stoch et al., 2000; Tanahashi et al., 1996). This film possibly to act as a further physical barrier against titanium ion

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diffusion through titanium oxide layer into the electrolyte (Sousa & Barbosa, 1996; Wever et al., 1998).

Titanium prostheses have been chemically modified by applying bioactive calcium phosphate coating, using a plasma spray process to promote osseointegration. Nevertheless, the high operating temperatures involved in this technique make it impossible to coat heat sensitive substrates. Decomposition of metastable calcium phosphate phases, and incorporation of drugs and proteins in the calcium phosphate coating for enhancing prostheses survival are also not possible using this approach (Kokubo, 1996; Rey et al., 1991). The line-of-sight nature and the complexity of the calcium phosphate adhesion mechanism during plasma spray process makes it not suitable for coating implants with complex geometry/porous structure or where thin coatings (few microns) are to be applied (Barrere, 2002; Liu et al., 2001; Stigter et al., 2002).

The biomimetic process is an alternative technique developed to cater for the above-mentioned applications. This process imitates, or ‘mimics’, the natural biomineralisation during bone formation in humans and animals. Biomimetic calcium phosphate coatings are deposited on the substrates by immersion in SBF (Kim et al., 2001), at physiological pH and temperature (Furuzono et al., 2000; Li & Ducheyne, 1998; Takadama et al., 2001; Tanahashi et al., 1996).

The essential criterion for a successful biomimetic coating process is the substrate’s capability to initiate calcium phosphate nucleation on its surface (Kokubo et al., 1999). Biomimetic calcium phosphate formed varied with the substrate’s surface topography, surface chemistry and with SBF solutions ion concentrations. These calcium phosphates phases possess structures and compositions close to those of natural bones, which are mainly carbonated HA (Kokubo et al., 2003; Takadama et al.,

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2001; Yan et al., 1997). There is also speculation that these are OCP, since the Ca:P ratios in bones are rarely exactly 1.67, as in the case of hydroxyapatite (Turek, 1984). Bone was found to deposit directly onto commercially pure-titanium plate biomimetically coated with calcium phosphate without any intervening soft tissue (Tanahashi et al., 1996).

The formation of biomimetic calcium phosphate coatings takes up to forty-eight days. Studies have been done to reduce the immersion time: by increasing the concentration of the SBF solutions (Barrere et al., 2002a; Turek, 1984); introducing a two-step process by immersion in two separate SBF solutions of different concentrations (Tanahashi et al., 1996); and surface chemical modification by alkali and heat-treatment (Kokubo et al., 1999; Takadama et al., 2001; Yan et al., 1997).

Titanium alloys still undergo electrochemical exchange (corrosion) and release metallic ions to the physiological environment. Traces of metals have been found in the fibrous tissue enveloping a retrieved Ti6Al4V prosthesis, and ions associated with Ti6Al4V were found to inhibit normal differentiation of bone marrow stroma cells to mature osteoblast *in-vitro*. It is therefore important to minimise such metal ion release through surface modification. The *in-vitro* corrosion resistance of titanium alloy, with or without modified surfaces, can be assessed by electrochemical corrosion tests in simulated physiological fluids. Potentiodynamic polarisation curves (Kao et al., 2002; Valereto et al., 2002) and electrochemical impedance spectroscopy (EIS) (Ibris & Rosca, 2002; Hukovic et al., 2003) are two common and useful tests employed.

Anodised Ti6Al4V samples were found to have thicker TiO₂ than polished ones. They also exhibit better passive behaviour in simulated fluid than both polished and thermally oxidised samples (Velten et al., 2002). Some reported that NiTi exhibits lower pitting resistance than Ti6Al4V, and pits initiated continue to propagate even

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when the applied potential was reversed (Aziz-Kerrzo et al., 2001). Others have revealed that NiTi exhibits a passive behaviour *in-vitro* and better pitting resistance than ANSI 316L (Wever et al., 1998). Contradicting reports of NiTi's corrosion resistance in physiological fluids may be a result of different surface treatments, presence of defects, metallic composition, non-uniform or ruptured surface film due to poor surface finishes (Es-Souni et al., 2002; Kuphasuk et al., 2001; Trepanier et al.,

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Procedures developed to produce duplex coating on Ti6Al4V and NiTi plates can be conveniently divided into two sections. The first portion, covered in sections 3.1 and 3.2, cover the selection and evaluation of process route and parameters for the production of a primary titanium dioxide (TiO₂) barrier coat. Effectiveness of the surface modification process in enhancing surface oxide stability, and the consequential reduction in metal ion release (enhanced corrosion resistance) *in-vitro/vivo*, was assessed by electrochemical methods. Topography and chemical characteristics of the surface oxide were studied microscopically and spectroscopically.

Sections 3.3 and 3.4 describe the second portion of the work, the deposition of a final biocompatible calcium phosphate (CaP) coating on the initial barrier coating. A thin layer of calcium phosphate was grown on top of the initial barrier layer by a biomimetic route at or near to physiological conditions. Material characterisations were carried out to study changes in the coating's surface morphology and chemical composition with immersion duration.

3.1 Production of Initial Barrier Titanium Oxide Coating

To enhance titanium alloys' biocompatibility, dissolution of metal must be eliminated/minimised by enhancing the stability and uniformity of its passivating surface oxide layer (Marino et al., 2001). Anodising is a relatively simple process, which can be employed to increase thickness of the titanium dioxide layer on the titanium alloy's surface. Depending on the electrochemical parameters employed, properties of the oxide layer formed, such as thickness, structure and composition can be varied (Sul et al., 2001). Using polished and freshly etched samples as the anode and a 316L stainless steel mesh as cathode, various voltages were applied using a DC

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power source, while partially immersing the two electrodes in a chosen electrolyte. Parameters such as applied voltage, current density, type of electrolyte, electrolyte concentration, anode to cathode surface ratio and temperature affected the properties of the anodic oxide film formed.

Twenty specimens (60x27x3mm³) were prepared using commercially available Ti6Al4V plates for the anodising process. NiTi plates (A.M.F., orthopaedic grade) were purchased in two different sizes, 60x25x3mm³ and 30x30x2mm³, with the latter employed for corrosion test experiments. These specimens were polished progressively using 360, 600, 1000 to 1200-grit Sic emery papers to remove macro-level surface defects and contamination. Polished samples were ultrasonically cleaned with ethanol and rinsed with de-ionised water before leaving to dry overnight in air at room temperature (25°C).

3.1.1 Anodisation of Ti6Al4V

NaOH and H₂SO₄ solutions are commonly used as electrolyte to anodise titanium and its alloys. They are commercially available and are generally, when used in moderate and low concentrations (below one molar), easy and cheap to dispose of. Polished and cleaned specimens were etched in hydrofluoric acid solution (5ml HF in 100ml de-ionised water + 5ml HNO₃ in 100ml de-ionised water) and rinsed in de-ionised water to expose fresh titanium base metal just before the anodisation process. An electrochemical cell, containing either 0.5M NaOH or 0.5M H₂SO₄ as electrolyte, was prepared. Etched specimens were partially immersed in the electrolyte, (a total of five specimens each in NaOH_{aq} and H₂SO_{4aq}), and oxidised individually at 20V, 30V, 50V, 60/70V and 80V for approximately one minute. Figure 12 gives a schematic illustration of the cell set-up in an anodising bath.

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Figure 12. An anodising cell set-up.

Anodised samples were rinsed with de-ionised water and samples were left to dry overnight in air at room temperature. The anodisation procedure was repeated on a separate set of plates to access the repeatability of the process. Anodised samples were labelled according to the electrolyte type and anodising voltage, as summarised in Table 8. Care was taken not to introduce organic contamination on anodised samples (gloves and tweezers were used all the time). Samples for electrochemical tests (30x30x2mm³) and for deposition of calcium phosphate (10x10x3mm³) were prepared using using a diamond cutter. Samples of etched (un-anodised) Ti6Al4V were prepared for reference purposes. All experiments were carried out at room temperature.

Table 8. Matrix of Ti6Al4V anodised at 20V, 30V, 50V 60/70V and 80V in two separate electrolytes, 0.5M sodium hydroxide and 0.5M sulphuric acid, for 60 seconds at room temperature.

Electrolyte/ Voltage (V)	Initial Set					Repeatability Check				
	20	30	50	60/70	80	20	30	50	60/70	80
0.5M NaOH	*B20	B30	B50	B60	B80	2B20	2B30	2B50	NA	NA
0.5M H ₂ SO ₄	#A20	A30	A50	A70	A80	2A20	2A30	2A50	NA	2A80

*B20= alkaline-anodised, 20V, #A20=acid-anodised, 20V.

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Samples of as received, etched and nitric acid passivated (20 minutes at 80°C in 30ml 37%HCl + 100ml H₂O) NiTi were prepared for reference purposes. All experiments were carried out at room temperature.

3.2 Micro Topography (WYKO/SEM)

Topography measurement was performed using an interference microscope (WYKO NT2000 optical profiler) equipped with several exchangeable magnification objectives. Vertical resolution ranged from 0.1nm to several millimetres, with a roughness measurement resolution better than one angstrom. A 10x objective lens was used in this study. It produced a 0.603x0.459mm² field of view, with x-sampling of 0.820µm and y-sampling of 0.957µm. Measurements were taken only when greater than or equal to 98% of the collected data were valid. The roughness average, R_a, the root mean square (rms) average, R_q and the surface area (S.A.) of the measured samples were calculated using the instrument's software.

3.3 Corrosion Resistance Assessment of the Titanium Oxide Barrier Coating

Corrosion behaviour and resistance of anodic titanium oxide coatings were studied using electrochemical methods. A CPT Flexcell™ was used with plates of Ti6Al4V (26x26x3mm³) and NiTi (30x30x2mm³) as the working electrode. A saturated calomel electrode (SCE) was used as reference electrode, and a graphite rod as counter electrode. The backside of the plates was polished with 350-grit Si Cemery paper to expose fresh material and ensure proper electrical contact. Electrolytic SBFx1 solutions were prepared according to Kokubo's protocol (Kokubo et al., 1990) by varying the amount of dissolved NaCl, NaHCO₃, KCl, K₂HPO₄·3H₂O, MgCl₂·6H₂O, CaCl₂ and

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Na₂SO₄. The supersaturated solution was buffered to pH7.4 at 36.5°C using reagent grade 1M HCl and tris-hydroxymethyl aminomethane (TRIS, (CH₂OH)₃CNH₂) (Merck) (see Table 10 for ion concentrations). Solutions prepared were clear and free from precipitate. All salts used in preparation of the SBF solutions were reagent grade chemical (Merck). Prior to each preparation, the pH meter electrodes (Metrohm) were calibrated with pH4.00 and pH7.00 standard buffer solutions (AJAX Chemicals).

Electrochemical measurements were performed using a Solartron SI 1287 potentiostat, and Electrochemical Impedance Spectra were recorded with a Solartron SI 1260 Impedance/Gain-phase Analyser. Measurement and analysis of impedance and potentiodynamic data was carried out using CorrWare™ and ZPlot™ softwares. Samples were allowed to stabilise for 30 minutes, during which time the open circuit potential was monitored, before commencing the test. All experiments were carried out at room temperature (25°C). One set of measurements was made for Ti6Al4V but measurements were repeated three times for NiTi, which is the main material of interest.

3.3.1 CPT Flexcell™

The CPT Flexcell™ (Gamry Instruments, USA) (Gamry, 2003) used for corrosion resistance test is an electrochemical test cell designed specially to counter crevice corrosion around the specimen seal, a problem commonly faced by flat substrates, and especially samples of passive materials under test.

Samples are mounted facing upwards, allowing for close but unobtrusive placement of a counter electrode and a reference electrode. Unlike traditional cells, localised corrosion at the seal between the sample and its holder is avoided by having a flooded gasket seal design, shown in Figure 13.

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As in the case of conventional cells, the test area of the sample is in contact with the electrolyte. However, a plastic barrier or O-ring does not seal the active area of the test cell. Instead, a fibre washer is placed on test area of the metal sample into which de-ionised water is allowed to slowly diffuse. The fluid in the fibre washer is nearly pure de-ionised water, and is thus not sufficiently conductive to initiate/sustain corrosion processes. The consequence is a conductivity gradient between the electrolyte and the de-ionised water defining the test specimen's active area instead of initiating crevice corrossions (Gamry, 2003).

The Flexcell™ can be heated externally with a wrap around heater or internally with insertion heaters. The only materials in contact with the test solution are glass, filter paper, and PTFE Teflon™. This cell is usable with a wide variety of test solutions, including non-aqueous media.

Figure 13 Cross section of the flooded gasket seal (Gamry, 2003).

3.3.2 Electrochemical impedance spectroscopy (EIS)

For impedance tests, the amplitude of the sinusoidal signal was set to 30mV and the frequency was ranged from 10^{-2} to 10^4 Hz.. Polarisation resistance (R_p) was determined by fitting the obtained impedance data with a Randles cell model, adapted

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from Garcia-Alonso et al. (Garcia-Alonso et al., 2003), with R_s being the electrolyte resistance, CPE corresponding to the double layer capacitor (TiO_2) and R_p to the charge transfer resistance of the passive layer.

3.3.3 Potentiodynamic polarisation

The potential of the test plate was swept from -200mV (versus open circuit potential) to 10V at a scan rate of 0.5mV/sec. The sweep was terminated once a current greater than 5×10^{-5} Amp was detected. The pitting corrosion potential, E_{pitt} was determined from the potential dynamic curves as the point where a slight increase in potential lead to a rapid increase in current density or when the current density exceeded 10^{-6} Amps/cm², a sign of loss in passivity for titanium alloys (Hoar & Mears, 1966).

3.4 Composition Studies of the Titanium Oxide Barrier Coating

Chemical composition of the barrier coating, mainly titanium dioxide, was studied using electron spectroscopy for chemical analysis (ESCA), also term X-ray Photoelectron Spectroscopy (XPS). This is an expensive ultra high vacuum based technique, and is increasingly used to provide information on the elemental composition, chemical state and functional groups present on surfaces (Skoog et al., 1997).

Surfaces of the prepared specimens (10x5x3mm³) were cleaned with ethanol just before mounting onto the sample holder. However, some atmospheric organic material is expected. XPS analysis was done in the ultra-high vacuum chamber of a Kratos Ultra X-ray photoelectron spectroscopy system (Kratos company, UK) equipped with a dual anode (Al/Mg $K\alpha_{1,2}$), a monochromatised Al $K\alpha_{1,2}$ X-ray source, and a hemispherical electron energy analyser. The base pressure of the chamber was 2×10^{-9}

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Torr. Samples were exposed to X-ray radiation from a monochromated AlK_α radiation source with photon energy of 1486.7 eV and operating at 150 W (15 kV, 10 mA). A survey scan was followed by narrow scans for Ti_{2p} , O_{1s} , C_{1s} , Al_{2p} , V_{2p} (Ti6Al4V) and Ni_{2p} (NiTi). Photoelectrons induced by X-ray were filtered by the hemispherical analyser, and finally recorded by the eight-channeltron multi-detector (for XPS spectrum). A mixed Gaussian-Lorentz (30%) function was employed for peak fitting routine to identify elemental species in various oxidation states. The relative atomic concentrations (atm. %) of Ti_{2p} , Al_{2p} , V_{2p} , O_{1s} and C_{1s} were calculated by region. The measured binding energy shifts for each sample were referenced to the energy position of C_{1s} (285 eV), when necessary.

A one-time etch (120 seconds) was performed on the samples to remove the top carbonaceous layer (contaminants) and to assess the chemical composition immediately underneath the surface TiO_2 layer. The etching and analysis process were performed in the ultra-high vacuum chamber of the XPS. During etching, the argon pressure in the chamber was approximately 1×10^{-7} Torr. The argon-etching rate was calibrated, using Ta_2O_5 on Si substrate, and estimated to be approximately 4 nm/min. Scans were again performed to collect the post-etching XPS spectra. The core level binding energy peaks were compared against reference standards (Moulder et al., 1995), and with values reported in the literature (Pouilleau et al., 1997; Takadama et al., 2001; de Sena et al., 2002; Schreckenbach & Marx, 1999; Sul et al., 2002a; Barrère et al., 2003).

3.5 Production of the Final Biocompatible CaP Coating

Plates (10x10x3 mm³) were prepared from anodised Ti6Al4V (50V in 0.5M H_2SO_4) and NiTi (1.5V in 0.1M HNO_3) with highest measured corrosion resistance (E_{pitt}). Samples of alkaline-anodised Ti6Al4V (50V in 0.5M NaOH) and control samples were prepared for reference purposes. Plates were ultrasonically cleaned in

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ethanol (70%) for ten minutes before being rinsed with de-ionised water. 12 Ti6Al4V (4 acid-anodised at 50V, 4 alkaline-anodised at 50V and 4 unanodised) and 24 NiTi plates (6 anodised at 1.5V, 6 passivated, 6 as received and 6 etched) were prepared.

SBFx3 solution was prepared according to Kokubo's protocol (Kokubo et al., 1990) by tripling the amount of dissolved NaCl, NaHCO₃, KCl, K₂HPO₄·3H₂O, MgCl₂·6H₂O, CaCl₂ and Na₂SO₄ (Table 10). The supersaturated solution was buffered to pH7.0, and the final solution was clear and free from any precipitate.

NiTi and Ti6Al4V plates were suspended, using nylon strings, and immersed in SBFx3 solution contained in polyethylene bottle (Figure 14). The incubation temperatures were 38.5±0.1°C and 39.5±0.1°C respectively. Each plate was exposed to approximately 30ml of SBFx3 solution. One plate from each Ti6Al4V sample group was retrieved at 24, 48, 72 and 96 hours for analysis. Three plates from each NiTi sample group were retrieved at 24 and 96 hours. The SBFx3 solution was refreshed every 48 hours to minimise partial carbonate loss and hence pH fluctuation (Dorozhkina et al., 2003). Polypropylene film was used to prevent/minimise concentration variations due to evaporation.

All samples retrieved from the various SBF solutions were visually inspected for a 'rainbow appearance', an indication of the presence of a thin calcium phosphate coating (Barrere et al., 2002a; Liu et al., 2003b; Stigter et al., 2002), prior to further analysis.

Table 10. The ionic concentrations (mM) of SBFx1 and SBFx3 solutions.

Solution	Na ⁺	K ⁺	Mg ²⁺	Ca ²⁺	Cl ⁻	HCO ₃ ²⁻	HPO ₄ ²⁻	SO ₄ ²⁻
SBFx1	142	5.0	1.5	2.5	147.8	4.2	1.0	0.5
SBFx3	426	15.0	4.5	7.5	443.4	12.6	3.0	1.5

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Figure 14. Titanium alloy plates suspended in SBFx3 solution using nylon strings.

Plates retrieved from the SBFx3 solution were carefully rinsed with de-ionised water, and left to dry in air at room temperature (25°C) for 24 hours. Samples were labelled as shown in Table 11.

Table 11. The matrix of Ti6Al4V and NiTi plates named according to its process history.

<i>Ti6Al4V</i>: Electrolyte (Forming voltage, V)/ Immersion time (hr) in SBFx3	0 hr	24 hr	48 hr	72 hr	96hr
0.5M H₂SO₄ (50V)	A50	A5024	A5048	A5072	A5096
0.5M NaOH (50V)	B50	B5024	B5048	B5072	B5096
Unanodised	UA	UA24	UA48	UA72	UA96

A5096 : Acid-anodised, 96 hours immersion, B: Alkaline-anodised, UA: Unanodised

<i>NiTi</i>: Forming voltage, V, or process/Immersion time (hr) in SBFx3	0hr	24hr	96hr
1.5V in 0.1M HNO₃	1.5V	N1.5V24	N1.5V96
Passivated	P	NP24	NP96
As-etched	E	NE24	NE96
As-received	AR	NAR24	NAR96

3.6 Compositional and Structural Studies of the CaP Coatings

3.6.1 Phase identification

Final coatings were analysed using Fourier-transform infrared spectroscopy (FTS 3000) equipped with a reflectance stage. An attenuated total internal reflection (ATR) attachment, with Ge crystal, was used to enhance the signal. Spectra were collected using specular reflection over the range from 700-4000 cm^{-1} at a nominal resolution of 4.00. The number of scans was equal to 32. Average readings collected from three different locations for each sample were used to plot the FTIR spectra. Calibration was performed in air prior to each run.

Further analysis of calcium phosphate coatings produced after 96 hours immersion was undertaken using ESCA (XPS). Settings used were as described in section 3.4. A survey scan was followed by narrow scans for Ti_{2p} , Ni_{2p} , C_{1s} , O_{1s} , Ca_{2p} , P_{2p} and Mg_{2p} , and the Ca:P ratio of individual samples was determined.

The core level binding energy peaks were compared against reference standards (Moulder et al., 1995). Values reported in several relevant publications (de Sena et al., 2002; Schreckenbach & Marx, 1999; Takadama et al., 2001; Chung et al., 2003; Furuzono et al., 2000) were also used to confirm the chemical state of the elements detected at the coating's surface.

3.7 Structural Study of Ti Oxide and CaP Coatings

The crystalline structures of titanium oxide and calcium phosphate layers deposited were investigated using thin film-X-ray diffraction (TF-XRD) equipped with a Rigaku system. X-rays were produced by a monochromatic source (CuK_{α} ,

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$\lambda=1.5405\text{\AA}$, 40KV, 30mA). Entire XRD patterns were recorded with scan range of $2\theta=15.00$ to 50.00° , at scan speed of $2\theta=1.00^\circ/\text{min}$, scan step of 0.05° and 0.5° glancing angle.

3.8 Morphological Study of the CaP Coating

In order to observe both nucleation and the final calcium phosphate coating, samples prior immersion (0 hour), were compared with those after 24 hours and 96 hours immersion. Samples were gold sputtered and observed microscopically with a Field Emission Scanning Electron Microscope (FESEM, JEOL, JSM-6340F, 20keV) and Scanning Electron Microscope (SEM, JEOL, JSM-5410, 15-20keV), both equipped with Energy Dispersive for X-ray Analysis (EDX, Philips).

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4.1 Production of TiO_2 Barrier CoatingTi6Al4V

The change in interference colour exhibited by plates of Ti6Al4V anodised at various forming voltages (E_f), in 0.5M H_2SO_4 and 0.5M NaOH, are shown in Table 12. Samples from the repetitive run are laid side by side with those of the first run.

NiTi

Results from quick preliminary experiments conducted to determine suitable anodising voltage range and electrolyte for NiTi are shown in Figure 15.

Figure 15 Nyquist (left) and potentiodynamic polarisation (right) plots for NiTi in 0.5M NaOH, 0.1M H_2SO_4 and 0.1M HNO_3 .

Table 12 Pictures of anodised samples using 20V, 30V, 50V, 60/70V and 80V in two separate electrolytes, 0.5M sodium hydroxide and 0.5M sulphuric acids, for 60 seconds at room temperature.

ELECTROLYT E/ VOLTAGE (V)		Batch 1/Batch2				
		20	30	50	60/70	80
Sodium Hydroxide, (NaOH) _{aq} , 0.5M (colour/estimated oxide thickness)		B20/2B20 Blue (50nm)	B30/2B30 Light blue (80nm)	B50/2B50 Yellow (120nm)	B60 Orange	B80 Magenta
	Sulphuric acid, (H ₂ SO ₄) _{aq} , 0.5M	A20/2A20 Indigo/Navy blue	A30/2A30 Light blue	2A50 Yellow	2A70 Magenta (170nm)	A80/2A80 Cyan/Greenish blue

A=acid electrolyte, B=alkaline electrolyte, 2A20= (batch 2, 20V, acidic electrolyte)

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4.2 Topography study of Anodised Ti Alloys

Ti6Al4V

Changes in average surface roughness (R_a), root mean square roughness (R_q) and surface area (S.A.) of Ti6Al4V samples with different surface treatments are shown in Figure 16.

Figure 16 Changes of a) average surface roughness (nm), R_a , b) root mean square surface roughness (nm), R_q and c) surface area (μm^2), S.A., of Ti6Al4V with anodising voltages and electrolyte types.

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A three-dimensional axonometric plot for Ti6Al4V anodised at $E_f=50V$ in 0.5M H₂SO₄ is shown in Figure 17

Figure 17 3-D axonometric plot of Ti6Al4V sheet anodised in 0.5M H₂SO₄ at 50V.

NiTi

R_a , R_q and S.A. values for differently treated NiTi are shown in Figure 18.

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Figure 18 Changes of a) average surface roughness (nm), R_a , b) root mean square surface roughness (nm), R_q and c) surface area (mm²) of NiTi samples: as received (AR), etched (E), passivated (P), anodised at 1.0V (1), 1.5V (1.5) and 3.0V (3).

4.3 Corrosion resistance of TiO₂

Polarisation resistance (R_p) and pitting corrosion potential (E_{pitt}) determined from the measured spectra are summarised in Table 13. Note that the figures reported for NiTi are average values from three repetitive runs.

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4.3.1 Electrochemical impedance spectroscopy (EIS) investigationTi6Al4V

Nyquist plots from EIS measurements for Ti6Al4V are shown in Figure 19.

The changes in R_p of Ti6Al4V with E_f and electrolyte-type employed are shown in Figure 20.

Table 13 Polarisation resistance ($M\Omega cm^2$), R_p , and pitting corrosion potential (V), E_{pitt} , of control and anodised Ti6Al4V and NiTi in SBFx1 solution. NiTi is the mean of 3 repetitive runs. Refer to Table 8 and Table 9 (section 3.1.2) for details of sample abbreviation and treatment.

Sample (Ti6Al4V)	R_p ($M\Omega/cm^2$)	E_{pitt} (V)	Sample (NiTi)	R_p ($M\Omega/cm^2$)		E_{pitt} (V)	
				Mean	SD ($Er \pm$)	Mean	SD ($Er \pm$)
A20V	1.9	11.2	P				
A30V	3.5	12.6		1.0×10^8	1.7×10^8	1.00	0.09
A50V	4.3	13.1	E	0.6	0.4	1.00	0.07
A80V	1.4	12.1	1V	3.2×10^6	2.3×10^6	1.01	0.09
B20V	0.8	7.1	1.5V	1.4×10^{10}	1.6×10^{10}	1.05	0.04
B30V	1.3	12	3V	2.5	1.3	1.08	0.03
B50V	1.9	8.3	AR	0.5	0.7	0.32	0.10
NA	0.4	2.8					

NiTi

The R_p of as received (AR), etched (E), passivated (P) and NiTi anodised at different applied E_f (1.0, 1.5 and 3.0V) are shown in Figure 21.

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Figure 19 Nyquist plots of Ti6Al4V anodised in a) 0.5M H_2SO_4 with 20,30, 50 and 80V, and b) in 0.5M $NaOH$ with 20,30 and 50V, versus control sample in SBFx1 solution.

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Figure 20 Changes in polarisation resistance (R_p) of Ti6Al4V plates with anodising parameters.

Figure 21 Changes in polarisation resistance (R_p) of NiTi plates with different surface treatment (graph before the scale break is in log scale). AR= as received, E=etched, P=passivated, 1V, 1.5V and 3V= NiTi anodised in 0.1M HN03 at E_f =1.0, 1.5 and 3.0V.

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4.3.2 Potentiodynamic polarisation*Ti6Al4V*

Figure 22 shows the potentiodynamic polarisation curves for anodised and unanodised Ti6Al4V.

Changes in corrosion potential, E_{pitt} , with applied voltage, E_f , in 0.5M H_2SO_4 and 0.5M NaOH , are presented graphically in Figure 23.

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Figure 22 Polarisation curves of Ti6Al4V a) reference control sample; b)-d) anodised in 0.5M NaOH; and e)-h) anodised in 0.5M H₂SO₄(refer to Table 8 and Table 12 for details).

Figure 24 shows potentiodynamic polarisation curves for the three-repetitive run for each NiTi sample group.

SEM picture of the large pits observed on tested as received (AR) NiTi samples, which become obvious upon grinding with 320-grit SiCemery paper, is shown in Figure 25

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Changes of NiTi's E_{pitt} , in SBF, with different surface treatment is shown in Figure 26.

Figure 23 Changes in pitting corrosion resistance (V), E_{pitt} of Ti6Al4V plates with E_f (20V, 30V, type (0.5M H_2SO_4 and 0.5MNaOH).

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Figure 24 Polarisation curves (3-repetitive runs denoted by 1, 2 and 3) of NiTi a) as received (AR), b) etched (E), c) passivated (P), d) anodised at $E_f=1.0\text{V}$ (1V), e) at $E_f=1.5\text{V}$ (1.5V) and f) at $E_f=3.0\text{V}$ (3V), in SBFx1 solution.

4.4 Composition and structural studies of TiO₂

Ti6Al4V

Table

ion etching

Figure

The au

ion etching are

Table 14 Percent

Large pits

New pits or process-induced surface defects

Figure 25 Large pit observed on as received NiTi (after grinding with 320-grit emery paper).

Figure 26 Changes in pitting corrosion resistance (E_{pitt}) of NiTi plates with type of surface treatment (graph presenting the mean and standard deviation from 3-repetitive runs). AR-as received, (E)-etched, (P)-passivated, anodised in HNO₃ at E_f =1.0V (1V), 1.5V (1.5V) and 3.0V (3V).

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4.4 Composition and structural studies of TiO₂

Ti6Al4V

Table 14 summarises the relative chemical composition (atm.%) of the acid-anodised, alkaline-anodised and control Ti6Al4V samples determined by XPS prior to ion etching.

Figure 27 shows deconvoluted high-resolution spectra of the O_{1s} and C_{1s} peaks.

The survey scan and high-resolution XPS spectra of Ti acquired before and after ion etching are shown Figure 28.

Table 14 Percentage elemental composition detected by XPS before etching.

Atomic%/sample	Ti _{2p}	O _{1s}	Al _{2p}	V _{2p}	C _{1s}
UA	11.69	38.61	2.32		45.74
A20	8.27	32.26	1.25		58.21
A30	7.47	28.98	1.08	0.23	62.24
A50	3.74	23.16			73.10
A80	10.29	30.20	0.85		58.66
B20	5.66	27.26		0.42	66.67
B30	3.42	19.46	2.33		74.79
B50	7.73	32.12	1.36		58.78

Figure 27 Deconvoluted spectra of a) O 1s region and b) C 1s region of sample A20 before Ar⁺ ion etching.

Ti 2p i) Unanodised

ii) Acid-anodised, 20V

iii) Acid-anodised, 30V

Ti_{2p} iv) Acid-anodised, 50V

v) Acid-anodised, 80V

vi) Alkaline-anodised, 20V

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Ti_{2p} vii) Alkaline-anodised, 30V

viii) Alkaline-anodised, 50V

ix) Survey scan

Figure 28 XPS spectra of Ti narrow scan before (denoted by 'Before') and after (denoted by 'After') 120 seconds Ar ion etching, of i) control sample; ii) – v) acid-anodised Ti6Al4V and vi) – viii) alkaline-anodised Ti6Al4V. The survey scan of all samples are similar to that shown in ix).

NiTi

The chemical compositions (atm.%) of the NiTi samples as determined by XPS prior to ion etching are summarised in Table 15.

XPS survey and narrow (Ti and Ni) scans spectra before and after 120 seconds Ar ion etching of as received (AR), etched (E), passivated (P) and NiTi plates anodised at $E_f = 1V$, $1.5V$ and $3V$ are shown in Figure 29.

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Table 15 Relative quantities of elements detected on NiTi by XPS before etching.

Atomic%/ Sample	Ti _{2p}	Ni _{2p}	O _{1s}	C _{1s}	Ti:Ni
AR	8.3	1.2	29.5	61.0	6.9
E	6.5	1.0	24.0	68.4	6.5
P	4.3	0.7	16.3	78.8	7.2
1.0V	7.5	0.6	29.6	62.3	12.5
1.5V	7.3	0.8	28.1	47.8	9.1
3.0V	8.0	1.0	38.2	60.9	8.0

ai)

ii)

bi)

iii)

iv)

Figure 29 XPS survey and narrow (Ti and Ni) scans spectra before (denoted by 'Before') and after (denoted by 'After') 120 seconds Ar ion etching, of a) as received, b) etched, c) passivated d) anodised (1V), e) anodised (1.5V) and f) anodised (3V) NiTi plates.

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4.5 Materials Characterisation of Final Biocompatible CaP Coating

4.5.1 FTIR

Ti6Al4V

FTIR spectra, showing changes of the Ti6Al4V surface with immersion period in SBFx3 (38.5°C), are shown in Figures 30 to 32.

Figure 30 FTIR of unanodised Ti6Al4V control samples after 24,48,72 and 96 hours immersion in SBFx3 at 38.5°C.

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Figure 31 FTIR of Ti6Al4V samples, anodised at 50V in 0.5M H₂SO₄ after 24, 48, 72 and 96 hours immersion.

Figure 32 FTIR of Ti6Al4V samples, anodised at 50V in 0.5M NaOH, after 24, 48, 72 and 96 hours immersion.

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NiTi

FTIR spectra collected from as received (AR), etched (E), 1.5VNiTi, passivated (P) NiTi after 24 and 96 hours immersion (in SBFx3, at 39.5°C) are shown in Figures 33-36 respectively.

Figure 33 FTIR of AR (as received) NiTi after 24 and 96 hours immersion in SBFx3 at 39.5°C.

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Figure 34 FTIR of E (etched) NiTi after 24 and 96 hours immersion in SBFx3 at 39.5°C.

Figure 35 FTIR of 1.5VNiTi (NiTi anodised at $E_f=1.5\text{V}$ in 0.1M HNO_3) after 24 and 96 hours immersion in SBFx3 at 39.5°C.

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Figure 36 FTIR of P (passivated) NiTi after 24 and 96 hours immersion in SBFx3 at 39.5°C.

4.5.2 XPS Analysis

The survey and high-resolution C_{1s} , Ti_{2p} , Ca_{2p} , P_{2p} and Mg_{2p} XPS spectra of the calciudphosphorous-rich film formed on Ti6Al4V and NiTi, after 96 hours immersion in SBFx3, are shown in Figures 37 and 38 respectively.

Table 16 presents the determined relative atomic percentage (atm. %) of Ca_{2p} and P_{2p} on the Ti6Al4V and NiTi plates after 96 hours immersion.

a) Survey i. Unanodised

ii. Alkaline-anodised

iii. Acid-anodised

b) Ti 2p i. Unanodised

ii. Alkaline-anodised

iii. Acid-anodised

c) **C 1s**

i. Unanodised

ii. Alkaline-anodised

iii. Acid-anodised

d) **Mg 2p**

i. Unanodised

ii. Alkaline-anodised

iii. Acid-anodised

(* - unknown peak)

Figure 37 XPS spectra of i) unanodised control sample, ii) alkaline-anodised and iii) acid-anodised Ti6Al4V before and after 96 hours immersion in SBFx3.

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ci)

ii)

iii)

iv)

v)

vi)

vii)

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Figure 38 XPS spectra of a) as received (AR), b) etched (E), c) anodised (1.5VNiTi) and d) passivated (P) NiTi after 96 hours immersion in SBFx3.

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Table 16 Relative quantities of elements determined by XPS after 96 hours immersion in SBFx3.

Atomic%/Ti6Al4V sample	Ti 2 _{p3/2}	Ca 2 _p	P 2 _p	Ca:P
*UA96	Nil	56.1	43.9	1.28
A5096	Nil	53.33	46.67	1.14
B5096	Nil	54.82	45.18	1.21

*3UA96 = (SBFx3)(Unanodised)(96 hours), A=Acid-anodised, B=Alkaline-anodised.

Atomic%/NiTi sample	Ti 2 _p	Ni 2 _p	Ca 2 _p	P 2 _p	Ca:P
AR96	Nil	Nil	61.5	38.5	1.60
E96	Nil	Nil	56.9	43.1	1.32
P96	Nil	Nil	57.2	42.8	1.33
1.5V96	Nil	Nil	60.8	39.2	1.55

*AR96 = (SBFx3)(As-received)(96 hours), E= Etched, P= Passivated, 1.5V= anodised at 1.5 Volts.

4.5.3 TF-XRD

Ti6Al4V

XRD diffraction pattern of 96 hours immersed (SBFx3, 38.5°C) unanodised (NA96), acid-anodised (A5096) and alkaline anodised (B5096) Ti6Al4V are shown in Figure 39.

NiTi

Figure 40 presents the TF-XRD diffraction pattern of 96 hours immersed (SBFx3, 39.5°C) etched (E), as received (AR), passivated (P) and anodised (1.5V in 0.1M HNO₃) NiTi.

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Figure 39 TF-XRD diffraction pattern of unanodised (3NA96), acid-anodised (3A5096) and alkaline anodised (3B5096) Ti6Al4V at 96 hours immersion (SBFx3, 38.5°C).

Figure 40 XRD diffraction pattern of etched (E), as received (AR), passivated (P) and anodised (1.5V in 0.1M HNO_3) NiTi after 96 hours immersion (SBFx3, 39.5°C). (Note: * - apatite)

4.6 Morphological study of CaP Coating

Ti6Al4V

SEM/FESEM pictures of Ti6Al4V samples after 0, 24 and 96 hours immersion in SBFx3 are shown in Figures 41 (unanodised and acid anodised) and 42 (alkaline anodised).

NiTi

SEM/FESEM pictures of etched (E) and as received (AR) NiTi plates, after 0, 24, and 96 hours immersion in SBFx3 at 39.5°C, are shown in Figure 43.

Figure 44 presents the SEM/FESEM pictures of anodised (1.5V) and nitric acid passivated NiTi plates after 0, 24, and 96 hours immersion in SBFx3 at 39.5°C.

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Figure 41 SEM/FESEM pictures of i) un-anodised and ii) acid-anodised Ti6Al4V plates after a) 0 hour, b) 24 hours, and c) 96 hours immersion in SBFx3 at 38.5°C.

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Figure 42 SEM/FESEM pictures of basic-anodised Ti6Al4V plates after a) 0 hour, b) 24 hours, and c) 96 hours immersion in SBFx3 at 38.5°C.

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Figure 43 SEM/FESEM pictures of i) etched and ii) as received NiTi plates after a) 0 hour, b) 24 hours, and c) 96 hours immersion in SBFx3 at 39.5°C.

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Figure 44 SEM/FESEM pictures of i) anodised (1.5V) and ii) passivated NiTi plates after a) 0 hour, b) 24 hours, and c) 96 hours immersion in SBFx3 at 39.5°C.

Chapter 5: Discussion I-Ti6Al4V

Assessing *in-vitro* corrosion resistance, surface chemical compositions and topography are essential in determining the possible biological response towards metallic implants.

In the next two chapters, structure and chemical composition of the coating are analysed. The effects of different surface treatments applied to NiTi, Ti6Al4V plates on its topography; surface oxide thickness and *in-vitro* corrosion resistance in SBF are discussed. An attempt to relate variations in morphology, chemical composition and relative thickness of the final calcium phosphate coating, with immersion temperature (38.5 and 39.5°C), immersion time (24 and 96 hours), substrate-type (Ti6Al4V and NiTi) and surface treatments (as received, etched, passivated, anodised) are made.

5.1 Anodisation of Ti6Al4V

Studies on structure, growth of anodic oxide on titanium and Ti6Al4V revealed that thickness of the films is a linear function of the applied voltage (Blondeau et al., 1977b). This growth law holds even when modification in the crystallographic structure in the film occurs at higher voltages (>50V). Recent studies, using ellipsometry (Blackwood et al., 1989; Velten et al., 2002) and electrochemical methods (Badekas & Panagopoulos, 1987; Ibris & Rosca, 2002), on anodic oxide of titanium and its alloys support these findings.

5.1.1 Effect of applied voltage on the anodic titanium oxide thickness

The interference colour of anodic titanium oxide corresponding to their thickness (E_f) has been consistent between related studies (Sul et al., 2001; Velten et

Chapter 5: Discussion I-Ti6Al4V

al., 2002) (see Appendix D). It is therefore viable to estimate thickness of anodic oxide by its colour

From Table 12 it is observed that, with time as a fixed parameter, colour of anodic oxide formed on Ti6Al4V is similar, at E_f up to 50V, in both NaOH and H₂SO₄. This indicates similarity in the thickness of the oxide formed anodically in the two different electrolytes. Reproducibility of the electrochemical anodisation process was demonstrated with a second batch of samples (Table 12). The thickness of the titanium oxide formed anodically on, Ti6Al4V at various applied E_f , was estimated from their interference colour by referencing to those reported in related studies as summarised in Appendix D (Sul et al., 2001; Velten et al., 2002).

The thickness of the anodic oxide is observed to increase with increasing applied E_f , and estimated to be from a minimum of 50nm ($E_f=20V$) to a maximum of 120nm ($E_f=50V$). The variations in the corresponding anodic oxide film colour, at a given anodising voltage, from those reported in the literature (Sul et al., 2001; Velten et al., 2002), are likely due to differences in the electrolyte's concentration and chemical composition of the test materials used.

Gas evolution observed during anodising of Ti6Al4V at $E_f \leq 50V$ is likely to be H₂, released with the formation of an anodic oxide layer as per Equation 1 (section 2.2.4.1). Vigorous effervescence is accompanied by greyish appearance on the sample's surface when anodised at $E_f > 50V$ in 0.5M NaOH electrolyte. Such phenomenon was not observed for samples anodised in 0.5M H₂SO₄ at E_f up to 80V.

Ti6Al4V has lower anodic oxide forming ability in alkaline electrolyte compared to acid electrolyte. The respective E_f corresponding to steady state i.e. dynamic equilibrium between the oxide formation rate and field-assisted/chemical dissolution rate is much lower in alkaline electrolyte (Marino et al., 2001; Sul et al.,

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2001). The vigorous effervescence observed at $E_f > 50V$ in 0.5M NaOH is caused by oxide dissolution rate becoming prominent. At the same E_f , anodic oxide formation rate of Ti6Al4V in 0.5M H₂SO₄ far exceed its dissolution rate, and therefore only gas evolution was observed.

5.1.2 Effect of electrolyt-type and anodising voltage on topography of Ti6Al4V

Titanium alloy anodised in acid media, below the breakdown potential, is known to produce surfaces with reduced roughness when compared to those anodised in alkaline media (Critchlow & Brewis, 1995; Oliveira et al., 2000).

Alkaline electrolyte (NaOH)

Surface roughness (R_a , R_q) and surface area (S.A) of alkaline-anodised Ti6Al4V samples are observed to decrease with increasing applied E_f (Figure 16). Lowest surface roughness is observed for sample anodised at $E_f = 30V$. Further increase in E_f to 50V resulted in an increase in measured topography. Sample anodised at $E_f = 50V$ has measured roughness (R_a , R_q) and area (S.A.) similar to those of the unanodised control samples.

Acidic electrolyte (H₂SO₄)

Acid-anodised Ti6Al4V samples have measured roughness and surface area decrease with increasing voltage. Surface roughness is at minimum for samples anodised at $E_f = 50V$, which again increases when E_f is increased to 80V.

The initial reduction in roughness and surface area of samples is a consequent of the preferential dissolution of sharp groove edges (from sample preparation), and growth of a compact homogeneous oxide throughout the whole surface (Marino et al.,

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2001). At high E_f values (50V in NaOH and 80V in H₂SO₄), anodising process is at or close to the steady state i.e. dynamic equilibrium between the oxide formation and dissolution, where dissolution of oxide becomes prominent (Sul et al., 2001). The competitive processes of oxide formation and dissolution processes at the substrate surface resulted in increased roughness and surface area, and therefore the increased in measured topography at high E_f values (Figure 16).

5.2 Corrosion Resistance of Ti Oxide-coated Ti6Al4V in SBF

The impedance spectra can be fitted well with a Randles cell equivalent circuit containing one time-constant (Figure 10 in sub-section 2.4.2). This suggests that the titanium oxide formed is single, compact and barrier-type layer with no major defects or diffusion paths within the layer (Da Silva et al., 2002; Garcia-Alonso et al., 2003; Krupa et al., 2003). Such compact oxide layers are desirable in terms of biocompatibility, as they exhibit enhanced corrosion resistance compared to thick porous ones (Trepanier et al., 1998).

5.2.1 Effectiveness of oxide formed anodically in enhancing corrosion resistance

Stability of Ti oxide-coated Ti6Al4V

Blackwood et al. investigated the reactivation of anodic oxide film formed on titanium in acid solution (Blackwood et al., 1988). They show that stability of the oxide depends on the rate at which the oxide film had been formed, as well as on solution composition and temperature. Blondeau et al. investigated influence of electrolyte-type, pH and substrate's compositions on the structure of anodic oxide formed (Blondeau et al., 1977b). Their results show that with increase pH and presence of alloying elements

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delay the transformation of amorphous (microcrystallites with anatase symmetries) titanium oxide to crystalline anatase. Anodic titanium oxide film potentiodynamically formed on titanium in phosphate buffered solutions, with pH varied from 1 to 5, were also found to be stable in the acidic phosphoric media. The surface was revealed to be compact, homogeneous and practically free of pores (Marino et al., 2001).

It is evidence from Figures 19 and 20 that acid-anodised Ti6Al4V samples exhibit higher polarisation resistance (R_p) in SBF than alkaline-anodised ones. The R_p of acid-anodised Ti6Al4V samples increase with anodising voltage, and reaches a maximum at $E_f = 50V$ ($4.3M\Omega/cm^2$) before declining to a minimum at $E_f = 80V$ ($1.4M\Omega/cm^2$). Alkaline-anodised Ti6Al4V samples also have R_p values that increase with increasing applied E_f , reaching a maximum at $E_f = 50V$ ($1.9M\Omega/cm^2$) and dropped to a minimum at $E_f = 20V$ ($0.8M\Omega/cm^2$). The control sample exhibits lowest R_p value ($0.4M\Omega/cm^2$).

Pitting resistance Ti oxide-coated Ti6Al4 V

Acid-anodised Ti6Al4V plates are observed to exhibit higher pitting potential, E_{pitt} , than alkaline-anodised ones (Figures 23). Changes in E_{pitt} with applied E_f of acid-anodised Ti6Al4V samples are consistent with their R_p . They increase with the applied E_f , reaching a maximum at $E_f = 50V$ ($E_{pitt}=13.1V$), and decline to a minimum at $E_f = 20V$ ($E_{pitt}=11.2V$). Such consistency is not observed for alkaline-anodised Ti6Al4V plates, whose E_{pitt} reaches a maximum at $E_f = 30V$ ($12.0V$), and minimum at $E_f = 20V$ ($7.1V$). Unanodised Ti6Al4V again exhibits the lowest E_{pitt} ($2.8V$). Although the anodic oxide interference colours of samples anodised at higher voltages (golden for 50V in NaOH and green for 80V in H_2SO_4) indicate a greater oxide thickness, a drop in pitting corrosion potential has been experienced ($8.3V$ and $12.1V$ respectively).

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Results from the electrochemical tests on Ti6Al4V match well with those reported in literature (Sul et al., 2001). Anodic oxide formed in H₂SO₄ is likely to be different from those formed in NaOH (Blondeau et al., 1977b). Titanium oxide films formed in acid are probably more stable than those in alkaline electrolyte, therefore exhibiting enhanced corrosion resistance in SBF. However, this hypothesis was not quantified as, though XPS analysis (sub-section 5.3.1) has confirmed the presence of titanium oxide, the corresponding diffraction peaks were not detected during TF-XRD (sub-section 5.4). These indicate the titanium oxide is either amorphous, or partially crystallised with microcrystallites of anatase and/or rutile (Blondeau et al., 1977a), whose sizes are beyond sensitivity of the instrument (TF-XRD) used (Badekas & Panagopoulos, 1987; Klug & Alexander, 1974).

5.2.2 Influence of oxide thickness and topography on pitting resistance

Ibris and Rosca studied the effect of alloying elements on stability of titanium alloy's oxide film in Hank's solution. They proposed that alloying elements enhances titanium's corrosion resistance and the diffusion of titanium ions. This leads to formation of thicker, single-layered and compact oxide film i.e. more protective, during anodisation (Ibris & Rosca, 2002). Velten et al. assessed the corrosion resistance of thermally oxidised, anodically oxidised and sol-gel processed titanium and Ti6Al4V in 0.9% NaCl. They concluded that anodised and sol-gel coated samples exhibit lower current density in the passive range over polished sample with air-formed oxide, and that the corrosion resistance can be improved by thickening the oxide thickness (Velten et al., 2002).

Accompanying the increasing E_f is the growth of a compact homogeneous oxide replacing the grooves from sample preparations. The results of such growth are: 1) increased oxide thickness (Blackwood et al., 1988; Ibris & Rosca, 2002; Velten et al.,

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2002) as indicated by the oxide interference colours (Table 12), and 2) reduced measured topography (Es-Souni et al., 2002; Marino et al., 2001) (sub-section 5.1.2). This explains the initial improvement in corrosion resistance of anodised Ti6Al4V.

Es-Souni et al. evaluated the corrosion resistance and biocompatibility of NiTi samples with different surface finishes and impurities residue in Ringer's solution. He reported that poor surface finish, surface impurities have negative effect on the passive layer, leading to highly localised corrosion rate and large pits on tested samples (Es-Souni et al., 2002). At high E_f values (50V in NaOH and 80V in H₂SO₄) dissolution of oxide becomes prominent (Sul et al., 2001), resulting in samples with increased surface roughness and area (Figure 16). This explains the reduction in corrosion resistance of samples anodised at higher E_f , despite the presence of a thicker titanium oxide layer (as estimated from its oxide interference colour).

5.2.3 Repassivation capability of anodised Ti6Al4V

Figure 22 show the potentiodynamic polarisation curves of anodised Ti6Al4V. With the exception of samples anodised at $E_f = 20V$ in NaOH, all samples exhibit large hysteresis loops and high current density (exceeding 10^{-1} Amp/cm²) at applied potentials above E_{pitt} . The corrosion current density remains high until a sufficiently low potential has been reached in the reverse scan, whereupon repassivation occurs once more. This indicates poor resistance to pitting because once the passive film is broken, it will not repassivate easily (Trethewey & Chamberlain, 1995).

The unanodised Ti6Al4V exhibit lowest corrosion resistance ($R_p = 0.4$ M Ω /cm², $E_{pitt} = 2.8V$), which is expected with its measured high surface roughness, large surface area (Figure 16) and thin, air-formed oxide layer (Figure 28i).

Overall, anodised of Ti6Al4V samples exhibit four-fold improvement in its corrosion resistance over unanodised samples. The enhancement in corrosion is a result

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of increased thickness of the compact, single-layered non-porous titanium oxide film. A drop in Ti6Al4V's corrosion resistance observed at higher E_f values is likely because of increased topography. Although anodised Ti6Al4V samples exhibit good passivity, repassivation does not occur readily if the oxide layer ruptures at potential above its E_{pitt} . Anodised Ti6Al4V is expected to exhibit good passivity within the measured body potential (0.2-0.6V) (Hoar & Mears, 1966), which is well below its measured E_{pitt} , provided no rupture in the titanium oxide layer is induced by mechanical wear, fretting, abrasion etc.

5.3 Chemical Characterisation of the Ti Oxide Layer

5.3.1 XPS analysis

Many studies have been done to characterise the anodic oxide of titanium alloy (Asami et al., 1993; Badekas & Panagopoulos, 1987; Blondeau et al., 1977b; de Sena et al., 2002; Pouilleau et al., 1997; Krupa et al., 2003; Marino et al., 2001; Oliveira et al., 2000; Sul et al., 2002a; Sul, 2003; Velten et al., 2002; Yang et al., 2004). Their results revealed that titanium dioxide, TiO_2 , is the main constituent of anodic titanium oxide layer.

Surface titanium oxide

All Ti6Al4V samples exhibit similar survey-scan spectra, before and after ion etching (Figure 28ix). Results from the survey scan prior to ion etching are analogous with those reported for oxidised titanium in related studies (de Sena et al., 2002; Filip et al., 2001; Schreckenbach & Marx, 1999). The main elements detected are Ti_{2p} , O_{1s} and C_{1s} , indicating that the component of analysed surface is titanium oxide. Regardless of the surface treatments employed, all samples prior to etching have Ti spectra (Figures

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28i-viii) with binding energies of 459eV ($\text{Ti}_{2p3/2}$) and 464.5eV ($\text{Ti}_{2p1/2}$), which are assigned to TiO_2 (Armitage & Grant, 2003). However, extra peaks at lower binding energies (~454.4eV and 460.5eV) corresponding to metallic titanium (Moulder et al., 1995) are observed on unanodised Ti6Al4V sample (Figure 28i). From the high-resolution Ti spectra, it is clear that titanium is always present in the form of TiO_2 , with binding energies (BEs) of approximately 459 and 464.5eV regardless of the surface treatment.

Influence of applied voltage on thickness of the oxide layer (Ti6Al4V)

Prior to ion etching, peaks from metallic titanium (Filip et al., 2001; Moulder et al., 1995) and lower oxidation states of Ti i.e. Ti^{3+} , Ti^{2+} and Ti^+ (–454.4 to 460.5eV) (Sul et al., 2002a), are observed on the unanodised control sample (Figure 17i). These indicate that the air-formed titanium oxide is no thicker than 60-80Å (Moulder et al., 1995; Ponsonnet et al., 2002) i.e. since signal from underlying metallic substrate can be picked up. Detection of peaks corresponding to titanium sub-oxides also indicates the outermost TiO_2 layer is very thin.

No peaks from metallic or lower oxidation states of titanium are observed for all anodised samples (Figures 28ii-viii), indicating that titanium is found only in the form of TiO_2 at the sample's surface. It is known that titanium oxide thickness increase linearly with anodisation voltage (Badekas & Panagopoulos, 1987; Blondeau et al., 1977b; Blackwood et al., 1989; Ibris & Rosca, 2002; Velten et al., 2002). The top TiO_2 layer is also likely to be thicker than those on unanodised Ti6Al4V. Results from XPS analysis of Ti6Al4V verified that anodised samples have enhanced titanium oxide with thicker top TiO_2 layer than unanodised sample.

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Titanium oxide underneath the topmost TiO_2 layer

A three-layer model, with intermediate sub-oxide layer between titanium substrate, and an outermost layer of TiO_2 , has been proposed (Blackwood et al., 1989; Pouilleau et al., 1997) for passive anodic titanium oxide films. This model fits well with the results from Sul et al. (Sul et al., 2002a), who characterised the outer oxide layer of electrochemically oxidised titanium implants. The oxide layer on anodised titanium alloys can be amorphous, anatase or mixture of anatase and rutile, depending on the applied forming voltage, E_f , electrolyte and substrate's compositions (Blondeau et al., 1977a).

After 120 seconds of argon ion etching, the initially defined Ti peaks for the unanodised Ti6Al4V sample were replaced by a broad spectrum, spreading from 454.5eV to 467eV. These binding energies corresponds to titanium in lower oxidation states: Ti^0 (Ti-Ti), Ti^{2+} (TiO), Ti^{3+} (Ti_2O_3) and Ti^{4+} (TiO_2) (Moulder et al., 1995; Pouilleau et al., 1997). The result indicates presence of a mixture of titanium sub-oxides and relatively high amount of metallic titanium underneath the top most TiO_2 layer.

Ti spectra of anodised samples all appear similar, but asymmetric to those obtained prior etching. Titanium in the oxide layer is still present mainly as TiO_2 . A small shoulder is observed on the lower energy side of both $Ti_{2p3/2}$ (~457.5eV) and $Ti_{2p1/2}$ (~463.4eV) peaks. These indicate that there is a mixture of TiO_2 and Ti_2O_3 underneath the top TiO_2 oxide layer. These results match well with the three-layer oxide model proposed during oxide analyses of anodic titanium oxide in related studies (Blackwood et al., 1989; Pouilleau et al., 1997; Sul et al., 2001). The lack of metallic peaks, in contrast to those of unanodised samples, indicates that anodic titanium oxide is thicker than those formed in air.

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Incorporation of alloying elements (V and Al) into the oxide layer

The XPS spectra for V is hardly detectable ($<1.0\text{atm. \%}$), and that for Al, when observed, has concentration below three atomic percent ($<2.5\text{ atm. \%}$). The detection of V and Al peaks indicates that these materials have been incorporated into the anodic titanium oxide layer. Their exact compositions are unable to be determined because of the low peak intensities. However, these alloying elements are believed to exist as oxides due to their high affinity for oxygen (Blondeau et al., 1977b).

Figure 27a shows deconvoluted high-resolution spectra of the O_{1s} peak. The three components have binding energies of metallic oxides, Ti-O (-529.8eV), hydroxides, Ti-OH (-531.6eV) and water, H_2O (533.0eV), which have been observed for anodised titanium (Pouilleau et al., 1997; Takadama et al., 2001). Upon ion etching, the O_{1s} spectra was reduced to one single asymmetric peak, with the main peak corresponds to oxide species (529.8eV , Ti-O) and a smaller shoulder to the hydroxide group (531.6eV , Ti-OH) (de Sena et al., 2002).

Carbon, one of the main elements detected, can be deconvoluted into two components (Figure 27b) with binding energies corresponding to C-C (285.0eV) and C-H (286.3eV) bonds (Moulder et al., 1995). These are from a carbonaceous overlayer, which is frequently encountered in surface analysis, where examined surface is contaminated with adsorbed components from the atmosphere (hydrocarbons) (Asami et al., 1990; de Sena et al., 2002; Filip et al., 2001). Even in a vacuum environment, the sample surface could be covered with monolayer of gas molecules in just a matter of seconds (Skoog et al., 1997).

5.4 Structural Study of Ti Oxide Layer

Electron diffraction studies on anodic oxide film formed on titanium and Ti6Al4V revealed that films formed at low voltages ($\leq 50\text{V}$) are composed of microcrystallites of 15-25 atoms (Blondeau et al., 1977a). These “quasi-amorphous” microcrystallites have anatase symmetries, shaped like platelets or polyhedral aggregates, which transformed to crystalline anatase or mixture of anatase and rutile at higher voltages (Blondeau et al., 1977b). Electrolyte-type, pH and substrate's composition influence voltage at which such transformation occur and structure of final oxide.

Presence of alloying elements has also been reported to hinders crystallisation of amorphous anodic TiO_2 at higher anodising voltages (20% crystalline structure at $E_f=90\text{V}$) (Velten et al., 2002). Sul et al. anodised commercially pure titanium in 0.1M acetic acid and did not detect a crystalline TiO_2 structure up to anodising voltage of 200V (Sul et al., 2002a). Pouilleau et al. anodised titanium at 20V in 0.5M H_2SO_4 and observed weak anatase peaks (together with prominent peaks of metallic substrate) only after heat-treating the sample at 600°C (Pouilleau et al., 1997). XRD analysis of titanium anodised in phosphoborate electrolyte at 48V shows that no diffraction plane of titanium oxide has been detected even though crystallisation of amorphous titanium oxide is expected (Badekas & Panagopoulos, 1987). It has been concluded that 1) the outer layer of air formed and anodic titanium oxide is composed of amorphous TiO_2 or 2) poorly crystalline anodic film with ultra-fine crystallites beyond the sensitivity of the equipment used. Another explanation given for the lack of detectable TiO_2 structure was the presence of a non-continuous oxide network formed at the titanium/ TiO_2 interfaces. Non-stoichiometric compounds with continuously varying composition do

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not give rise to X-ray diffraction peaks since they behave more like a molecular layer rather than a crystalline structure.

The lack of characteristic rutile or anatase peaks in the TF-XRD spectra of Ti6Al4V is analogous with those reported in related studies (Badekas & Panagopoulos, 1987; Pouilleau et al., 1997; Sul et al., 2002a; Velten et al., 2002). XPS analysis (sub-section 5.3.1) for the oxide layer of Ti6Al4V shows that it consists of a titanium top TiO_2 layer, with underlying titanium sub-oxides and metal (Ti^0 , Ti^{2+} , Ti^{3+} and Ti^{4+}). These results indicate that the surface TiO_2 layer is very thin, amorphous or poorly crystallised with a continuous variation of composition of oxide film along the whole thickness (three-layer model).

5.5 Chemical Characterisation of the Calcium Phosphate (CaP) Layer

It has been estimated that up to 50% of young, newly formed bones are 'amorphous' calcium phosphate solid. However, the main calcium phosphate solid is thought to be a poorly crystalline hydroxyapatite (Turek, 1984). Calcium phosphate coatings formed on bioactive surfaces in SBF are found to have a composition and structure analogous with those in bones (Kokubo et al., 1999). They contain carbonate ion substitutions at phosphate ion lattice sites. These coatings are therefore calcium deficient-carbonated apatite exhibiting lower calcium-to-phosphorous (Ca:P) ratio.

Hench & Wilson found that the amorphous calcium/phosphorous-rich layer formed instantaneously on surface of 45S5 Bioglass-type glass, during *in-vitro* test using Tris-buffered solution i.e. distilled water buffered with tris-hydroxymethyl aminomethane (Hench & Wilson, 1984).

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Analysis of NaOH and heat-treated bioactive Ti6Al4V in SBF solution revealed that titania undergo ion exchange process to formed titania hydrogel with negative surface charge (Takadama et al., 2001). Electrostatic attractions of positively charged calcium ions and subsequent negatively charged phosphate ions lead to nucleation and deposition of amorphous calcium phosphate.

Frauchiger et al. observed a spontaneous layer of calcium phosphate on titania surface, whose thickness increase with immersion time in Hank's solution. Composition studies of the absorbed calcium phosphate layer indicate that only phosphate and calcium ions were specifically adsorbed on the TiO₂ surface. However, contradictory findings from AES study of the apatite layer formed indicate that the first layer formed on the TiO₂ surface was phosphate followed by binding of calcium ions (Frauchiger et al., 1999). An EIS and photoelectrochemical studies also reported that phosphate ions in SBF solution appeared to react faster with the semiconductor TiO₂ film than calcium ions. Such interactions were found to have no effect on the semiconductivities of the film, i.e. does not degrade the TiO₂ film (Hodgson et al., 2002).

Ti6Al4V samples retrieved at 24,48,72, 96 hours immersion in SBFx3 solution were studied in detail. All samples retrieved, except those at 24 hours, exhibit a multicoloured interference fringe. This is an indication for the presence of a uniform calcium phosphate layer, reported to be <1 to 3pm thick (Barrere et al., 2002a; Liu et al., 2003b; Stigter et al., 2002), which diffracts natural light to give samples a 'rainbow' appearance.

5.5.1 FTIR analysis

FTIR spectra for calcium-deficient carbonated apatite precipitated from SBF solution are typified by featureless broad characteristic bands of ν_1 and ν_3 PO_4^{3-} vibration modes (Dorozhkina et al., 2003). Deviations from ideal tetrahedral structure were reported to be due to lack of crystallinity (Barrere et al., 2002a), or small crystal size and defects in the precipitates of apatite (Stoch et al., 2000). This is accompanied by a lack of absorption band for OH- around 3570cm^{-1} , which suggest that the coating is amorphous (Stoch et al., 2000). Broad bands attributed to CO_3^{2-} (around 1400 and 870cm^{-1}) and HPO_4^{2-} (around 870cm^{-1}) are also characteristic of deficient-carbonated apatite precipitates in SBF solution as these ions substitute PO_4^{3-} ions in the apatite lattice (de Sena et al., 2002; Furuzono et al., 2000). Substitution of CO_3^{2-} for PO_4^{3-} leads to B-type carbonate with characteristic bands around 1420 and 1470cm^{-1} . A band indicating carbonate replacement for the OH (A-type carbonate) can be found at around 1545cm^{-1} (Layani et al., 2000).

Calcium phosphates deposited on Ti6Al4V samples exhibit FTIR spectra characteristic of bone-like apatite deposited in SBF at physiological pH and temperature (Barrere et al., 2002a; Chou et al., 2004; Dorozhkina et al., 2003; Furuzono et al., 2000; Layani et al., 2000; Li et al., 1997; Stoch et al., 2000). The featureless nature of the characteristic bands (carbonate, phosphate, hydrogen phosphate) reveals that calcium phosphate deposited is amorphous or defective with small crystal size.

Changes in composition of the Ca Player composition with immersion time

At 24 hours immersion, unanodised Ti6Al4V sample (Figure 30) exhibits a broad single band around 1099cm^{-1} , which increases in intensity and shifts gradually to 1041cm^{-1} at 96 hours immersion. These bands are assigned to the ν_3 P-O vibration

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mode in HPO_4^{2-} and PO_4^{3-} respectively (Slosarczyk et al., 1997). Small band around 869cm^{-1} , dedicated to P-OH bond in HPO_4^{2-} or ν_2 vibration mode in CO_3^{2-} group (de Sena et al., 2002; Dorozhkina et al., 2003; Slosarczyk et al., 1997), appears after 48 hours immersion. Accompanying it is small shoulder around 960cm^{-1} corresponds to ν_1 vibration mode of PO_4^{3-} . The band spreading from 1419 to 1463cm^{-1} is assigned to C-O stretching (Layani et al., 2000; Oliveira et al., 2003b) or to the ν_3 mode of CO_3^{2-} (Barrere et al., 2002a; Stoch et al., 2000). The broad single bands at 3000 - 3400 and 1650cm^{-1} originate from O-H bond stretching/bending of H_2O (Barrere et al., 2002a; Oliveira et al., 2003b). Band corresponding to O-H bond of HA (around 3600cm^{-1}) is not observed.

The strong HPO_4^{2-} band (around 1099cm^{-1}) for 24 hours immersed unanodised Ti6Al4V indicates calcium-deficiency calcium phosphate nuclei, due mostly to HPO_4^{2-} substitution for PO_4^{3-} , has been deposited. The presence of PO_4^{3-} , HPO_4^{2-} and carbonate (-1419 and 1463cm^{-1}) bands for final coating formed at 96 hours immersion indicate a calcium-deficient B-type carbonated apatite coating (de Sena et al., 2002; Deng. et al., 2003; Layani et al., 2000; Khor et al., 2003a). The change in deposited apatite may be induced by changes in surface of unanodised Ti6Al4V with immersion period. Upon immersion, the air formed titanium oxide in contact with SBF solution may favour and stabilise HPO_4^{2-} over PO_4^{3-} . However, formation of the initial calcium phosphate layer change the surface chemistry, favouring stabilisation of both HPO_4^{2-} and PO_4^{3-} to produce coating with higher PO_4^{2-} content (Osaka et al., 1991).

Calcium phosphate deposited onto alkaline anodised Ti6Al4V (Figure 32) differs slightly from those of unanodised samples. A broad single band assigned to ν_3 mode of HPO_4^{2-} (around 1109cm^{-1}), together with another prominent band centred at around 826cm^{-1} , attributed to HPO_4^{2-} or ν_2 vibration in CO_3^{2-} group, are observed at at

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24 hours immersion. Centre of the HPO_4^{2-} band shifts to around 1058 cm^{-1} (96hr) at 96 hours immersion, which is assigned to the ν_3 vibration mode for PO_4^{3-} . Ratio of the $\text{HPO}_4^{2-}/\text{CO}_3^{2-}$ ($-780\text{--}860\text{ cm}^{-1}$) to PO_4^{3-} (cm^{-1}) bands decreases with increasing immersion period. Spectra of the final coating has a small shoulder around -960 cm^{-1} corresponding to ν_1 vibration mode of PO_4^{3-} (de Sena et al., 2002). Small band at 1437 cm^{-1} observed is attributed to C-O stretching of the CO_3^{2-} ion. Water is again observed from the spectra at around $3000\text{--}3400$ and 1650 cm^{-1} (Barrere et al., 2002a; Stoch et al., 2000). Band corresponding to O-H bond of HA (around 3600 cm^{-1}) (Osaka et al., 1991) is again not observed.

FTIR spectra of acid-anodised samples (Figure 31) are very similar to those of the alkaline-anodised ones (Figure 32). The only difference is that the ratio of $\text{HPO}_4^{2-}/\text{CO}_3^{2-}$ band and PO_4^{3-} bands is almost equal as early as at 72 hours, instead of at 96 hours.

The detection of an addition $\text{HPO}_4^{2-}/\text{CO}_3^{2-}$ band (around 826 cm^{-1}) at 24 hours immersion indicates calcium phosphate nucleus deposited on the anodised Ti6Al4V are likely to have higher degree of calcium deficiency than those in the unanodised sample (Barrkre, 2002). Decrease in the intensity ratio of $\text{HPO}_4^{2-}/\text{CO}_3^{2-}$ (-860 cm^{-1}) to PO_4^{3-} (1058 cm^{-1}) bands indicates a less calcium-deficient coating with increase immersion period. These observations are analogous with the findings of Frauchiger et al., who reported a decrease in calcium-deficiency of apatite with increasing immersion time, i.e. increasing Ca:P ratio (Frauchiger et al., 1999). The comparatively higher intensity ratio of $\text{HPO}_4^{2-}/\text{CO}_3^{2-}$ to PO_4^{3-} bands also shows that final calcium phosphate coating (96 hours) on anodised Ti6Al4V has higher degree of calcium deficiency than those on unanodised ones.

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Effect of surface treatments on degree of carbonate and hydrogen phosphate substations for phosphate in the final CaP layer

Deviations in calcium phosphate composition deposited on anodised Ti6Al4V and unanodised samples are likely due differences in the titanium oxide layer.

Air formed titanium oxide is known to be amorphous (Sul et al., 2002a). Those of anodised samples can be amorphous, partial/fully crystallised anatase or rutile (Blondeau et al., 1977b; Pouilleau et al., 1997), although it is normally not detectable by XRD technique (Badekas & Panagopoulos, 1987; Pouilleau et al., 1997; Velten et al., 2002; Wang et al., 2003b).

Titanium oxide surface carries a weak negative charge at physiological pH (Jones, 2001). However, depending on the oxide structure and solution's ionic strength, acidic and basic Ti-OH can be formed (Chang & Lee, 2002; Connor et al., 1999; Jones, 2001). Composition of apatite precipitated in SBF is known to be strongly pH dependent (Chou et al., 2004; Li et al., 1997; Rey et al., 1991). Hydration of anodic titanium oxide may have caused localised variation in pH at the oxide/SBF bulk solution interfaces, stabilising both HPO_4^{2-} and CO_4^{3-} ions at the oxide surface (Osaka et al., 1991). However, subsequent nucleation and formation of calcium-deficient apatite changes the surface in contact with the bulk SBF solution. At the final pH or conditions, PO_4^{3-} ions increasingly stable over HPO_4^{2-} at surface of the first apatite layer formed. Therefore, subsequently deposited apatite exhibits lower calcium-deficiency. Broad bands from chemisorbed water are again detected and lack of the characteristic O-H band of HA again indicate amorphous nature of deposited apatite.

FTIR spectra of calcium phosphate deposited onto anodised Ti6Al4V samples are also characteristics of amorphous, calcium-deficient B-type carbonated-apatite. However, higher intensity of $\text{HPO}_4^{2-}/\text{CO}_3^{2-}$ around 826 cm^{-1} show that the final apatite

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coating has higher degree of calcium deficiency compared to those of unanodised samples.

5.5.2 XPS analysis

Biological apatites such as bone and tooth apatite are known to deviate in composition from stoichiometric hydroxyapatite (Aoki, 1994). They are partially carbonated, calcium deficient and contains additional ions, e.g. Mg^{2+} , K^+ and Na^+ . Calcium phosphate apatite precipitated from SBF is also carbonated with composition similar to those of natural bone (Li et al., 1997; Liu et al., 2004).

Survey spectra of calcium phosphate deposited on Ti6Al4V (Figure 37) correspond well with those reported in the literature (Chung et al., 2003; de Sena et al., 2002; Furuzono et al., 2000; Gu et al., 2003; Gu et al., 2004; Krupa et al., 2003; Takadama et al., 2001). Main peaks identified are attributed to different chemical states of Ca, P, O and C. The lack of detectable Ti and Ni (for NiTi samples only) peaks indicates the continuous nature of the apatite layer deposited (Chung et al., 2003).

Ca spectra of apatite coated Ti6Al4V and NiTi revealed the Ca_{2p} p-level spin doublet with binding energies at approximately 347.4eV ($\text{Ca}_{2p_{1/2}}$) and 351.1eV ($\text{Ca}_{2p_{3/2}}$), which agree well with those reported for hydroxyapatite (Chung et al., 2003; de Sena et al., 2002; Takadama et al., 2001). These peaks do not match the binding energy of CaCO_3 (346.7-346.9eV) (Chung et al., 2003; Moulder et al., 1995).

Partial substitution of calcium by magnesium

It is known that apatitic structure, with its hexagonal lattice, has a strong tendency to form a solid solution, accepting numerous substitutions (Aoki, 1994). Magnesium, detected at very low intensity with binding energy around 50.5eV (Figures 37di-iii), is likely to exist in the apatite structure by partially substituting divalent Ca^{2+} .

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Partial substitution of phosphate by carbonate and hydrogen phosphate

The observed high intensity P-XPS peak has binding energy of 133.1eV. This is in good agreement with that of HA (Chung et al., 2003; Takadama et al., 2001). A shoulder is observed at higher binding energy (134.2eV) on the asymmetric P peak, indicating that P atoms also exist in another chemical state. The same has been observed by Chung et al. (Chung et al., 2003), but an explanation was given by Schreckenbach and Marx (Schrecke Marx, 1999) who suggested the simultaneous presence of isolated $[\text{PO}_4]^{3-}$ tetrahedral (133.5eV) and hydrogen phosphate groups $[\text{HPO}_4]^{2-}$ (134.4eV). This is consistent with the observed high intensities of HPO_4^{2-} peaks in FTIR spectra of apatite coatings deposited (Figures 30 to 32) on Ti6Al4V.

Carbon exists mainly as a hydrocarbons from atmospheric contamination (as discussed in sub-section 5.3.1) (Skoog et al., 1997). An extra, but distinctive, C peak is observed at higher binding energy around 288.7eV for apatite deposited. This peak is ascribed to metallic carbonate (MCO_3) groups (Chung et al., 2003; de Sena et al., 2002) as its binding energy does not correspond to carbide (281-283eV) (Moulder et al., 1995), and the absence of CaCO_3 is confirmed from analysis of Ca spectra. Together with the results from FTIR analysis, this peak is likely to originate from carbonate groups incorporated into the apatite structure by substitution of phosphate, producing calcium-deficient B-type carbonated apatite (Aoki, 1994; Barrere et al., 2002a; Chung et al., 2003; de Sena et al., 2002).

Binding energy of the main O_{1s} peak shifts from around 529.8eV (Ti-O) to 531eV, which is assigned to the P-O bonds of HA (Chung et al., 2003; de Sena et al., 2002; Takadama et al., 2001). The small shoulder with higher energy around 533eV

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originates from adsorbed water, which is normal for an immersion process (Chung et al., 2003).

Effect of surface treatments on the degree of calcium-deficiency in the CaP layer

The calculated calcium-to-phosphorous ratios (Ca:P) for coatings on Ti6Al4V samples varies from 1.14 to 1.28 (Table 16). These Ca:P ratios are lower than those of stoichiometric hydroxyapatite (1.67) and amorphous calcium phosphate (1.50), but close to those of octacalcium phosphate (1.33). The low values indicate a calcium-deficient apatite system, a phenomena often observed when lacuna are created by replacement of PO_4^{3-} with $\text{HPO}_4^{3-}/\text{CO}_3^{2-}$, and also the partial substitution of Ca^{2+} by Mg^{2+} (Krupa et al., 2003; Oliveira et al., 2003b). These ratios are supported by the results of FTIR (section 5.5.1) and XPS (section 5.5.2) analyses of 96 hours immersed Ti6Al4V, which show that calcium phosphate deposited is calcium-deficient with HPO_4^{2-} and CO_3^{2-} substituting PO_4^{2-} .

The variation in the Ca:P ratios, between sample groups, could be due to different surface treatments received by the samples. As discussed in sub-section 5.5.1, variations in titanium oxide formed by different surface treatments influence its interaction with SBF (Chang & Lee, 2002; Jones, 2001; Connor et al., 1999). This in turn affects surface pH and thus chemical composition of apatite deposited (Chou et al., 2004; Li et al., 1997; Rey et al., 1991).

Results from XPS analysis show that calcium phosphate deposited on Ti6Al4V is calcium-deficient carbonated apatite with calcium-to-phosphorous ratio between that of octacalcium phosphate and hydroxyapatite. This is favourable as it has been shown that, when suspended in an aqueous medium with Ca^{2+} and PO_4^{3-} ions, calcium phosphate compounds with low calcium-to-phosphorous ratio (Ca:P) exhibited a greater affinity for Ca^{2+} than those with higher Ca:P (Pak & Skinner, 1968). This may

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be favourable in terms of bioactiveness as it analogue maturation of poorly crystallised mineral at the mineralisation front, which is essentially due to physiochemical processes (Magne et al., 2001).

5.6 Structural study of the Calcium Phosphate (CaP) Coating

Biomimetically formed apatite is known to be amorphous or poorly crystalline in structure (Kokubo et al., 1999). Lattice defects induced by carbonate and hydrogen phosphate substitutions, together with the inhibitory effect of magnesium ions stabilise the amorphous phases (Barrere et al., 2002b). In *in-vitro* experiments using Tris-buffered solution, an amorphous calciudphosphorous-rich layer deposited initially on surface of 45S5 Bioglass-type glass only transformed to crystalline apatite after 7-10 days immersion (Hench & Wilson, 1984). Takadama et al. (Takadama et al., 2001) also reported that amorphous calcium phosphate was formed on the calcium titanate, before converting to crystalline calcium phosphate SBF.

5.6.1 Effect of surface treatment on structure of the final CaP coating

Peaks from Ti6Al4V substrate dominate the X-ray diffraction patterns with no peaks for apatite, anatase or rutile observed for all Ti6Al4V samples (Figure 39). Since presence of an apatite layer has been confirmed by FTIR and XPS analyses, this indicates that the apatite deposited is either amorphous in nature or defective with small crystal size beyond the sensitivity of the equipment used. This result is consistent with the featureless characteristic bands (carbonate, phosphate, hydrogen phosphate) observed on their FTIR spectra (Figures 30-32), and low Ca:P ratios calculated (Table 16), both indicating that the calcium phosphate deposited is amorphous or defective with small crystal size (Barrere et al., 2002a; Chou et al., 2004; Dorozhkina et al., 2003; Furuzono et al., 2000; Layani et al., 2000; Li et al., 1997; Stoch et al., 2000).

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Results from chemical analyses of calcium phosphate deposited revealed the incorporation of CO_3^{2-} , HPO_4^{2-} (FTIR and XPS) and small quantity of Mg^{2+} (XPS) into the apatite structure (calcium deficient B-type carbonated apatite). Structural analysis shows that, the apatite deposited on all Ti6Al4V samples is amorphous or defective with small crystal-size calcium phosphate.

5.7 Morphological study of the Calcium Phosphate (CaP) Layer

Kokubo et al. immersed glass-ceramic A-W (containing crystalline oxyfluoroapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OF}_2)$) and β -wollastonite ($\text{CaO} \cdot \text{SiO}_2$) in an MgO - CaO - SiO_2 glassy matrix) in SBF at 36.5°C . They reported transformation of amorphous calcium/phosphorous-rich layer into crystalline apatite (Kokubo et al., 1990). Calcium phosphate deposited after 7 days is composed of linked globules $0.1\text{-}0.5\mu\text{m}$, which transformed into scaly particles or plate-like minerals at 30 days immersion.

Similar calcium phosphate globules have been reported in literature (Barrere et al., 2002a; Barrère, 2002; de Sena et al., 2002; Li & Ducheyne, 1998; Oliveira et al., 2003b; Wang et al., 2003a; Wei et al., 2002). The variations in size of the globules are likely due to differences in experimental parameters such as SBF ion concentrations, surface composition of substrates, incubation temperatures etc.

5.7.1 Effect of surface treatment on the morphology of Ti6Al4V

Criss-crossed grooves and ridges observed in all samples are artefacts from sample polishing prior to etching. The 'flat-edged hills' and 'dimples' on the unanodised sample at 0 hour, prior to immersion (Figure 41a) are a result of etching (in HF , HNO_3 and H_2O). The sharp edges of these 'flat-edged hills' are removed during anodisation (oxide formation and dissolution), leaving behind smaller and smoother humps (Figures 40a and 42a). These SEM results, together with the single time constant

obtained in the equivalent circuit for EIS fitting; show that anodic oxide layer on all samples is a non-porous, compact and single TiO_2 barrier layer.

5.7.2 Influence of variation in Ti oxidation produced by different surface treatment on the subsequent CaP deposit

Clusters or globules approximately 0.1–0.5 μm diameter are observed on surface of the anodised Ti6Al4V samples (Figure 41bii and Figure 42b) at 24 hours immersion. EDX identified these clusters as calcium/phosphorous-rich phase. However, the intensity is very low due to the strong background titanium metal signal. These globules and clusters, when observed under high magnifications (Figure 41aii), appear to be composed of smaller nuclei. These globules and nuclei scatter over the substrate's surface at localised areas, and appear to adhere well to the substrate's surface. This observation suggests heterogeneous nucleation and growth of calcium phosphates (Barrere et al., 2004; Cuisinier, 1996). This could be stimulated by electrostatic interactions between the hydrated titanium oxide with the SBF solution (Frauchiger et al., 1999; Jones, 2001; Kokubo et al., 1999) and/or driven by the high ionic concentrations in SBFx3 solution (Barrkre, 2002). The exact mechanism of how calcium and phosphate ions accumulate in localised areas is not known (Cuisinier, 1996). The lack of sign of obvious calcium phosphate nucleation on unanodised sample, UA24 (Figure 24b) indicates a less favourable nucleation condition. As discussed in sub-section 5.5.1, this is probably due to difference in structure between air-formed and anodic titanium oxide, therefore influencing the degree of hydrolysis and number of acidic/basic Ti-OH formed when the substrate is immersed in SBF (Jones, 2001). Conditions of hydrolysed air-formed titanium oxide is likely to be less favourable, than anodic ones, as the rate of calcium phosphate deposition is strongly

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influenced by number of surface functional group available (Le. Ti-OH, nucleation sites) (Liu et al., 2002b) and pH (Chou et al., 2004).

5.7.3 Influence of the presence of magnesium on CaP deposit

Barrere et al. (Barrere et al., 2002b) and Oliveira et al. (Oliveira et al., 2003b) studied the influence of Mg^{2+} ions in SBF on calcium phosphate coating formation. They revealed that the inhibitory effect of Mg^{2+} delays Ca-P precipitation, changing the structure of the precipitate into amorphous or poorly crystallised apatite, producing Ca-P coating composed of evenly distributed small calcium phosphate globules (Barrere et al., 1999). Evidence of this effect is obtained from high magnification SEM pictures of the fine calcium phosphate globules on the surface of Ti6Al4V and passivated NiTi (Figures 41 and 42). The globules observed, 0.1 to 0.5 μm in diameter, are much smaller than those reported by Barrere et al. (Barrere et al., 2002a) and Stoch et al. (Stoch et al., 2000) (1- μm). This is due to differences in the SBF solution's concentration and immersion time (Barrere, 2002). However, the size of these globules matches well with those reported during a nano-scale study of calcium phosphate nucleation and growth on Ti6Al4V plates (Barrere, 2002).

5.7.4 Formation of the CaP coating

At 96 hours immersion, all samples are covered with a thin but apparently dense film (Figure 41ci-cii and Figure 42c). EDX analysis shows that this film is calcium/phosphorous-rich. The film is composed of linked globules, a phenomenon that matches the model proposed by Cuisinier (Cuisinier, 1996) where islands of amorphous calcium phosphate globules grow to form a dense layer.

The globules and nuclei observed at 24 hours appear to have progressively increased in number, but with no apparent increment in size, and linked together

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forming a dense coating. The high number of nucleation sites controls their size, and smaller globules are observed with increased nucleation sites due to impingement effect (Cuisinier, 1996). This indicates the possibility of evolution of new multiple nucleation sites between 24 and 96 hours. It is apparent from high magnification pictures that, in all cases, new globules of similar size form on top of the underlying globules. Cracks observed on calcium phosphate coatings are likely a result of dehydration of the water absorbed in the layer.

Overall, a thin, dense layer of calcium/phosphorous-rich phase film is deposited onto Ti6Al4V samples after 96 hours immersion in SBFx3 solutions with no observable delamination. Calcium phosphate film deposited is composed of two entities: 1) Linked globules with diameter approximately 0.1-0.5mm and 2) Globules that are formed by clustering of smaller nuclei. The size of the nuclei is observed to be approximately tens of nanometre by Barrere et al. (Barrere, 2002).

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6.1 Anodisation of NiTi

6.1.1 Selection of electrolyte for anodisation of NiTi

Oxygen containing anions such as SO_4^{2-} , NO_3^- , CO_3^{2-} and OH^- are known as oxide supplying anions. Nickel and their alloys were reported to form mainly barrier-type films when exposed to fused nitrates (Tzvetkoff & Gencheva, 2003). It was found that the kinetics of oxide film formation and the extent of passivation in nitrate media bear close resemblance to those in aqueous media. In alkaline melt however, formation of soluble species e.g. NiO_2^{2-} is possible, accelerating metal corrosion instead of passivation. These findings indicate that nickel and their alloys are likely to form stable oxide film in acid media (especially those with nitrate content) than in alkaline ones.

The results from the preliminary experiment, being consistent with the above literature, revealed that NiTi exhibits best corrosion resistance (R_p , E_{pitt}) in 0.1 M HNO_3 , compared to in 0.1M H_2SO_4 and 0.5M NaOH (Figure 15).

6.1.2 Effect of alloying element (Ni) on the breakdown potential

The workable anodising range was estimated to be 0.2 to a maximum of 1.5V (Figure 15). Presence of alloying elements has been reported to greatly affect growth rate and resistance of oxide film formed on titanium alloys (Ibris & Rosca, 2002). As surface Ni is removed during etching process, the oxide layer is mainly TiO_2 formed by diffusion of titanium ions. Such diffusion may be retarded by presence of Ni, henceforth lowering their anodic oxide forming ability. Titanium is a valve metal whose surfaces are usually covered by a thin barrier-type oxide film. This film formed spontaneously in air and electrolyte, where the thickness grows irreversibly by field-

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assisted migration of ions through the film when anodically polarised (Blackwood et al., 1988; Oliveira et al., 2000). Nickel is a noble metal highly resistant to oxidation and corrosion and a thin, stable, and barrier-type oxide covers its surfaces (Trethewey & Chamberlain, 1995; Tzvetkoff & Gencheva, 2003). These differences between titanium and nickel are also a likely reason for the separate workable E_f range from Ti6Al4V.

6.1.3 Effect of applied voltage on the anodic titanium oxide thickness

XPS analysis of oxide film of NiTi samples anodised in sulphuric acid revealed that, like titanium and their alloys, thickness of the resultant oxide film increased linearly with potential (Asami et al., 1990; Asami et al., 1991). Anodised NiTi exhibit passive current comparable to those of titanium until a potential where primary passivity of nickel breaks down and successful evolution of oxygen.

The anodisation of NiTi at $E_f = 1.0$ and $1.5V$ did not produce brilliant coloured coatings as was the case for Ti6Al4V. The anodic oxide formed at these voltages is likely to be similar to air-formed oxide on etched NiTi i.e. 10nm or less (Blackwood & Chooi, 2002), therefore exhibiting no interference colour. A slight tinge of golden colour and gas formation observed on samples anodised with $E_f = 3.0V$ is a result of enhanced oxide thickness with E_f , as confirmed with XPS analysis (see sub-section 5.3.2). The thickness of this oxide layer, when reference to Appendix D, is estimated to be 10nm to 25nm.

6.1.4 Effect of surface treatment and anodising voltage on topography

As received (AR) NiTi samples, despite the mirror-like finish, has high surface roughness and surface area (Figure 18). Reduction in the measured topography for etched (E) NiTi is probably due to removal of pre-existing surface defects and growth of a fresh thin oxide film during sample preparations (polishing followed by etching)

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(Marino et al., 2001). Unlike Ti6Al4V, subsequent surface treatments (anodisation and passivation) evidently resulted in increase surface roughness again.

The workable anodising voltage range for NiTi (0.2-1.5V) is much lower than those for Ti6Al4V (at least 50V). The E_f values used to anodise NiTi (1.0V, 1.5V and 3.0V) are likely to be close to or at steady state, where dissolution of oxide film becomes prominent. With increasing applied E_f , accompanying growth of oxide thickness is an increase in extent of the surface “roughening”. Similar to Ti6Al4V samples anodised at high E_f values, NiTi samples anodised at these voltages have increased measured topography.

6.2 Corrosion Resistance of Ti Oxide-coated NiTi in SBF

The impedance spectra, similar to those from Ti6Al4V, can be fitted well with a Randles cell equivalent circuit containing one time-constant (Figure 10 in sub-section 2.4.2). This suggests that the oxide formed is single, compact and barrier-type layer having no major defects or diffusion paths within the layer (Da Silva et al., 2002; Garcia-Alonso et al., 2003; Krupa et al., 2003). This property is desirable in terms of NiTi’s biocompatibility, as they exhibits enhanced corrosion resistance, minimising metal ions release (Trepanier et al., 1998).

6.2.1 Effectiveness of anodic oxide in enhancing corrosion resistance of NiTi

Stability of Ti oxide-coated NiTi

Trepanier evaluated the E_{pitt} of differently treated NiTi stents in Hank’s solution and concluded that oxide uniformity has significantly greater influence on corrosion resistance over oxide thickness. Samples with the thinnest but uniform oxide layer

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demonstrated enhanced corrosion resistance compared to those thicker but porous ones (Trepanier et al., 1998). A preferential oxidation of titanium is observed during electropolishing and passivation processes, which is favourable for biocompatibility due to its stability and protective nature. Wever et al. compared the corrosion properties relating to surface characteristics of NiTi and AISI 316L LVM in the same solution (Wever et al., 1998). They revealed that NiTi is more corrosion resistance than AISI 316L LVM, a well-established implant material, due to presence of a protective passive oxide film with TiO_2 as main constituent.

The high value for standard deviation within each sample group (Table 13 and Figure 26) is likely a result of inconsistency in sample preparation method, which has a great effect when using surface sensitive techniques like EIS. However, differences between sample groups are pronounced, allowing results to be used for relative comparison between processes, but not for quantifying the material's absolute corrosion resistance.

With exception of NiTi anodised with $E_f=3.0\text{V}$, anodised NiTi plates exhibit good R_p in SBFx1. As observed in Figure 21, R_p of anodised samples increases with the applied E_f , and reaches a maximum at $E_f=1.5\text{V}$ ($R_p=1.4\times 10^{10}\text{M}\Omega/\text{cm}^2$) before declining to a minimum at $E_f=3.0\text{V}$ ($R_p=0.5\text{M}\Omega/\text{cm}^2$). The R_p of passivated (P) NiTi ($R_p=1.0\times 10^8\text{M}\Omega/\text{cm}^2$) is similar to those anodised at $E_f=1.5\text{V}$. The etched (E) and as received (AR) NiTi have much lower R_p values (0.6 and $0.5\text{M}\Omega/\text{cm}^2$ respectively), which are very close to those of unanodised Ti6Al4V sample ($R_p=0.4\text{M}\Omega/\text{cm}^2$).

Pitting resistance Ti oxide-coated NiTi

It is obvious from Figure 26 that anodised, passivated and etched NiTi have similar E_{pitt} (about 1.0V), a three-fold improvement over as received orthopaedic grade

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NiTi ($E_{\text{pitt}}=0.32\text{V}$). Except for as received samples, changes in E_{pitt} NiTi with different surface treatment are not consistent with their respective R_p .

From the enhanced R_p (coating stability) and E_{pitt} (pitting resistance), anodisation has proved to be as effective as passivation process in enhancing NiTi's corrosion resistance in SBFx1.

Nitric acid passivation, heat treatment and aging in boiling water are simple, inexpensive and effective processes to surface-modified NiTi implants of various shapes and geometries (Shabalovskaya et al., 2003; Trepanier et al., 1998). Passivated NiTi has been reported to exhibit enhanced corrosion resistant over heat-treated, mechanical and electropolished samples when exposed to simulated physiological fluids (Cissé et al., 2002; O'Brien et al., 2002; Thierry et al., 2002; Trepanier et al., 1998). Anodised NiTi plates are therefore, expected to exhibit enhanced corrosion properties over conventional heat-treated, electropolished and mechanical polished samples.

6.2.2 Effect of surface roughness and removal of pre-existing surface defect

The benefits of removing deep surface defects (Figure 43ai) and reduced surface roughness (Figure 8), through polishing and etching processes, on NiTi's *in-vitro* corrosion resistance are obvious from the results for etched NiTi samples. Increase in the surface rou; mess and area during subsequent anodisation or passivation process has no detrimental effect on their corrosion resistance ($R_p=2.5\text{-}1.4\text{E}10\text{M}\Omega/\text{cm}^{-2}$ and $E_{\text{pitt}}=1.01\text{-}1.08\text{V}$) in SBF solution. These results not only demonstrate the effectiveness of the protective oxide formed, by anodisation and passivation, but also

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highlighted the adverse effect of pre-existing manufacturing surface defects on *in-vitro* corrosion resistance of NiTi.

6.2.3 Repassivation capability of anodised NiTi

Similar to those for Ti6Al4V, cyclic polarisation plots (Figure 24) of as received NiTi have relatively large hysteresis loops and high current densities at potentials above E_{pitt} . Corrosion current density remains high (approaching 10^{-2} Amp/cm²) until a sufficiently low potential has been reached in the reverse scan. This again indicates poor resistance to pitting, explaining the large pits observed on tested as received NiTi samples (Figure 25). The comparatively inferior corrosion resistance of as received NiTi ($R_p=0.5 \text{ M}\Omega/\text{cm}^2$ and $E_{\text{pitt}}=0.32\text{V}$) is likely due to the preexisting surface defects in the oxide layer (Kuphasuk et al., 2001; Trepanier et al., 1998). This is confirmed by observation of deep surface defects on as-received NiTi's surface under SEM (Figure 44a_{ii}), together with the high surface roughness and surface area measured (Figure 18).

Anodised, etched and passivated NiTi have polarisation curves with much smaller/narrower hysteresis loops. The corrosion current density reduces quickly during reverse scans with values much closer to those observed during the forward scans when compared to as received NiTi and anodised Ti6Al4V. At low potential (<1.0V), treated NiTi plates have good pitting corrosion resistance, as pits initiated are quickly repassivated, eliminating local active sites. These results are favourable in terms of biocompatibility, as the determined E_{pitt} is well above the measured body potential (0.2-0.6V) (Hoar & Mears, 1966; Velten et al., 2002). Within this potential range, treated NiTi is expected to exhibit good passivity with current density <10⁻⁶ Amp/cm²; and rupture in the titanium oxide layer, due to mechanical abrasion or pitting, is expected to be readily repaired. The measured E_{pitt} (average of 1.0V) values are also well above the

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potential region where where oxygen reduction reaction is feasible (Aziz-Kerrzo et al., 2001). Pitting corrosion events initiated at these high potentials is non-spontaneous, since the accompanying reduction reaction is not favourable.

Overall, it has been shown that anodisation can be employed as an effective and simple alternative process to enhance NiTi's corrosion resistance in SBF. Anodised NiTi samples exhibit three-fold improvement in corrosion resistance over as received orthopaedic grade samples. Enhancement in corrosion properties is a result of removal of macro surface defects and growth of a compact, single-layered, anodic titanium oxide film.

6.3 Chemical Characterisation of the Ti Oxide Layer

6.3.1 XPS analysis

Photoelectrochemical and ESCA/XPS analyses show that oxide thickness of NiTi, like titanium, increased linearly, up to few nanometres with potential when anodised in sulphuric increased (Asami et al., 1990; Asami et al., 1993). The anodic oxide was found to be titanium-rich, and a layer distribution model of $\text{Ti}^{4+}/\text{Ti}^{3+}/\text{Ti}^{2+}$ underneath the surface TiO_2 , similar to those for titanium and their alloys, has been proposed. Detection of titanium in lower oxidation states has also been reported for mechanical, electropolished (Armitage & Grant, 2003), heat-treated (Firstov et al., 2002) and oxygen plasma surface modified (Tan & Crone, 2002) NiTi samples.

Surface oxide composition

From the survey scans (Figures 29ai-fi) of NiTi samples prior to ion etching, the main XPS peaks presences are O_{1s} , C_{1s} and Ti_{2p} . This indicates that the main component of the oxide layer is titanium oxide. High-resolution Ti spectra, before

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argon etching (Figures 29a_{ii}-f_{ii}), show that the main $Ti_{2p3/2}$ peak of the p level spin doublet of the NiTi samples (459eV), as in the case for Ti6Al4V, corresponds to TiO_2 . This is desirable as treated NiTi samples exhibiting improved corrosion resistance and biocompatibility are reported to have similar surface chemistry (Filip et al., 2001; Firstov et al., 2002; Ponsonnet et al., 2002).

The main binding energy of the low intensity (51.0 atm.%) Ni peak detected for anodised samples is 856eV (Figures 29a_{iii}-f_{iii}), which corresponds to NiO (Belhomme et al., 2001) or Ni_2O_3 (Moulder et al., 1995; Shabalovskaya et al., 2003). As received and etched NiTi have slightly higher Ni content (1.2 and 1.0 atm.%) and binding energy of main XPS peak at approximately 854eV (assigned to NiO) (Moulder et al., 1995) or metallic Ni (Armitage & Grant, 2003). However due to the low spectra intensity, it is difficult to determine the exact oxidation state of Ni in the top most oxide layer. Determination of the Ni composition in the top oxide layer using the high-resolution oxygen spectra prove to be ineffective, as the binding energies of Ni in various oxidation states (NiO-529.6eV) (Shabalovskaya et al., 2003) overlapped with and shadowed by those of TiO_2 (529.9-530.5eV) (Shabalovskaya et al., 2003; Trepanier et al., 1998). Another possibility is the top oxide layer is so thin, that signals from underlying metallic material has been detected.

Composition of immediate underlying oxide

After 120 seconds of argon ion etching, additional main peaks from Ti_{2s} , Ni_{2p} , and Ni_{3s} were observed. This suggests that the top TiO_2 layer of the titanium oxide is very thin.

Except for NiTi anodised at 3.0V, the main $Ti_{2p3/2}$ peak of the p levels spin doublet of all samples shifted to 454eV (Figure 29i_{ii}), which is assigned to metallic titanium. Shoulders of higher binding energy appear on the shifted Ti peaks, and the

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whole spectra spread approximately from approximately 454.4eV to 464.8eV. Results show that the titanium oxide layer on these samples is very thin, assuming a distribution of $Ti^{4+}/Ti^{3+}/Ti^{2+}$ between the surface TiO_2 and the metallic substrate (Asami et al., 1990). For the 3.0V NiTi (Figure 29fii), the main $Ti_{2p3/2}$ peak remains at around 459eV with shoulders observed at lower binding energies. This indicates that only TiO_2 and some amount of titanium sub-oxides are present underneath the top TiO_2 layer. Together with the lack of metallic Ti peaks, results indicates enhancement of the titanium oxide layer thickness with increasing E_f .

The main Ni photoelectron peaks of all samples shifted to lower binding energy of 853eV, and their shake-up line patterns match well with those of metallic Ni (Armitage & Grant, 2003; Moulder et al., 1995).

Influence of applied voltage on thickness of the oxide layer (NiTi)

Figures 29aiii-fiii show the high-resolution spectra of Ni before and after etching. XPS experiment yields peak area relative to quantity of detected elements (Moulder et al., 1995; Skoog et al., 1997). The relatively lower metallic Ni intensity of the 3.0V anodised sample (Figure 29fiii), and the lack of metallic peak in the respective Ti high-resolution spectra (Figure 29fii), indicate presence of a titanium oxide layer thicker than those produced by lower E_f or different treatments. This increase in thickness of the oxide layer did not result in significant improvement in their *in-vitro* corrosion resistance, due to the corresponding increase in their surface roughness and area (sub-section 6.2.2).

Incorporation of Ni in the oxide layer

Kuphasuk et al. tested NiTi's corrosion resistance in Ringer's solution and demonstrated the importance of preferential removal of surface nickel and prevention

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of it from being incorporated into the surface oxide (Kuphasuk et al., 2001). They identified a mix of TiO_2 and $\text{Ti}_4\text{Ni}_2\text{O}$ in oxide film during TEM studies of failed NiTi samples, indicating nickel was incorporated into the surface oxide film. They recommended the use of chemical treatment, to selectively dissolve only nickel from NiTi surface; or administering an oxidation-passivation treatment to the alloy under controlled oxygen partial pressure before use, to prevent incorporation of Ni into the surface oxide.

From Table 15, it is obvious that the Ni content of all treated samples is less than 1.0 atomic percent. These values are much lower than those reported for differently treated NiTi, indicating the effectiveness of the surface treatment processes in reducing surface nickel (Firstov et al., 2002; Shabalovskaya et al., 2003).

All treated samples have titanium-to-nickel ratio (Ti:Ni) (7.2-12.5), except for etched NiTi (6.5), greater than those of as received NiTi (6.9). These values are well above 1.0 expected of the equiatomic NiTi, again demonstrating the effectiveness of the introduced processes in enhancing titanium oxidation and nickel removal. The anodisation and nitric acid passivation processes have shown to reduce, but not eliminate the incorporation of Ni into the titanium oxide layer.

Overall, the oxide formed on NiTi samples is found to be titanium-rich, with nickel presence in oxidised state and in less than 1.0 atomic percent. Nickel incorporation in the titanium oxide layer can be reduced, but not eliminated by anodisation nor passivation process. Thickness of titanium oxide on NiTi can be enhanced by anodisation, and increases with increasing voltage. Except for 3.0V anodised NiTi samples, a mixture of titanium sub-oxides and metallic Ni exist immediate underneath the top TiO_2 layer. Results from XPS analyses of titanium

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spectra show that titanium oxide of NiTi, like those of Ti6Al4V samples, assumes a layer distribution of $Ti^{4+}/Ti^{3+}/Ti^{2+}$ underneath the surface TiO_2 (Asami et al., 1993).

The high initial intensity of C_{1s} peaks again originated from hydrocarbons of atmospheric contamination, which is hardly visible after the etching process. Oxygen, as in the case of Ti6Al4V (sub-section 5.3.1), exists mainly as metallic oxide (Ti-O), metallic hydroxide (Ti-OH) and water (adsorbed moisture).

6.4 Structural Study of Ti Oxide Layer

As discussed in sub-section 5.6, the lack of characteristic rutile or anatase peaks in the TF-XRD spectra of NiTi indicates that the surface TiO_2 layer is likely very thin, amorphous or poorly crystalline anodic film with ultra-fine crystallites beyond the sensitivity of the equipment used.

Presence of a non-continuous oxide network formed at the titanium/ TiO_2 interfaces could also be a cause for such lack of titanium oxide peak. Non-stoichiometric compounds with continuously varying composition do not give rise to X-ray diffraction peaks since they behave more like a molecular layer rather than a crystalline structure (Pouilleau et al., 1997). XPS analysis of the oxide layer on NiTi (sub-section 6.3.1) is analogous with the layer model, revealing presence of titanium sub-oxides and metal (Ti^0 , Ti^{2+} , Ti^{3+} and Ti^{4+}) underneath the top TiO_2 layer.

6.5 Chemical Characterisation of the Calcium Phosphate (CaP) Layer

NiTi samples retrieved at 24 and 96 hours immersion in SBFx3 solution were studied in detail. Samples at 24 hours immersion and passivated samples at 96 hours exhibit a multicoloured interference fringe. This indicates the presence of a thin

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(reported to be <1-3mm thick) uniform layer of CaP that diffracts natural light to give the samples a 'rainbow-like appearance (Barrere et al., 2002a; Liu et al., 2003b; Stigter et al., 2002). As received and anodised NiTi samples were found to have a whitish coating, a phenomena observed on samples with thicker CaP coatings (Barrere et al., 1999).

6.5.1 FTIR analysis

Calcium phosphate deposited on NiTi samples in, SBFx3 at 39.5°C, exhibits FTIR spectra analogous with those reported for bone-like apatite deposited in SBF (Barrere et al., 2002a; Chou et al., 2004; Dorozhkina et al., 2003; Furuzono et al., 2000; Layani et al., 2000; Li et al., 1997; Stoch et al., 2000). The featureless nature of the characteristic bands (carbonate, phosphate, hydrogen phosphate) reveals that calcium phosphate deposited is amorphous or defective with small crystal size.

Changes in composition of the CaP layer with immersion time

All NiTi samples at 24 hours immersion exhibit similar FTIR spectra. The broad single band around 1120 cm^{-1} is attributed to the ν_3 P-OH vibration mode in HPO_4^{2-} (Barrère, 2002). At 96 hours immersion time, the centre of this band shifts to around 1020-1040 cm^{-1} corresponding to the ν_3 vibration mode for PO_4^{3-} . A significant increase in intensity of the initially small $\text{HPO}_4^{2-}/\text{CO}_3^{2-}$ band ($\sim 860 \text{ cm}^{-1}$) and bands attributed to C-O stretching (Layani et al., 2000; Oliveira et al., 2003b) or the ν_3 mode of CO_3^{2-} (Barrere et al., 2002a; Stoch et al., 2000) (1420 cm^{-1} and 1470 cm^{-1}) is also observed for final calcium phosphate coatings. FTIR bands corresponding to PO_4^{3-} and CO_3^{2-} are much sharper for anodised and as received NiTi, than those of etched or passivated samples. Water is again evident from the broad single bands at 3000-3400

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and 1650 cm^{-1} (Barrere et al., 2002a; Oliveira et al., 2003b). Band corresponding to O-H bond of HA (around 3600 cm^{-1}) (Osaka et al., 1991) is still not observed.

Effect of increase in the immersion temperature (1°C) on CaP nucleation rate

NiTi samples at 24 hours immersion exhibit much higher spectra intensity over those of Ti6Al4V (Figures 33-36 verses Figures 30-32), indicating that a shorter immersion time is required to deposit calcium phosphate onto NiTi samples. Lin et al. studied the effect of temperature of SBF on apatite deposited on 316L stainless steel (Lin et al., 2002). They showed that increasing the immersion temperature (to 80°C) enhances deposition of apatite. Faster nucleation and growth rate is, therefore due to increase in incubation temperature for NiTi samples (39.5°C verses 38.5°C for the Ti6Al4V samples).

Influence of substrate's chemistry on composition of CaP nucleated

The strong HPO_4^{2-} band at 1120 cm^{-1} observed for NiTi samples at 24 hours immersion (Figures 33-36) shows that calcium phosphate layer is calcium deficient, with high mainly HPO_4^{2-} substitution for PO_4^{3-} . Deviation in calcium phosphate composition, at 24 hours immersion, between anodised NiTi and Ti6Al4V could be due to differences in the anodic titanium oxide. Presence of alloying elements plays a role in determining the interactions that ensue between surface oxide and aqueous environment upon exposure to SBF solution (Hodgson et al., 2002). The high nickel content in NiTi is likely to influence the hydration and pH of this anodic titanium oxide. This may cause stabilisation of HPO_4^{2-} but not CO_3^{2-} , as in the case of anodised Ti6Al4V, at the titanium oxide/SBF solution interfaces. As discussed in sub-section 5.5.1, changes in substrate's surface after nucleation of first calcium-deficient apatite lead to subsequent deposition of apatite with increasing PO_4^{2-} and CO_3^{2-} content. The

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FTIR spectra of the final CaP coating are characteristic of a calcium-deficient B-type carbonated apatite.

Effect of surface treatments on crystallinity final CaP layer

Amorphous calcium phosphate deposited on in SBF is known to transform into crystalline apatite with increasing immersion time (up to 10 days for 45S5 Bioglass and 5 days for NaOH-treated Ti6Al4V) (Hench & Wilson, 1984; Takadama et al., 2001). Sharper PO_4^{3-} and CO_3^{2-} bands observed, for 96 hours immersed anodised (Figure 35) and as received (Figure 33) NiTi samples, indicate apatite with a higher degree of crystallinity. As discussed for Ti6Al4V (see sub-section 5.5.1), differences in structure, roughness and number of surface defects of surface titanium oxide are induced by various surface treatments (Chang & Lee, 2002; Connor et al., 1999; Jones, 2001; Osaka et al., 1991). It has been reported that anodic titanium oxide produced at low voltages is amorphous, or contain only small amount of anatase (Blondeau et al., 1977a; Velten et al., 2002). The slight differences in surface oxide between differently treated NiTi apparently affect their interactions with SBF solution (Hodgson et al., 2002; Li et al., 1997), encouraging fast maturing (crystallisation) of apatite deposited. A band corresponding to an O-H bond of in HA (around 3600 cm^{-1}) is not observed.

Overall, FTIR spectra of calcium phosphate deposited onto NiTi samples are characteristic of calcium-deficient B-type carbonated-apatite. However, sharper characteristic bands of PO_4^{3-} , HPO_4^{2-} and CO_3^{2-} , for anodised and as received NiTi samples, show that the final apatite coating has higher crystallinity over those of etched and passivated ones.

6.5.2 XPS analysis

Survey spectra of calcium phosphate deposited on NiTi (Figure 38) in SBFx3 are similar to those collected for TiAl4V (Figure 37). The spectra match well with those reported in literature, having main peaks attributed to different chemical states of Ca, P, O and C (Chung et al., 2003; de Sena et al., 2002; Furuzono et al., 2000; Gu et al., 2003; Gu et al., 2004; Krupa et al., 2003; Takadama et al., 2001). The lack of detectable Ti and Ni peaks indicates the continuous nature of the apatite layer deposited (Chung et al., 2003).

Ca spectra of apatite coated NiTi revealed the Ca_{2p} p-level spin doublet with binding energies at approximately 347.4 eV ($\text{Ca}_{2p_{1/2}}$) and 351.1 eV ($\text{Ca}_{2p_{3/2}}$), which agree well with those reported for hydroxyapatite (Chung et al., 2003; de Sena et al., 2002; Takadama et al., 2001). These peaks do not match the binding energy of CaCO_3 (346.7-346.9 eV) (Chung et al., 2003; Moulder et al., 1995).

Partial substitution of calcium by magnesium

As for Ti6Al4V (sub-section 5.5.2), magnesium was detected at very low intensity with binding energy around 50.5 eV (Figures 38av-dv), and likely to exist in the apatite structure by partially substituting divalent Ca^{2+} (Aoki, 1994).

Partial substitution of phosphate by carbonate and hydrogen phosphate

The high intensity P-XPS peak can again be deconvoluted into two components with binding energies of 133.1 eV and 134.2 eV. The peak at lower binding energy is assigned to the $[\text{PO}_4]^{3-}$ bond in HA (Chung et al., 2003; Takadama et al., 2001). A shoulder at higher binding energy (134.2 eV) suggests presence of hydrogen phosphate groups $[\text{HPO}_4]^{2-}$ (Schreckenbach & Marx, 1999). This is consistent with the observed

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high intensities of HPO_4^{2-} peaks in FTIR spectra of apatite coatings deposited (Figures 33 to 36) on CaP coated NiTi samples.

Carbon exists mainly as hydrocarbons from atmospheric contaminations as previously discussed in sub-section 5.4 (Skoog et al., 1997). An extra, but distinctive, C peak is again observed at higher binding energy around 288.7eV. This peak is ascribed to metallic carbonate (MCO_3) groups (Chung et al., 2003; de Sena et al., 2002) as the binding energy does not correspond to carbide (281-283eV) (Moulder et al., 1995) and the absence of CaCO_3 is confirmed from analysis of Ca spectra. Together with the results from FTIR analysis, the apatite produced is likely calcium-deficient B-type carbonated apatite with substitution of phosphate by carbonate group (Aoki, 1994; Barrere et al., 2002a; Chung et al., 2003; de Sena et al., 2002).

Binding energy of NiTi and Ti6Al4V samples' main O_{1s} peak shifts from around 529.8eV (Ti-O) to 531eV, which is assigned to the P-O bonds of HA (Chung et al., 2003; de Sena et al., 2002; Takadama et al., 2001). Water is again detected, which is normal for an immersion process (Chung et al., 2003).

Effect of surface treatments on the degree of calcium-deficiency in the CaP layer

The calculated calcium-to-phosphorous ratios (Ca:P) for the NiTi samples (1.32-1.60) are higher than those for the Ti6Al4V samples (1.14 to 1.28) (Table 16).

Etched or passivated NiTi (1.32 and 1.33 respectively), similar with those calculated for Ti6Al4V samples (1.14-1.28), have lower Ca:P ratios than those of stoichiometric hydroxyapatite (1.67), amorphous calcium phosphate (1.50), but closer to those of octacalcium phosphate (1.33). As discussed in sub-section 5.5.2, the presences of HPO_4^{2-} and CO_3^{2-} peaks in their FTIR (Figures 34 and 36) and XPS spectra (Figures 38b and 38d) indicate $\text{HPO}_4^{3-}/\text{CO}_3^{2-}$ substitutions for PO_4^{3-} . The resultant lacuna created in the apatite lattice, together with the partial substitution of Ca^{2+} by

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Mg^{2+} , therefore produce a calcium-deficient apatite system (Krupa et al., 2003; Oliveira et al., 2003b).

Increase in Ca:P ratio of SBF deposited apatite approaching that of hydroxyapatite (1.67) (Frauchiger et al., 1999), and subsequent crystallisation of amorphous calcium phosphate (Hench & Wilson, 1984; Takadama et al., 2001) have been reported to occur with increasing immersion time. These observation analogue with results from FTIR investigation on maturation of nonstoichiometric apatites in mineralised tissue (horse dentin) (Magne et al., 2001). It was reported that maturation (with time) of the poorly crystallised young mineral close to mineralisation front resulted in gain of crystallinity, decrease in HPO_4^{2-} and increase in CO_3^{2-} .

The higher Ca:P ratio of the anodised and as received NiTi (1.55 and 1.60) are expected from their respective XPS and FTIR spectra, which revealed a lower degree of Mg^{2+} (for Ca^{2+}) and $\text{HPO}_4^{2-}/\text{CO}_3^{2-}$ (for PO_4^{3-}) substitutions. The sharp FTIR bands collected from these two samples also revealed that apatite deposited has higher degree of crystallinity. As discussed in sub-section 6.5.1, higher immersion temperature of NiTi samples enhances calcium phosphate deposition (Lin et al., 2002). Variations in titanium oxide formed by different surface treatments influence the interaction with SBF (Chang & Lee, 2002; Jones, 2001; Connor et al., 1999), affecting surface pH and thus producing apatite nuclei that is less stable i.e. encouraged crystallisation of amorphous CaP nuclei (Chou et al., 2004; Li et al., 1997; Rey et al., 1991).

Results from FTIR and XPS analyses show that calcium phosphate deposited on NiTi and is B-type carbonated calcium-deficient apatite, with Ca:P ratios between that of octacalcium phosphate and hydroxyapatite. As discussed in section 5.5.2, this is favourable as calcium phosphate compounds with low calcium-to-phosphorous ratio (Ca:P) exhibited a greater affinity for Ca^{2+} than those with higher Ca:P (Pak & Skinner,

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1968) i.e. enhanced bioactivity (Magne et al., 2001). Higher Ca:P ratios of anodised and as received NiTi, at 96 hours immersion, are likely driven by 1) higher immersion temperature and 2) composition and structure of titanium oxide produced induced amorphous apatite that is less stable (crystallised more readily).

6.6 Structural Study of the Calcium Phosphate (CaP) Coating

6.6.1 Effect of surface treatment on structure of the final CaP coating

Peaks from the NiTi substrate dominate the X-ray diffraction patterns with no peaks for apatite, anatase or rutile observed (Figure 40) for passivated or etched samples. As discussed in sub-section 5.6 for Ti6Al4V, since the presence of an apatite layer has been confirmed by FTIR and XPS analysis, the apatite deposited is either amorphous in nature or defective with microcrystallites of size beyond the sensitivity of the equipment used.

Small peaks at around $2\theta = 32^\circ$ and 26° observed for anodised and as-received NiTi correspond to (211) and (002) planes of SBF deposited apatite (denoted by “*” in Figure 40) (Kokubo et al., 1990). XRD peaks from NiTi substrate are not as distinct, or strong, for these samples, indicating an increase in thickness of the apatite coating. Broadness of these low intensity XRD peaks shows that the apatite deposited has small crystal size, and is defective or strained or a combination of all (Klug & Alexander, 1974).

It is interesting to note that the peak intensity ratio of apatite (002) crystal plane to (211) crystal plane, for anodised (1.5V96) and as received (AR96) samples, is about one. This is different from the standard hydroxyapatite powder XRD pattern (0.4) (JCPDS-ICDD, 1994). Similar phenomena have been reported for SBF deposited apatite (Barrere et al., 1999; Chen et al., 2003; Gil et al., 2002; Yang et al., 2004).

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Results indicate that apatite deposited on NiTi crystallised with a preferred orientation of growth in the (002) plane.

Detection of apatite peaks on as-received, anodised samples is expected from their sharp FTIR bands (sub-section 6.5.1) and high Ca:P ratios (sub-section 6.5.2). Increase in Ca:P ratio of SBF deposited apatite approaching that of hydroxyapatite (1.67) (Frauchiger et al., 1999), and subsequent crystallisation of amorphous calcium phosphate (Hench & Wilson, 1984; Takadama et al., 2001) have been reported to occur with increasing immersion time.

Results from chemical analyses of calcium phosphate deposited revealed the incorporation of CO_3^{2-} , HPO_4^{2-} (FTIR and XPS) and a small quantity of Mg^{2+} (XPS) into the apatite structure (B-type carbonated calcium deficient apatite). Structural analysis shows that, depending on the immersion temperature and the state of the substrate's surface oxide, apatite deposited can be amorphous or defective with small crystal-size calcium phosphate.

6.7 Morphological Study of the Calcium Phosphate (CaP) Layer

Kokubo et al. immersed glass-ceramic A-W (containing crystalline oxyfluoroapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OF}_2)$) and β -wollastonite ($\text{CaO} \cdot \text{SiO}_2$) in an MgO - CaO - SiO_2 glassy matrix) in SBF at 36.5°C. They reported transformation of an amorphous calciudphosphorous-rich layer into crystalline apatite (Kokubo et al., 1990). Calcium phosphate deposited after 7 days is composed of linked globules 0.1-0.5 μm , which transformed into scaly particles or plate-like minerals at 30 days immersion.

Similar calcium phosphate globules have been observed and reported in related studies (Barrere et al., 2002a; Barrère, 2002; de Sena et al., 2002; Li & Ducheyne, 1998; Oliveira et al., 2003b; Wang et al., 2003a; Wei et al., 2002). Plate-like calcium

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phosphate minerals are also commonly observed on biomimetic deposited apatite (Barrere et al., 1999; Chen et al., 2003; Choi et al., 2003; Chou et al., 2004; Liu et al., 2003b; Li et al., 1997). The variations in size of the globules are likely due to differences in experimental parameters such as SBF ion concentrations, surface composition of substrates, incubation temperatures etc. Composition of globules/precursor spheres, rate of phase transformation from amorphous globules to plate-like minerals, and composition of the final crystallised apatite are also reported to be strongly affected by pH (Chou et al., 2004).

6.7.1 Effect of variation in surface treatment on the morphology of NiTi

The effect of the different surface treatments on the NiTi plates can be observed from Figures 43ai-aii) and 44ai-aii). Under high magnification, evenly distributed small but deep defects found on as received NiTi sample, which has a shiny mirror-like appearance. Manufacturing processes used by the supplier have likely induced these defects. The etched (E) NiTi has criss-crossed grooves and ridges similar to that of unanodised Ti6Al4V, which are again artefacts from sample polishing and etching. These are replaced with smoother grooves and some process-induced (but relatively shallow) surface defects during anodisation in 0.1M HNO₃. Passivation of NiTi in concentrated HNO₃ acid roughens the surface but removes the grooves. Surface oxide on all samples is observed to be non-porous. Together with the single time constant obtained from EIS fitting (sub-section 6.2.1), it is clear that titanium oxide on all NiTi samples is non-porous, compact, single and barrier-type layer.

6.7.2 Effect of immersion temperature on the CaP nucleation rate

At 24 hours immersion, a nucleated layer, formed from linked clusters or globules of calcium phosphate, covered all NiTi samples. This layer follows the

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substrate's topography closely, and the globules are similar, but smaller (0.1-0.2 μm) compared to those of Ti6Al4V samples (0.1-0.5 μm). Apparently, nucleation and growth of calcium phosphate onto NiTi is faster than for Ti6Al4V. This observation is expected from their FTIR analysis, which reveal high intensities bands characteristic of apatite over those for Ti6Al4V. SBF solution is itself a supersaturated system (Kokubo et al., 1999). Increase in immersion temperature, even though by 1°C, could have enhanced calcium phosphate nucleation and growth rate (Lin et al., 2002). The result is formation of a nucleated layer with smaller globules that fully cover surfaces of NiTi samples within 24 hours.

6.7.3 Effect of immersion time on the morphology of the CaP coating

Morphological changes of the calcium phosphate deposited onto NiTi are observed at 96 hours immersion. Morphology of the final calcium phosphate coating varied with the surface treatments applied to the NiTi substrates. The coating deposited onto as received, etched and anodised NiTi is believed to be much thicker than those on passivated NiTi, as topography of the underlying substrates is no longer apparent. Etched and anodised NiTi are continuously covered by many spheroidal particles less than 1 μm in diameter. Higher magnification reveals that these spherulites are composed of flakes or plate-like minerals (Figures 43ci and 44ci). The as received NiTi surface is covered with a layer of calcium phosphate with developing spherulites (Figure 43cii). Only calcium phosphate deposited onto passivated NiTi exhibits similar morphology with those observed for Ti6Al4V samples (Figure 44cii).

6.7.4 Influence of variation in Ti oxidation on crystallisation of amorphous CaP globules

The observed spherulites composed of plate-like minerals are crystallised amorphous calcium phosphate globules/precursor spheres. As discussed in sub-section 5.5.1 for Ti6Al4V, titanium oxide formed on NiTi samples from different surface treatments interact with SBF differently (Jones, 2001; Connor et al., 1999). Depending on the final conditions (e.g. pH, number of nucleation sites etc.) at the oxide/fluid interfaces, deposition and phase transformation (i.e. crystallisation) of apatite may be enhanced or hindered (Chang & Lee, 2002; Chou et al., 2004; Li et al., 1997; Rey et al., 1991). Amorphous calcium phosphate deposited in SBF has been reported to crystallised into plate-like minerals with increasing immersion time (Hench & Wilson, 1984; Takadama et al., 2001). This rate of this crystallisation is found to be dependent on stability of the amorphous globules/precursor spheres nucleated, which in turn is dependent strongly on pH (Chou et al., 2004).

Variations in surface titanium oxide produced by different surface treatments has likely influenced the stability (number of precursor spheres comprised of unstable cluster $[\text{Ca}_3(\text{PO}_4)_2]_n$ arrangement) of amorphous calcium phosphate globules/precursor spheres nucleated (Chou et al., 2004). Globules/precursor spheres nucleated onto titanium oxide, produced by etching, anodising and heat-treatment (as received NiTi), apparently required shorter time to crystallise (i.e. less stable). Calcium phosphate globules/precursor spheres initiated on passivated NiTi are likely to differ slightly in composition. Being more stable, these globules delayed in transforming into crystallised plate-like apatite, explaining the final morphology observed for passivated NiTi samples.

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Overall, a dense layer of calciudphosphorous-rich phase film is deposited onto NiTi with no observable delamination. Only the calcium phosphate film deposited onto passivated sample has similar morphology with those of Ti6Al4V. Other samples have thicker and poorly crystallised calcium phosphate coating composed of two entities: 1) spherulites approximately 1 μ m in diameter and 2) spherulites are formed by flakes or plate like minerals.

Chemical, structural and morphological analyses show that calcium phosphate deposited in SBF is near to biological apatite. Biomimetic deposition of bone-like apatite on anodised NiTi; Ti6Al4V can be an important step towards direct bonding of implants with bone. However, one drawbacks of this method was the samples covered in apatite were susceptible to contamination by bacteria. SBF at around 37°C, a typical immersion temperature, is an ideal medium for bacterial growth unless strict measures are taken to ensure aseptic conditions (autoclave, UV radiation, etc.) (Gil et al., 2002).

Chapter 7: Conclusions

1. A duplex titanium oxide/calcium phosphate coating was successfully applied on plate-form NiTi, Ti6Al4V by first anodising in 0.1M HNO₃, 0.5M H₂SO₄/NaOH respectively to form an initial titanium oxide coating, and subsequent immersion in simulated body fluid with three times the standard concentration.
2. Alloying elements and electrolyte-type strongly influence the range of applicable anodising voltages and stability of the anodic oxide layer. The high nickel content (50 atomic percentage) of NiTi results in lower range of applicable anodising voltages (around 3.0V) compared to those of Ti6Al4V (up to 80V). The anodising potential of NiTi is limited by the potential where primary passivity of nickel breaks down. Even though anodised NiTi samples exhibit much lower pitting potential (1.0V when compared to anodised Ti6Al4V (up to 14V), both materials demonstrated excellent passivity (current density $\leq 10^{-6}$ Amp/cm²) within the measured human body potential (0.2-0.6V). Polarisation tests also indicate that anodised NiTi exhibits good repassivation capability within this potential range.
3. Enhancement in anodic oxide thickness, while maintaining their compactness, improves NiTi and Ti6Al4V's *in-vitro* corrosion resistance in SBF solution. The anodic titanium oxide formed on NiTi in 0.1M HNO₃, is as protective as those formed during nitric acid passivation, and is therefore expected to perform better than thermally oxidised samples with regards to corrosion resistance. Anodic oxide thickness increases with increasing anodising voltage, and layers are thicker than the air-formed oxide of etched samples. XPS analysis shows that beneath an outermost TiO₂ stratum there exists an intermediate sub-oxide (TiO_{2-x}) phase above the metal substrate. Non-

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destructive EIS analysis and SEM micrographs show that this film is compact and barrier-type oxide for both NiTi and Ti6AlV samples.

4. Increase in immersion temperature has shown to enhance the rate of initial deposition and growth rate of calcium phosphate in simulated body fluid. However, variation in properties of titanium oxide, produced by different treatments, influences the morphology of the final apatite during longer periods of immersion. This study shows that rate of crystallisation from globules amorphous calcium phosphate to plate-like crystalline apatite (morphology) can be controlled by modifying the titanium oxide layer.
5. Apatite deposited has structure, chemical composition and calcium-to-phosphorous ratios (Ca:P) closer to those of biological apatite than stoichiometric hydroxyapatite. Calcium phosphate deposited biomimetically is amorphous or defective/poorly crystallised apatite. FTIR and XPS analyses show that this calcium phosphate deposited is a calcium-deficient B-type carbonated apatite system with substitutions of PO_4^{3-} by $\text{CO}_3^{2-}/\text{HPO}_4^{2-}$ and of Ca^{2+} by Mg^{2+} . The lower Ca:P ratios also indicate a higher affinity for Ca^{2+} than stoichiometric HA when immersed in an aqueous medium, which may be favorable in term of bioactiveness as crystal maturation is essentially due to physiochemical processes.
6. Deposition of bone-like apatite on anodised NiTi can be an important step towards direct bonding of implants with bone. However, strict measures have to be taken to ensure aseptic conditions (autoclave, UV radiation, etc.) to prevent susceptibility to bacterial contamination during immersion in SBF at physiological temperature (around 37°C).

Chapter 8: Recommendations for Future Work

1. Higher E_f could be employed during anodisation of NiTi in determining the breakdown potential (where E_{pitt} starts to decline or titanium oxide dissolution begins to dominate). Diluted H_3PO_4 (<0.5M) or calcium containing mixed electrolytes for anodisation of Ti6Al4V and NiTi could be considered to incorporate P or Ca in the anodic titanium oxide formed (Schreckenbach & Marx, 1999; Sul, 2003). It has been reported that incorporation of these ions in the titanium oxide induced stronger bone reactions (higher bone to metal contact) than those without or with other ions e.g. S incorporated from H_2SO_4 (Sul, 2003). Frauchiger et al. revealed that there is a selective interaction of Ca and P ions in the SBF solution with TiO_2 (Frauchiger et al., 1999). The presence of Ca or P incorporated within the titanium oxide layer may enhance the interaction and therefore the nucleation and growth of calcium phosphate when immersed in SBF.
2. The thickness of the anodic oxide formed could be determined either by depth profiling technique using the XPS, SIMs or by non-destructive ellipsometry analysis. However, the accuracy of these techniques depends on the calibration material used, the anodised metal's surface finished and so forth. Additional information regarding the change of chemical composition across the thickness of the titanium oxide layer may provide a better understanding of the differences in their *in-vitro* corrosion resistance and the possible mechanism behind the anodic oxide formation for each alloy. Total removal of metallic content (nickel and titanium) from the implant's surface would be the main objective as metal ions release, in-particularly nickel, are suspected to contribute to loosening of hip and knee prostheses and to negative tissue response.

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3. The thickness of the biomimetically-coated calcium phosphate could be determined by depth profiling technique using the XPS or SIMs. Information on the predominant components at the CaP/TiO₂ interface could be obtained, to aid understanding of the calcium phosphate nucleation mechanism. This in turn may provide some insight into the influence of surface oxide modification (oxide thickness, topography, chemical and structural change) on the nucleation mechanism.
4. EIS monitoring of duplex-coated Ti6AlV and NiTi plates immersed in SBFx1 solutions, during long-term *in-vitro* test, could yield information on the coatings' stability in simulated physiological condition (37°C, pH 7.4). EIS is a surface sensitive technique, which is useful in both studying the polarisation resistance of duplex coated sample immediately after immersion and monitoring changes in the surface with immersion time (Hodgson et al., 2002; Ibris & Rosca, 2002). A new equivalent circuit with two time constants would likely be required to fit spectra obtained from the EIS data, due to the difference in compactness and nature of the TiO₂ and calcium phosphate coatings (Hukovic et al., 2003).

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Appendix A

Appendix A: EIS Nyquist Plot of Anodised and Unanodised Samples

a) Reference (unanodised, 0V)

b) B20 (Alkaline-anodised, 20V)

c) B30 (Alkaline-anodised, 30V)

d) B50 (Alkaline-anodised, 50V)

Appendix A

e) A20 (Acid-anodised, 20V)

f) A30 (Acid-anodised, 30V)

g) A50 (Acid-anodised, 50V)

h) A80 (Acid-anodised, 80V)

Appendix B: WYKO Three-dimensional Plot of Anodised and Unanodised Ti6Al4V Samples.

APPENDIX