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Abstract

Nitinol is an equiatomic binary alloy of nickel and titanium. It has been extensively used since 1970s due to its unique properties of shape memory effect and super-elasticity. Nitinol also has excellent physical and mechanical properties, and together with its good biocompatibility, Nitinol has been widely applied in biomedical areas. Nitinol is generally considered as biocompatible, as it is covered by naturally formed TiO₂ layer, which provides a protection shield to the bulk material. However, Nitinol contains ~50 at.% nickel: an element which is believed to be toxic and could induce allergic response if its concentration in human body exceeds a certain limit. Therefore, surface modifications are usually required applied on Nitinol to improve its biocompatibility for possible biomedical applications. Many different surface modification methods have been applied on Nitinol, and among those, electrochemical methods are known to be effective. This research employed two electrochemical processes, electrochemical etching and anodisation to treat and modify the surface of Nitinol, aiming to improve its biocompatibility and enhance the properties associated with biocompatibility.

Nitinol was electrochemically etched in an aqueous solution made up with 1M H₃PO₄ and 10 wt.% NH₄F. Etching parameters, including applied voltage and etching time were varied to investigate their influence on the resulting Nitinol surfaces. Rough surfaces with round nodules were generated on Nitinol after electrochemical etching. Higher etching voltage resulted in an increase in the size of round nodules generated on Nitinol surface, and longer etching time gave rise to the number of round nodules. The Ti/Ni molar ratio on Nitinol surface was greatly enhanced by electrochemical etching, indicating that the electrochemically etched Nitinol surface was mainly composed of protective TiO₂ layer, with depleted Ni content. Compared with the ground Nitinol surface, the electrochemically etched Nitinol surfaces exhibited improved wettability with simulated body fluid (SBF) and higher corrosion resistance in sodium chloride solution, suggesting enhanced biocompatibility.

Nitinol surface was anodised with a viscous ethylene glycol based electrolyte. The electrolyte compositions (water content and fluorine ion concentration) and anodising parameters (voltage and duration time) were investigated to assess their influence on Nitinol surface modification. Anodisation generally produced rough and porous Nitinol surfaces and enhanced the Ti/Ni ratio on Nitinol surface. Water content and fluorine ion concentration
exhibited profound effect on Nitinol surface morphology, with the former having greater influence than the latter. An increase in both water content and fluorine ion concentration enhanced the surface roughness. Water content and fluorine ion concentration also had impact on the Ti/Ni ratio on Nitinol surface, and the Nitinol anodised in the electrolyte containing 0.1 M fluorine ion and 2 vol.% water showed the highest Ti/Ni ratio. The roughness of Nitinol surface could also be enhanced by increasing anodising voltage and time, and a thicker oxide layer was obtained with prolonged anodising time. Even though the oxide layer was porous, it had little influence on the tensile properties of the bulk Nitinol. Anodised Nitinol surfaces were much more hydrophilic than the mechanically ground Nitinol surface and exhibited improved bacterial inhibition of *E. coli* growth in both water and SBF based media.

Anodised Nitinol surfaces were further evaluated for their biocompatibility, including Ni ion release into SBF, hydroxyapatite (HA) formation ability and direct cell-material interaction with L929 cell line. Ni ion release to SBF was governed by the surface morphology and oxide layer thickness, and more Ni ion released from a rougher and more porous Nitinol surface. Nevertheless, even the highest amount of Ni ion released from the anodised Nitinol surface was still below the tolerated concentration level. Anodised Nitinol surface exhibited much better HA formation ability than the ground Nitinol surface. Hydroxyapatite formed on the anodised Nitinol surface after immersion in SBF for three weeks, and more HA formed with longer immersion time. Anodised Nitinol possessed more suitable surface morphology for L929 mammalian cells to attach and proliferate. Anodisation was proved to be an effective way to improve the biocompatibility of Nitinol.

Electrochemical methods, electrochemical etching and anodisation were demonstrated to be effective approaches to enhance the biocompatibility of Nitinol surfaces. This thesis was therefore not only able to shed the light on optimal conditions to obtain modified Nitinol surface with improved biocompatibility, but also expanded the application of Nitinol in biomedical areas.
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Chapter 1  Introduction

1.1 Background

Nitinol is a near equiatomic binary alloy of nickel and titanium and attracts much attention due to its unique properties of shape memory effect and super-elasticity [1]. It was discovered in 1960s as part of a naval research project and was then commercially introduced in 1970s. Nitinol is also highly corrosion resistant and therefore generally considered to be biocompatible, as it is covered by naturally formed TiO$_2$ layer. Thanks to the desirable properties, Nitinol becomes a promising candidate for biomedical applications [2].

However, the high nickel content (~ 50 at.%) has hindered extensive applications of Nitinol in biomedical areas. Excessive surface corrosion and wear of Nitinol raise safety concerns, as nickel ions released to surrounding physiological environment can induce toxic and allergic responses, if its concentration exceeds a certain limit [3]. Even Nitinol is covered by native TiO$_2$ layer on the surface, this oxide layer is too thin (< 10 nm) to provide sufficient corrosion barrier [4]. Therefore, surface modification of Nitinol is needed when applied in biomedical areas.

Many surface modification methods have been explored, such as mechanical modifications and energy sources modifications, aiming to improve the biocompatibility of Nitinol. Among those, surface treatment methods that do not involve high temperature are generally favoured, as exposure of Nitinol to high temperature may alter the prescribed built-in properties [5]. Electrochemical processes, including electrochemical etching and anodisation, are known to be efficient for elimination of toxic nickel from the Nitinol surface and enhancing surface oxidation of protective TiO$_2$ layer. By adjusting the parameters and conditions of electrochemical processes, many different surface texture and surface chemistry could be achieved. Therefore, the process of electrochemical etching and anodisation on Nitinol will be studied in this research and the mechanism of the two electrochemical processes will be investigated.
1.2 Objectives

The objective of this research project is to modify the surface of Nitinol by electrochemical processes for potential biomedical applications. The surface modification of Nitinol is aiming at producing a rougher surface, eliminating Ni from the surface layer, suppressing Ni release from the surface in simulated body fluid (SBF) and improving its biocompatibility. Based on these modification results, electrochemical processing parameters and conditions will be altered to produce rough or even porous surface layer for better cell attachment after implantation. The approaches will be described in details as follows.

**Surface modification of Nitinol by electrochemical etching**

- To modify the surface of Nitinol by electrochemical etching in an electrolyte containing H$_3$PO$_4$ and fluorine ions.
- To investigate how etching parameters, including voltage and time, would affect the morphology and compositions of resultant Nitinol surfaces.
- To study the electrochemical etching process and its mechanism.

**Surface modification of Nitinol by anodisation**

- To modify the surface of Nitinol by anodisation in an ethylene glycol based electrolyte.
- To investigate the influence of electrolyte compositions on the resultant Nitinol surfaces.
- To investigate the influence of the anodising parameters (voltage and time) on the resultant Nitinol surfaces.
- To understand how the anodised Nitinol surface generated.

**Evaluation of the biocompatibility of anodised Nitinol**

- To analyse nickel ion released from anodised Nitinol surface over 28 days.
- To investigate the hydroxyapatite formation ability of the anodised Nitinol
- To evaluate the cell-material interaction on the anodised Nitinol.
1.3 Thesis Outline

This thesis is divided into eight chapters and part of the presented results have been published by the author of this thesis [6-8]. Chapter 1 generally introduces the entire project. Chapter 2 gives an introduction of Nitinol as a biomaterial, and its unique properties, and also reviews the related work on surface modification of Nitinol, especially on the electrochemical etching and anodisation techniques. Chapter 3 describes the experimental methods, processes and characterizations. Chapter 4 presents the surface pretreatments and the electrochemical etching process of Nitinol and investigates the influence of etching parameters on the resultant Nitinol surfaces. Chapter 5 studies the anodisation process of Nitinol in an ethylene glycol base electrolyte and investigates the effect of fluorine ion concentration and water content on the anodised Nitinol surfaces. Chapter 6 investigates how the anodisation parameters, including voltage and duration time, affect the modified Nitinol surfaces. Chapter 7 studies the biocompatibility of anodised Nitinol surfaces, including Ni ion release in physiological environment, hydroxyapatite formation ability and cell viability. Chapter 8 concludes the main findings of the research project and proposes future research directions.
Chapter 2   Literature Review

2.1 Introduction of Biomaterials

Biomaterials are category of materials that are applied in biomedical areas and are used associating with tissue engineering. Biomaterials mean differently at different times. From last century, biomaterials are defined as man-made materials that are used to direct, supplement, or replace the functions of living tissues of the human body [9]. Over the last century, surgical techniques and sterilization methods have been greatly developed and there are many new materials being synthesized. Nowadays, biomaterials are more than just implant materials and there is a large number of medical devices being utilized, such as probe and medicine slow-releasing system. Biomaterials, in both forms of implants and medical devices, are widely used to replace and/or restore the function of traumatized or degenerated tissues or organs, to assist in healing, to improve function, to correct abnormalities. With the help of biomaterials, the quality of life of the patients could be largely improved [10].

Biomaterial science is a newly developed area and it is multi-discipline involving tissue engineering, clinical medicine, biochemical science, material science and engineering. Even though biomaterial science is very new, the use of biomaterials dates back into ancient civilizations. Chinese and Indians used waxes, glues, and tissues in reconstructing missing or defective parts of the body [10]. In the early days, all kinds of natural materials were used as biomaterials on trial and error and this is the first generation of biomaterials. With the development of biomaterial science, natural materials are rarely used and are replaced with the second generation of biomaterials, which are designed and developed to meet the specific clinical and surgical requirements. Newly developed biomaterials include hydroxyapatite, collagen, fibrous protein, etc. Compared with natural materials, the mechanical properties and biocompatibility of the second generation biomaterials are greatly improved. Nowadays, thanks to the advancement of biomedical and material science, biomaterial science has entered into a new stage. The newest biomaterials not only provide support, but could also induce the regeneration of tissues. However, this new generation of biomaterials is still under research.

Biomaterials are expected to perform in the body’s internal environment, which is very aggressive. The pH value of body fluids in various tissues varies from 1 to 9. During daily
activities, bones are subjected to a stress of about 4 MPa; and the mean load on a hip joint is up to 3 times body weight. Moreover, these stresses are repetitive and fluctuating depending on the type of activities [9]. The host responses to these materials are extremely varied. Some materials are tolerated by the body whereas others are not. The same material could be tolerated in certain conditions, while it might be rejected in other conditions. Over the past 40 years, significant progress has been made in understanding the interactions between the tissues and the materials. “Biocompatibility” has been used by researchers to indicate the biological performance of materials [11]. Materials that are biocompatible are called biomaterials; and ‘biocompatibility’ is a descriptive term indicating the ability of a material to perform with an appropriate host response in a specific application [12]. This definition was later extended and distinguished between surface and structural compatibility of an implant. Surface compatibility presents the chemical, biological and physical suitability of an implant surface to the host tissues. Structural compatibility describes the mechanical properties, including elastic modulus, strength, implants design and load transmission at the implant/tissue interface, and refers to the adaptation to the mechanical behaviour of the host tissues [10].

Apart from the biocompatibility, the success of a biomaterial in the body also depends on many other factors, including surgical technique, health condition and activities of the patient. With the development of biomaterials during the past decades, there are many kinds of biomaterials produced which could be applied in all biomedical areas. Biomaterials could be classified by different ways. By considering the interaction of biomaterials with the surrounding physiologic environment, biomaterials could be described as bio-inert, bio-active and bio-degradable. According to the applications, biomaterials could be applied in the soft tissue engineering, hard tissue engineering or used as carrier material. In terms of the material itself, biomaterials could be made of ceramic, polymer, metal and composite; and this is the most common classification. However, clinical experience clearly indicates that not all the commonly used engineering materials are suitable for biomedical applications.

Generally, biomaterials cover all classes of materials: ceramics, polymers, metals and composites [13]. Ceramics are chemically stable and have excellent biocompatibility. Some ceramics, such as alumina and zirconia, also have good corrosion and wear resistance and have been widely used as hard tissue replacement. Some ceramics are bio-active and could induce the growth of bone cell. These ceramics include bioglass and hydroxyapatite. A large
number of polymers such as poly-ethylene (PE), polyacetal (PA), Polyurethane (PU), and polyethylene terephthalate (PET) are also used in various biomedical applications. Metals and alloys that are successfully used as biomaterials include: gold, tantalum, stainless steel, Co-Cr, Ti alloys and Nitinol. Each type of materials has its own positive aspects that are particularly suitable for specific applications. The application examples of biomaterials are shown in Table 2.1.

2.2 Nitinol

Nitinol is a nearly equi-atomic binary alloy of nickel and titanium. It was discovered as part of a Naval research project by Buehler and colleagues and reported in 1963[1]. It was given the name as the acronym for Nickel Titanium Naval Ordnance Laboratory. Nitinol was commercially introduced in 1970s and has been used as pipe couplings, glasses frames, earthquake dampeners and etc.

Nitinol has relatively low density, high fatigue strength, non-magnetic nature, good impact and heat resistance, with a melting point of about 1300 °C. Table 2.2 below lists some of the properties of Nitinol. The excellent malleability and ductility of Nitinol enable it to be manufactured in the form of wires, ribbons, tubes, sheets or bars. It is particularly useful for very fine small devices. Nitinol has a high corrosion resistance as well. The surface naturally forms a brown coating of TiO$_2$ without the presence of nickel oxides, and other coatings can be applied readily [14]. Apart from these great physical and mechanical properties, Nitinol has some unique properties, which common metals do not have, namely shape memory effect and super-elasticity.
Table 2.1 – Examples of different types of biomaterials [15, 16].

<table>
<thead>
<tr>
<th>Materials</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Metals:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stainless steel</td>
<td>High strength, fatigue resistance, wear resistance, easy fabrication, easy to sterilize, shape memory</td>
<td>High modulus, corrosion, metal ion sensitivity and toxicity, metallic looking</td>
<td>Hip joint replacement, dental implant, vascular implants, bone plate</td>
</tr>
<tr>
<td>Co-Cr alloy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ti and Ti based alloy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitinol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Polymers:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polyethylene</td>
<td>Low density and easy to fabricate, flexible and tissue equivalent density, good biocompatibility due to organic constituents.</td>
<td>Low mechanical strength and wear resistance, wetting characteristics are often not optimal.</td>
<td>Cardiovascular, ELISA dish surface, soft skeletal tissue; dental implants, bone cement, intraocular lens, catheters and tissue adhesive</td>
</tr>
<tr>
<td>Polyesters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polystyrene</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polyurethane</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Silicone Rubber</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Ceramics:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alumina</td>
<td>Good biocompatibility, corrosion resistance</td>
<td>Undesirable surface properties and special techniques required for fabrication</td>
<td>Improve biocompatibility as an interface</td>
</tr>
<tr>
<td>Zirconia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium Phosphates</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Composites</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibres</td>
<td>Designer physical or chemical properties, possible by varying components</td>
<td>Complicated synthesis</td>
<td>Assist regeneration of natural tissue</td>
</tr>
</tbody>
</table>
Table 2.2 – Typical properties of Nitinol [17].

<table>
<thead>
<tr>
<th>Property</th>
<th>55-Nitinol, austenite</th>
<th>55-Nitinol, martensite</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Physical</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Density (gm/cm$^3$)</td>
<td>6.35</td>
<td></td>
</tr>
<tr>
<td>Melting point (°C)</td>
<td>1310</td>
<td></td>
</tr>
<tr>
<td>Magnetic permeability</td>
<td>&lt;1.002</td>
<td></td>
</tr>
<tr>
<td>Coefficient of thermal expansion (×10$^6$/°C)</td>
<td>11.0</td>
<td>6.5</td>
</tr>
<tr>
<td>Electrical resistivity (ohm-cm)</td>
<td>100×10$^{-6}$</td>
<td>80×10$^{-6}$</td>
</tr>
<tr>
<td>Hardness 950°C (Furnace cooled)</td>
<td>89 R$_B$</td>
<td></td>
</tr>
<tr>
<td>Hardness 950°C (Quenched-R.T. water)</td>
<td>89 R$_B$</td>
<td></td>
</tr>
<tr>
<td><strong>Mechanical</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young’s modulus (GPa)</td>
<td>120</td>
<td>50</td>
</tr>
<tr>
<td>Yield strength (MPa)</td>
<td>379</td>
<td>138</td>
</tr>
<tr>
<td>Ultimate tensile strength (MPa)</td>
<td>690-1380</td>
<td></td>
</tr>
<tr>
<td>Elongation</td>
<td>13-40%</td>
<td></td>
</tr>
<tr>
<td><strong>Shape memory</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transformation temperature (°C)</td>
<td>-50 to +100</td>
<td></td>
</tr>
<tr>
<td>Latent heat of transformation</td>
<td>10.4 BTU lb$^{-1}$</td>
<td></td>
</tr>
<tr>
<td>Shape memory recoverable strain</td>
<td>6.4-8.5%</td>
<td></td>
</tr>
<tr>
<td>Super-elastic recoverable strain</td>
<td>up to 8%</td>
<td></td>
</tr>
<tr>
<td>Transformation fatigue life</td>
<td>several hundred</td>
<td></td>
</tr>
<tr>
<td>at 6% strain</td>
<td>cycles</td>
<td></td>
</tr>
<tr>
<td>at 2% strain</td>
<td>$10^5$ cycles</td>
<td></td>
</tr>
<tr>
<td>at 0.5% strain</td>
<td>$10^7$ cycles</td>
<td></td>
</tr>
</tbody>
</table>
2.2.1 Shape memory effect and super-elasticity of Nitinol

The first attempt to explain the shape memory effect is attributed to Zijderveld, who established in 1966 that the unique properties are due to crystalline transition induced by temperature change or application of stress.

The shape memory effect for Nitinol results from a temperature dependent phase change that occurs exclusively in the solid state, known as a thermoelastic martensitic transformation. When common metals are deformed, dislocations and repositioning of atomic planes within the crystal accumulate into tangles that resist further deformation. This process is called work hardening. Shape memory alloy (SMA) responds to deformation completely differently by undergoing transition in their crystal structures when heated or when stress is applied. At lower temperatures, SMA has a readily deformable crystalline arrangement termed martensite.¹ The unit cells are slightly tilted in bands which are described as a monoclinic configuration. As the martensite is deformed, these bands can move to accommodate the strain generated in the metal. In this way, no dislocation of the metallic lattice takes place and reversible plastic deformation could occur, so that the strain limits of the alloy are not exceeded [14].

As the crystal structure passes through a characteristic transformation temperature range (TTR), the realignment of atomic planes which has occurred in the SMA is exactly reversed. The crystal alters to a rigid and ordered cubic box-like configuration known as austenite, which is the most thermodynamically stable crystal structure at this temperature. Even though the metal deforms in martensite form, it rapidly recovers its original shape in the austenite form, in as short as 0.2 s. Fig. 2.1 represents the martensitic transformation and shape memory effect of Nitinol.

¹ Note that martensite in steel is usually less deformable.
Figure 2.1 – Diagrammatic representation of the martensitic transformation and shape memory effect of Nitinol [17].

For most engineering materials, stress increases in a linear relationship with strain within the material’s elastic deformation region. It is expressed by the Hooke’s law. Nitinol responds in a different way. As shown in Fig. 2.2, both loading and unloading curves show plateaus, along which large strains can be accommodated on loading, or recovered on unloading, with only a small change in stress. This behavior of Nitinol is much like natural tissues such as hair and bone, and results in a super-elastic ability. It is noted in Fig. 2.2 that, if a stainless steel is loaded to a similar degree of extension, it will have certainly deformed plastically, i.e. permanent (irrecoverable) deformation occurs.
Figure 2.2 – Schematic stress-strain curves of stainless steel, Nitinol (loading-austenite phase, unloading-martensite phase), and bone [18].

Nitinol has much better mechanical properties than other commonly used biomaterials and Table 2.3 lists the mechanical properties of some bio-metals and human hard tissues. Compared with commonly used metals, Nitinol has higher fatigue strength and similar elastic modulus with human hard tissues. Together with its super-elasticity, the recoverable strain of Nitinol is much higher than that of stainless steel. Stainless steel only recovers 0.8% of strain, as shown in Table 2.3. Once the strain exceeds the limit, stainless steel could not recover to its original state (due to plastic deformation), which gives patients additional pain if stainless steel is used as an implant. Due to the advantage of super-elasticity property, Nitinol could recover much more strain. When Nitinol is implanted into human body, Nitinol deforms under stress and restores its original shape when stress is removed, resulting in a better mechanical compatibility with human bones.
Table 2.3 – Mechanical properties of some biomaterials and human hard tissues [12].

<table>
<thead>
<tr>
<th>Materials</th>
<th>Young’s modulus (GPa)</th>
<th>Recoverable strain (%)</th>
<th>Fatigue strength (GPa)</th>
<th>Hardness (HV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitinol (A)</td>
<td>70 ~ 110</td>
<td>2</td>
<td>100 ~ 800</td>
<td>300 ~ 350</td>
</tr>
<tr>
<td>Nitinol (M)</td>
<td>21 ~ 69</td>
<td>8</td>
<td>50 ~ 300</td>
<td>-</td>
</tr>
<tr>
<td>316L (annealed)</td>
<td>176 ~ 196</td>
<td>0.8</td>
<td>343</td>
<td>170 ~ 200</td>
</tr>
<tr>
<td>Ti-6Al-4V</td>
<td>110</td>
<td>-</td>
<td>170 ~ 240</td>
<td>-</td>
</tr>
<tr>
<td>Enamel</td>
<td>84.3</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dentine</td>
<td>11.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cancellous bone</td>
<td>0.4</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

2.2.2 Applications of Nitinol

Nitinol has been utilized in many industries due to its excellent mechanical properties and its exceptional shape memory and super-elastic properties. The first recorded application of Nitinol was hydraulic coupling produced by the Raychem Corporation in 1969. These couplings were used in the US Navy’s F14 jet fighter and were so successful that this technology was soon employed across the military industries [19]. The super-elastic property of Nitinol is exploited in several high-volume applications, such as reading glasses. The improved properties of Nitinol over other biomaterials and its excellent biocompatibility lead to the major applications of Nitinol in the biomedical sector. Nitinol is one of few metals approved by Food and Drug Administration (FDA) for biomedical applications. In recent years, Nitinol has been applied in interventional cardiology, neurology; radiology and vascular surgery including drug and stent delivery systems, vascular closure devices, implantable devices, catheters and thrombectomy devices. Some related products are shown in Fig. 2.3. Nitinol is also used to repair broken bones. Cooled Nitinol, whilst in its martensitic shape, is stretched and wrapped around the bone section. As it heats up naturally in human body, Nitinol reverts to its original shape in the austenite phase and generates a
pressure, thus pushing the damaged bone section together. This technique is far more useful than using conventional stainless steel.

Figure 2.3 – (a) Nitinol stents; (b) Pre-shaped Nitinol orthodontic files; (c) Nitinol tubing with an internal actuation wire allows this 0.8mm diameter grasper to operate while tied in a knot; (d) Spinal vertebrae spacer shown in the martensitic state (left) and deployed super-elastic state (right).

2.3 Surface Modification of Nitinol

Biological reactions are frequently described as occurring in the solution phase, for example, the reaction of a soluble enzyme with its substrate. In fact, most reactions in biology occur not in solutions but at interfaces between a biomaterial and its host organism [20]. The surface region of a biomaterial is so important in considering the biological interactions, because (1) only the surface of a biomaterial would contact surrounding bioenvironment; (2) the morphology and chemical composition of surface layer is different from that of the bulk biomaterial; (3) the host tissue only biologically responds to the surface of a foreign material and (4) the mechanical stability of the interface between implant and tissue is governed by surface topography [21-25]. Therefore, the surface properties of materials in contact with
biological systems, including surface morphology, surface chemistry and surface energy, are extremely important in determining the outcome of biological materials interactions. In order to improve the biomaterials’ properties, including wear resistance, corrosion resistance, as well as biocompatibility, surface modifications are normally applied [26].

Nitinol is generally considered possessing good biocompatibility. It is also approved by the Food and Drug Administration (FDA) as a safe material for long-term biomedical applications [27]. The term ‘biocompatibility’ can be defined as the ability of a material to be accepted by the body. Since all materials generate a ‘foreign body reaction’ when implanted in the body, the degree of biocompatibility is related to the extent of this reaction [28]. Therefore, biocompatibility is directly related to the corrosion behaviour of the material in a specific solution and the tendency for the alloy to release potential toxic ions [29]. Under natural conditions, Nitinol surface is spontaneously covered by titanium dioxide. TiO$_2$ is preferentially formed thermodynamically, compared to nickel oxides. This TiO$_2$ oxide layer serves two purposes: (1) increasing the stability of the surface layers by protecting the bulk material from corrosion/oxidation; and (2) creating a physical and chemical barrier against Ni oxidation and modifying the oxidation pathways of Ni [28]. However, the formation of TiO$_2$ on Nitinol surface is not very consistent and depends on the purity and manufacturing conditions of the material [30]. Moreover, the oxides formed on the Nitinol surface always contain a certain fraction of Ni. Contrary to pure Ti and Ti6Al4V biomedical alloys, which repassivate after surface damage, the Nitinol oxides have lower self-healing ability in scratch tests, a lower resistance to localized corrosion [31].

Recent researches are aiming at improving the corrosion resistance of Nitinol as well as its biocompatibility. The biocompatibility of Nitinol in many clinical studies has been excellent [32]. However, there have been also conflicting results. The high content of Ni in this alloy (up to 50 at.% Ni) raises concerns. When Nitinol is exposed to body fluid, Ni is released due to corrosion. This element is known to be allergenic and toxic, though it is essential for the human body if the intake is minimal [33]. It has been shown in biological studies in vitro that the amount of Ni recovered may be either very low from the beginning or drops to an undetectable level after a short time [34, 35]. However, this toxic nickel keeps causing problems. The recent results obtained on commercial ready-to-use orthodontic wires showed that the Ni release varied in a wide range from 0.2 to 7 μg cm$^{-2}$ [36]. Moreover, it has been reported that the Ni release can significantly increase with time and maintains at a high level.
up to a few months [33]. As the nickel concern remains, a better understanding of the material is needed. Moreover, as an implant material, it is still far from perfect. Researchers have shown that even for successful titanium implants, the bone to implant contact ratio only have an average of 70 – 80% [37].

At present, mechanically polished, electrolytically polished, acid etched, as well as sandblasted Nitinol surfaces have been applied for implantation. These surfaces have thin oxide layers (≤ 10 nm) and their behaviour is not always neutral [38]. Thicker oxide layers (≥ 100 nm) have also been produced by heat treatments to ensure a more reliable barrier for Ni release from Nitinol. However, thick Ti external oxide layers lead to Ni accumulation underneath the surface and these Ni rich layers can be easily activated if the top TiO₂ surface is damaged. The damaged top TiO₂ surface may cause high and long lasting Ni release [39]. Thick oxides may also crack when strained in the super-elastic regime and would not repair after unloading [40]. Surfaces that are coated or modified using energy sources are also under development and these surfaces can reduce Ni release but only under static conditions without load [33]. A common problem of coatings for Nitinol is that their adhesion is worsened during phase transformation to martensite, as the surface topography is altered and the volume of the material increases slightly (≤ 0.6%).

Surface modification of Nitinol therefore frequently follows special demands concerning an enhanced passivity and corrosion protective layer to prevent Ni release. Traditional surface treatments for biomaterials include mechanical polishing, sandblasting, shot peening, heat treatments, electropolishing and chemical etching. Several acid solutions for electrochemical and chemical etching and different conditions for heat treatment have been explored. The proper surface modification techniques not only retain the excellent bulk attributes but also improve specific surface properties required by different clinical applications. In the following sections, surface modification methods applied on Nitinol will be described in details.

2.3.1 Mechanical modifications

Mechanical modifications are widely used to roughen or smooth a surface [41]. These methods apply physical forces to alter the surface condition, so that specific surface
topographies could be achieved [42]. Commonly used mechanical modifications include sandblasting, mechanical grinding and polishing.

Sandblasting is normally used to produce rougher surface, so that improved adhesion in bonding would be achieved, which is favoured for biomineralization due to increased surface area [43]. Li. et al. used irregular zirconia particles to sandblast pure titanium and their results showed an increased surface roughness (Fig. 2.4) [44].

![SEM micrograph of sandblasted Ti surface](image)

Figure 2.4 – SEM micrograph of sandblasted Ti surface [44].

Li et al. sandblasted Ti-based bulk metallic glass (BMG) with corundum of different grits. Their results showed that a rougher surface was obtained by sandblasting with a larger size of corundum (Fig. 2.5) and the rougher surface was favoured to improve MG 63 cell-material interactions (Fig. 2.6) [45].
Figure 2.5 – SEM images of Ti-based BMG, (a) untreated and sandblasted with (b) 180# grit and (c) 60# grit [45].

Figure 2.6 – MG63 cells morphologies on different Ti-based BMG samples for 4 h, 8 h, 12 h and 24 h [45].
However, sandblasting is unable to completely eliminate the original scale on a microscopic level and new surface defects are induced, resulting in irregular surface topography. Moreover, during the blasting process, impaction of abrasive particles against the surface occurs, which might induce contamination from the abrasive particles on the surface [46].

Mechanically ground or polished to luster surfaces are often used in biological studies or used as a substrate pre-treatment method. However, these surfaces are known for their poor reproducibility in corrosion resistance [31], and their biological performance is not always satisfactory. The elemental compositions of mechanically polished surfaces (1 µm finish) depend on the sample polishing, cleaning and handling procedures. The main contaminants on the surface, including Ca, Na, Mg, Si, P and Cl, are from polishing or cleaning solutions or calcium powdered gloves, and have concentrations varying from 1 to 8 at.% [33]. The concentration of contaminants affects the metal concentrations on the surface. Therefore, the concentrations of Ni and Ti on the surface vary. For example, the concentrations of Ni and Ti are in the 1 – 4 at.% and 8 – 13 at.% ranges on Nitinol respectively, determined by XPS analysis [30, 47]. Mechanical grinding or polishing also induces residual plastic deformation, which contributes to the inconsistent corrosion behaviour [33]. Therefore, mechanical modification may not be an optimal option to control the surface conditions. Additionally, for devices with complex shapes, mechanical modifications may be impractical [48].

2.3.2 Coatings

Coatings form a layer on top of the outer surface of Nitinol. The coating as a separate layer can provide more versatile modifications than other methods. Therefore, coating is more functional and can be applied for many specific applications.

Titanium oxide film is usually deposited on the Nitinol surface to enhance the corrosion protection by chemical vapour deposition (CVD) or ion beam assisted deposition (IBAD). By these methods, connective layer and graded interfaces are built up, so that stable adherence is promised [49]. Sol-gel deposition of TiO$_2$ has had wide technical applications [50]. Sol-gel TiO$_2$ films are rich in Ti-OH groups, which are bio-active, as they precipitate calcium phosphate in the form of hydroxyapatite from simulated body fluid. However, the interface between the substrate and the sol-gel TiO$_2$ film is incoherent, which tends to delaminate [51].
Hydroxyapatite (HA) coatings are normally applied on orthopedic or dental implants of titanium alloys. Hydroxyapatite is the mineral phase of bone and so is osteoconductive. Clinically, it accelerates the integration of an implant in bone. HA coatings could be applied by plasma spray, sputtering, ion beam assisted deposition, electro-deposition and sol-gel dip coating [52]. Jiang and Rong obtained HA coating on porous Nitinol treated in 32.5% HNO₃ and 1.2M NaOH solution and subsequently immersion in SBF [53]. Uniform HA layer formed not only on the surface and but also inside the pores. With HA coating, the Ni release from porous Nitinol was significantly reduced, as shown in Fig. 2.7 [53].

![Graph showing Ni release from untreated porous and solid Nitinol and porous Nitinol with HA coating](image)

**Figure 2.7** – Ni release from untreated porous and solid Nitinol and porous Nitinol with HA coating [53].

A common problem with coatings for Nitinol is their adhesion, which is worsened inevitably because of the alteration in surface topography and volume change caused by phase transformation to martensite. Another problem with coating is that if the underlying Nitinol substrate is strained to greater than 4%, coatings would crack [54, 55].
2.3.3 Chemical etching

Chemical etching has been applied to passivate the Nitinol surface. This method utilizes the preferential dissolution of Ni from the Nitinol surface to form a Ni-depleted layer. Chemical etching is known to be efficient for elimination of harmful Ni from the Nitinol surface and enhancing surface oxidation. Under natural conditions, the Nitinol surface is spontaneously covered by Ti dioxide, as the preferential oxidation of Ti always occurs. However, the oxides formed on the Nitinol surface always contain a certain fraction of Ni, with concentrations from ~2 to 7 at.%, depending on the conditions employed [33].

The standard chemical passivation of Nitinol is by dipping in 10% nitric acid (HNO₃) at room temperature according to ASTM-F86 standard [56]. The treatment modifies only about 3 nm of the outmost surface layer, but an amorphous state has been reported at a depth >50 nm [57], forming a uniform highly corrosion resistant TiO₂ layer. Chemical treatments have also been reported to be effective in modifying the surface of Ti and Ti-based alloys to favor the formation of bio-active apatite on the surface in simulated body fluid and prevent Ni ions releasing from Nitinol [58, 59]. Currently the most widely used etching solution is in aqueous HF + HNO₃ solutions [30, 47] and the best ratio is reported containing 1 part of HF, 4 parts of HNO₃ and the rest made of water [60].

Shabalovskaya et al. reported that chemically etched surfaces exhibit more consistent corrosion resistance, while mechanically polished and heat treated surfaces are prone to pitting [38]. Milošev and Kapun chemically etched Nitinol and the resultant surface had higher roughness compared with mechanically polished surface (Fig. 2.8). The chemically etched Nitinol surface also showed an improved resistance to localized attack [61].
Figure 2.8 – SEM images of Nitinol surfaces prepared by (a) polishing and (b) chemical etching [61].

2.3.4 Electrochemical modifications

Electrochemical modifications use an electrolytic process to treat metallic materials. An electrochemical process involves the use of an electrolyte, an anode and a cathode [62]. The anode is the metal piece to be modified, which is connected to the positive pole of a power supply (Fig. 2.9). When a voltage is applied, the surface of the metal piece dissolves, due to complex anodic reactions, so that desired surface finish would be obtained. Due to the current flow through the electrode surface, the local conditions at the interface between electrode surface and electrolyte could be altered by electrochemical treatments. By using this process, desired chemical reactivity could result [63].

Depending on the expected finish, the electrochemical process might be called electrochemical etching [63] or electropolishing [64]. Depending on the voltage applied, such an electrochemical process can be described as anodisation [65]. Electropolishing has been applied on Ti [66] and its alloys [67] to achieve mirror finish for industrial purpose or better corrosion resistant for biomedical applications. Anodisation has also been widely used to treat Ti and its alloys [68, 69]. TiO$_2$ nanotubes with high aspect ratio prepared on titanium by anodisation were reported [70, 71].
However, the electrochemical treatments of Nitinol have not been well explored and there have been no studies on electropolishing or anodisation of this material before 2004. In mid-2000s, many papers on electrochemical etching/electropolishing process in various electrolytes [5, 64, 72-74], and anodising in various solutions and voltage regimes [73, 75] have been published. Compared with chemical etching, electrochemical etching has a higher etching rate and lower side etching [76, 77]. Moreover, electrochemically etched ones are more heterogeneous than chemically etched, because all phases inherited from the alloy bulk could be retained during electrochemical etching process [33]. With electrochemical modifications, a stress induced martensite formation could be avoided, which is normally noticed on Nitinol surface modified with mechanical methods [73]. In the following sections, these two electrochemical modification methods, electrochemically etching and anodisation, will be described in detail.
2.3.4.1 Electrochemical etching

Researches have been focused on various surface treatments to decrease the Ni content in the surface layer and/or control Ni release rate from the bulk Nitinol [47, 61, 63]. Among all the surface modification methods mentioned above, such as mechanical polishing, thermal treatment and coatings, electrochemical etching is one of the most suitable and effective methods for decreasing the release of Ni and improving the biocompatibility and corrosion resistance of Nitinol [78]. Chu et al. compared chemical and electrochemical modifications on Nitinol surface and suggested that the electrochemically etched surface performed better with non-detectable Ni content in the surface and minimal Ni diffusion [78]. Miao et al. explored the effect of processing parameters on Nitinol electropolishing [74]. The parameters included the electrolyte, temperature, current density, time, and the space between anode and cathode [74]. Electropolished Nitinol alloy in their research was covered by a thin oxide film (about 3 nm), mainly consisting of Ti oxide which increased the corrosion resistance and biocompatibility [34, 79-81].

Electropolishing removes the outermost surface layer and forms a protective passive layer [33]. Quality and properties of the oxide layer influence the corrosion resistance [82]. The formed TiO₂ layer causes an increase in the corrosion resistance of Nitinol after electropolishing [82]. The balance between the formation of a passive layer and the dissolution of the surface region into the electrolyte is achieved during electropolishing [73]. The wear resistance and corrosion resistance was increased by electropolishing which removed the original oxide layer and constructed a new homogeneous oxide layer [73]. Cattarin et al. proposed electrochemical etching of Nitinol in a neutral fluoride solution [63]. A semi-lustrous rough Nitinol surface was produced as shown in Fig. 2.10. Their results indicated that a barrier film was formed and its thickness increased with increasing applied voltage.
Additionally, electropolished Nitinol surfaces exhibited better corrosion resistance than chemically etched, mechanical polished or thermally oxidised surfaces [83]. A thin Ti oxide film (about 10 nm) could be formed by electropolishing with depleted Ni content [61]. The research by Cissé et al. suggests that the amount of Ni on the Nitinol surface decreased after electropolishing and the corrosion resistance of Nitinol increased [84, 85]. Investigation on the corrosion performance of Nitinol after the surface treatment by mechanical polishing, electropolishing, electropolishing followed by chemical passivation, was conducted [85]. Their results showed that lower values of corrosion current density were obtained for electropolished Nitinol samples [38]. Liu et al. used a modified electrolyte (HClO$_4$-CH$_3$COOH-A-B) to electropolish a Ti-50.8at.% Nitinol alloys [86]. The results revealed that the electrochemical polished Nitinol alloys had improved corrosion resistance compared with the mechanical polished ones [86]. Trepanier et al. produced a thin and uniform oxide layer by electropolishing [87], which demonstrated a significantly improved corrosion resistance. A modified electrolyte (CH$_3$COOH-HClO$_4$-A-B) was used to electropolish Nitinol [84], and the work suggested that electropolished Nitinol displayed an improved corrosion resistance in Hanks’ solution (as shown in Fig. 2.11).
A thin Ti oxide layer formed on the Nitinol surface decides biocompatibility of Nitinol [34]. Nitinol is protected by the layer comprised mainly of TiO$_2$ [34]. The good biocompatibility depends on the formation of a dense Ti based oxide layer that prevents the release of Ni ion [64]. Chu et al carried out blood platelet adhesion test on chemically polished and electropolished Nitinol surfaces [78]. The thromboresistance of Nitinol was improved by electropolishing, as the platelets formed a single layer and were isolated, indicating that adhesion was greatly reduced (Fig. 2.12).

Electrochemical etching/electropolishing can reduce Ni release, which enhances the biocompatibility simultaneously with good mechanical properties. It can also provide better corrosion protection than other methods such as mechanical polishing. Electropolishing could also effectively mitigate out-diffusion of Ni ions from bulk Nitinol [78]. Ti/Ni ratio increased from 3.1 for the mechanical polished Nitinol sample to 27.6 for the electropolished sample [84]. Oxide film formed by electropolishing contained less Ni content than other surface modification methods, resulting in lower Ni release [83].
2.3.4.2 Anodisation

Due to the excellent physical and chemical properties as well as biocompatibility, Ti and Ti alloys with anodic tubular oxide layer have been attracting much attention as they could be applied as high performance biomedical materials [88-90]. In order to improve biocompatibility and enhance bone growth when contacting with the implant, TiO$_2$ nanotube layers with controlled structure and morphology could be applied. TiO$_2$ nanotubes can be produced by many means and among which, anodisation is the best way. Comparing with other methods like sol-gel and electrophoretic deposition, the highest adhesion strength between the oxide layer and the substrate was achieved by anodisation [91].
The typical TiO$_2$ nanotube arrays consist of single nanotubes of 80-150 nm in diameter and 0.5-25 µm in length (an example is shown in Fig. 2.13). By adjusting the processing parameters, such as the electrolyte compositions and the anodisation conditions, the physical and chemical properties of nanotube layer could also be controlled [91]. In fluorine ion containing electrolytes, TiO$_2$ nanotubes form as a result of two reactions: hydrolysis of Ti metal to form TiO$_2$ (Eq. 2.1) and chemical dissolution of TiO$_2$ (Eq. 2.2) [92-94].

\[
\text{Ti}^{4+} + 2\text{H}_2\text{O} \rightarrow \text{TiO}_2 + 4\text{H}^+ \quad (2.1)
\]

\[
\text{TiO}_2 + 6\text{HF} \rightarrow [\text{TiF}_6]^{2-} + 2\text{H}_2\text{O} + 2\text{H}^+ \quad (2.2)
\]

Figure 2.13 – FESEM micrograph of TiO$_2$ nanotubes formed by anodic oxidation of Ti in NaF-containing electrolyte (0.1 mol/L F$^-$), at pH 4.5, and constant potential of 20 V for 2 h [94].
The mechanism and kinetics of self-ordered TiO$_2$ nanotube formation is not fully understood, but the following three steps have been suggested by Crawford et al. [94].

(1) Initial barrier layer formation: in the initial stage of anodisation, current density decreases over time until a steady state is approached, a consequence of the increased electrical resistance caused by the compact oxide film.

(2) Formation of uniformly distributed pores: chemical dissolution of oxide layer takes place and current density increases during this stage. As a result of simultaneous oxidation and dissolution, self-organized pores are formed.

(3) Separation of interconnected pores into nanotubes: the current density again stabilizes, when an ordered nanotube structure is established. Beyond this point, extending anodisation time only increases the tube length.

The nanostructures on Ti alloys including Nitinol are different from those obtained on pure Ti substrate. Micro- and nano-scale hierarchical surfaces are obtained on Nitinol surfaces. By anodisation, a rough or porous Nitinol surfaces could be achieved, with reduced Ni content in the oxide layer and enhanced corrosion resistance. Moreover, biocompatibility of Nitinol could also be enhanced by anodisation. Siu and Man [95] conducted immersion test in SBF and found that anodised Nitinol could induce the formation of hydroxyapatite (HA) whereas HA could not form on the bare Nitinol. In vitro cell-material interactions with treated and non-treated Nitinol surfaces were investigated by Bernard et al. [96] and better osteoblast cell adhesion, growth and proliferation were found on the anodised Nitinol surface. As Nitinol alloys contain a high Ni content, the presence of Ni may well affect the mechanism and kinetics of the anodisation process on Nitinol. The mechanism and kinetics of Nitinol anodisation are still unclear and further investigation is needed. Table 2.4 below lists some anodisation conditions reported in the literature.
Table 2.4 – Reported conditions for anodising Nitinol.

<table>
<thead>
<tr>
<th>Electrolyte</th>
<th>Voltage (V)</th>
<th>Duration (min)</th>
<th>Type</th>
<th>Year [Ref.]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.6M Na$_2$SO$_4$ + 0.6M NaOH</td>
<td>80V, 50Hz</td>
<td>5, 10, 20</td>
<td>PEO</td>
<td>2013 [95]</td>
</tr>
<tr>
<td>0.04M Na$_3$PO$_4$</td>
<td>100, 200, 300</td>
<td>10</td>
<td>PEO</td>
<td>2012 [97]</td>
</tr>
<tr>
<td>0.15M NaAlO$_2$ + 0.01M NaPO$_2$H$_2$</td>
<td>100, 200, 300</td>
<td>10</td>
<td>PEO</td>
<td>2013 [96]</td>
</tr>
<tr>
<td>0.3M NaF + 1M H$_2$SO$_4$ + 0.02M citric acid</td>
<td>20</td>
<td>15 ~ 60</td>
<td>DC</td>
<td>2012 [98]</td>
</tr>
<tr>
<td>1M acetic acid</td>
<td>2 ~ 10</td>
<td>15 ~ 60</td>
<td>DC</td>
<td>2013 [99]</td>
</tr>
<tr>
<td>0.15 M NaAlO$_2$ and 0.01 M NaPO$_2$H$_2$</td>
<td>400</td>
<td>90</td>
<td>MAO</td>
<td>2011 [100]</td>
</tr>
<tr>
<td>0.075M HF</td>
<td>20</td>
<td>60</td>
<td>DC</td>
<td>2011 [101]</td>
</tr>
<tr>
<td>0.5M H$_3$PO$_4$</td>
<td>20</td>
<td>60</td>
<td>DC</td>
<td>2011 [102]</td>
</tr>
<tr>
<td>1M H$_2$SO$_4$</td>
<td>5A</td>
<td>60</td>
<td>DC</td>
<td>2011 [103]</td>
</tr>
<tr>
<td>1M acetic acid/ sodium acetate</td>
<td>20mA/cm$^2$</td>
<td>60</td>
<td>DC</td>
<td>2011 [104]</td>
</tr>
<tr>
<td>0.1M acetic acid/ sodium acetate</td>
<td>5 ~ 150</td>
<td>1 ~ 55</td>
<td>pulse</td>
<td>2011 [105]</td>
</tr>
<tr>
<td>0.1M ammonium pentaborate</td>
<td>5 ~ 150</td>
<td>1 ~ 55</td>
<td>pulse</td>
<td>2011 [106]</td>
</tr>
<tr>
<td>0.2 mol/L calcium acetate + 0.04 mol/L sodium b-glycerophosphate</td>
<td>rms 50</td>
<td>30</td>
<td>AC</td>
<td>2011 [107]</td>
</tr>
<tr>
<td>3 wt.% Na$_2$SO$_4$·10H$_2$O</td>
<td>10 ~ 40</td>
<td>120</td>
<td>DC</td>
<td>2011 [108]</td>
</tr>
<tr>
<td>0.12M NaNO$_3$ in Methanol (&lt;1% H$_2$O)</td>
<td>3mA/cm$^2$</td>
<td>180</td>
<td>DC</td>
<td>2011 [109]</td>
</tr>
</tbody>
</table>

**Effect of electrolyte**

Different kinds of electrolytes have been used to anodise Nitinol surface and rough or porous Nitinol surfaces were obtained. The appearance of modified Nitinol surfaces generally depends on the electrolyte compositions. These electrolytes could be categorized as salt solution/acid solution, fluric ion containing/non-fluric ion solutions or aqueous solvent/organic solvent. Anodisation in salt solution [95, 97] results in rough and porous
surface microstructure, but the pore distribution is not uniform [Fig. 2.14]. In contrast, anodisation in acid solutions gives rise to a more uniform surface texture [4, 98]. A dense layer with round nodules was produced in 1M acetic acid solution (Fig. 2.15). The fluorine ion containing solutions modify the Nitinol surface into evenly distributed pores (Fig. 2.16).

Figure 2.14 – Surface morphologies of the treated Nitinol at 80V for 20min [95].

Figure 2.15 – Nitinol film anodised in 1M acetic acid at 10V [98].
For the case of pure Ti, it has been confirmed that if the treatment is carried out in highly viscous and less aggressive organic solutions, such as ethylene glycol, longer and ridge-free TiO$_2$ nanotubes could be achieved [91]. There are not many reported results about anodisation of Nitinol in organic electrolytes. Cheng et al. [104] reported a thick oxide layer of more than 10 µm and free of cracks by using an anodising methanol containing 0.12M NaNO$_3$ and less than 1% of water. Shirkhanzadeh [105] postulated that TiO$_2$ is formed as a result of controlled hydrolysis and polycondensation of titanium methoxide in the presence of water. Thus the water content in organic electrolyte is an important parameter, as it affects the rate of oxidation.

**Effect of anodisation conditions**

Two main parameters associated with anodising process are voltage and duration. While the electrolyte composition influences the appearance of anodised Nitinol surfaces, the applied voltage and duration would influence the microstructure. In the literature, the applied voltage ranges from several volts to hundreds of volts. The process that uses very high voltage is often named as plasma electrolytic oxidation (PEO) or micro arc oxidation (MAO). By increasing the anodisation voltage, both the thickness of the oxide layer and roughness of the
resultant surfaces would be increased \([65, 98]\). As suggested by Yang et al. \([65]\), the roughness might be caused by different anodisation rate of titanium and nickel elements. Anodisation time is another important factor influencing the morphology of modified Nitinol surfaces. Bernard et al. \([96]\) reported that surface asperities such as pits/pores grow in both number and size by increasing duration. The same tendency was also observed by Siu and Man \([95]\), who pointed out that the thickness of oxide layer increased with anodisation time.

In summary, anodisation is a relatively simple and low temperature method to synthesize micro- or nano-structures on Nitinol surface. Research results indicate that by choosing specific type of electrolyte and controlling the applied anodising voltage and duration, the morphologies, including roughness, pore size and pore size distribution could be controlled. Such modified Nitinol surfaces are beneficial for biomedical applications, as the surface area has been increased and defined microstructure can improve the attachment between tissue and implant and hence enhancing the biocompatibility of Nitinol.

2.3.4.3 Existing problems

Electrochemical methods have been proved to be effective to modify the Nitinol surface for potential biomedical applications. However, there are still problems or limitations of these methods. Although the corrosion resistance of electrochemically polished Nitinol surface can be improved, smooth surface finish is not preferred for cell-material interaction. Therefore, a new electrolyte, which can generate a rough surface, and also improve the corrosion resistance of Nitinol, needs to be explored. On the other hand, anodisation has been extensively studied on Ti, but not much on Nitinol. Nitinol behaves differently from Ti during the anodising process. Therefore, directly employing the methods suitable for Ti to treat Nitinol does not always work. Moreover, a systematic study on the anodisation mechanism of Nitinol is missing in the literature.
2.4 Evaluation of the Biocompatibility of Nitinol

2.4.1 Ni ion release

In order to make Nitinol more suitable for biomedical applications, many techniques have been explored to modify the surface of Nitinol. The main purpose of surface modification of Nitinol is to improve its biocompatibility. In term of “biocompatibility” associated with a metallic alloy, two aspects should be considered. One is the biocompatibility of the alloy itself, which is essentially based on its corrosion resistance, and the other one is the biocompatibility of its by-products as a result of corrosion [106]. The latter one is more important, as absorption of corrosion by-products by patient may induce systemic reactions [107]. As one major component of Nitinol, nickel has drawn much attention, because this element could induce allergenic reactions [108-111]. In Europe, 10~15% of female adults and 1~3% of male adults are allergenic to Ni [107]. Moreover, acute and chronic hand eczema could be developed as a result of Ni contact dermatitis, which is found in 40~70% adult patients [112-114].

Due to the concerns of allergic Ni element, the European Union (EU) has accordingly decreed two directives:

(1) The Ni release from parts in direct and prolonged contact with the skin must be lower than 0.5 µg/cm²/week [112].

(2) All metallic parts that are inserted into pierced ears and other parts of the human body must not have a Ni release rate greater than 0.2 µg/cm²/week [113].

To improve the biocompatibility of Nitinol for biomedical applications, researchers aim to improve its corrosion resistance. The amount of Ni ion released into surrounding corrosive environment defines corrosion resistance to some extent and so biocompatibility. In the reported studies, immersion test in a simulated physiologic environment is used to evaluate Ni ion release behaviour, including both the total Ni ion release amount and the release rate. The studied Nitinol samples could be in the shape of plate [115, 116] or wire [107, 115]. The solutions used in immersion tests include conventional simulated body fluid [115], artificial saliva [107] or 0.9% NaCl solution [39]. The Ni ion release behaviour could be evaluated for short-term (less than a month) [117, 118] or long-term (longer than a month) period [39, 117], and most of the reported data so fat are based on short-term Ni ion release.
The reported Ni ion release data vary significantly, even when the studied Nitinol materials have similar surface conditions and this leads to the question of the Ni source and the mechanisms and kinetics of Ni release. Two major Ni release sources are generally accepted: one is in the surface oxide layer [119, 120] and the other is the Ni-rich layer underneath the outermost oxide layer [39, 121]. Briceño et al. [107] studied the Ni ion release of different Nitinol archwires in artificial saliva and found that the presence of martensitic phase in the microstructure reduced the ion release by 50%. Bernard et al. [96] anodised laser processed Nitinol alloy in electrolytes with different pH values and found that Ni ion release could be reduced if the electrolyte pH value was lower.

2.4.2 Apatite formation

When artificial materials are implanted into defective bone, they are generally encapsulated by a fibrous tissue, so that the implant could be isolated from the surrounding bone. However, in 1972, Hench et al. [122] reported that Na₂O-CaO-SiO₂-P₂O₅ glasses spontaneously bond to living bone without forming surrounding fibrous tissue. This result implies that the implant material has bone-bonding ability. Since then the question of how to evaluate the bone bioactivity has been raised. Later in 1991, Kokubo [123] studied the heat treatment of a MgO-CaO-SiO₂-P₂O₅ glass and concluded that the formation of a bone-like apatite layer on the surface of an implanted material is the essential requirement for that material bonding to living bone. As the *in vivo* apatite formation could be reproduced in a solution with ion concentration nearly equal to those of human blood plasma, it provides a way to predict and evaluate the *in vivo* bone bioactivity of an implant material. That solution is so called simulated body fluid (SBF) and has the chemical compositions shown in Table 2.5 [124].

When bioglass 45S5, a Na₂O-CaO-SiO₂-P₂O₅ glass, was implanted in the body environment, it bonded to living bond through a Ca₃(PO₄)₂ layer [125]. This glass later showed its ability to form apatite on its surface in SBF [126]. Another Na₂O-CaO-B₂O₃-Al₂O₃-SiO₂-P₂O₅ system also showed its ability to form calcium phosphate layer on their surfaces in SBF and bond to living bone when implanted [127]. In addition to glass-ceramics, some metallic oxide gels also have had apatite formed on the surface when immersed in SBF, such as titania [128]. Kokubo and Takadama [124] suggested that it was the Ti-OH group in the titania gel that induced the formation of apatite, and hence Ti and its alloys would be expected to have the ability to form apatite in SBF and bond to living bone *in vivo*, if these metals are properly
Surface treatments of Nitinol aim to convert bio-inert Nitinol surface into bio-active. Generally, after surface treatments, a layer of TiO$_2$ is produced and the resultant Nitinol surface showed better bioactivity of improved apatite forming ability in SBF [129, 130].

Table 2.5 – Nominal ion concentrations of SBF in comparison with those in human blood plasma [124].

<table>
<thead>
<tr>
<th>Ion</th>
<th>Ion concentration (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SBF</td>
</tr>
<tr>
<td>Na$^+$</td>
<td>142.0</td>
</tr>
<tr>
<td>K$^+$</td>
<td>5.0</td>
</tr>
<tr>
<td>Mg$^{2+}$</td>
<td>1.5</td>
</tr>
<tr>
<td>Ca$^{2+}$</td>
<td>2.5</td>
</tr>
<tr>
<td>Cl$^-$</td>
<td>147.8</td>
</tr>
<tr>
<td>HCO$_3^-$</td>
<td>4.2</td>
</tr>
<tr>
<td>HPO$_4^{2-}$</td>
<td>1.0</td>
</tr>
<tr>
<td>SO$_4^{2-}$</td>
<td>0.5</td>
</tr>
<tr>
<td>pH</td>
<td>7.40</td>
</tr>
</tbody>
</table>

2.4.3 Cell-material interaction

Even though the quantity of Ni ion released is below a critical concentration, long-term inflammatory responses could also be induced and cells behaviour could be altered. Sun et al. [131] studied the effect of Ni$^{2+}$ ion (with concentration ranging from 38~608 µmol/L) on osteoblast metabolism and differentiation, where a significant decrease in Alkaline Phosphatases Activity (ALP) and DNA synthesis was observed even at sublethal concentrations. In vitro studies give a more direct evaluation of the biocompatibility of Nitinol and so far there have been some reports on the cell response to Nitinol. However, the reported results
are not consistent or even conflict. The difference in the cell-material interaction results may be caused by the different cell types used, testing methods and Nitinol surface conditions.

El Medawar *et al.* [132] reported the cytocompatibility assessment of Nitinol, elemental Ni and commercially pure Ti. Cell proliferation tests with human epithelial embryonic cells (L132) and human embryonic palatal mesenchymal cells (HEPM) and viability tests with L132 cell line were used to evaluate the cytocompatibility of these three materials. It has been shown that Nitinol exhibited good biocompatibility as Ti, even though Ni was cytotoxic [132]. A similar result was obtained by Cui *et al.* [133] who studied the biocompatibility of chemically treated Nitinol with 3T3 human fibroblast cell. Pure Ni showed serious cytotoxicity to 3T3 cell line, as the cells could not adhere to pure Ni [133]. On the other hand, Nitinol did not show any inhibition effect on 3T3 cells, indicating a good biocompatibility [133].

With surface modifications, the biocompatibility of Nitinol could be further improved. A microporous Nitinol surface was obtained by Zheng *et al.* [134] who used a method combining sandblasting and acid etching. The modified Nitinol surface showed enhanced biocompatibility with osteoblast-like cell line MG63 [134]. Jin *et al.* [135] coated Nitinol with a thin layer of TiN, which demonstrated a slower apoptosis rate of L929 cell line and enhanced cell adhesion and proliferation, indicating better biocompatibility than bare Nitinol surface.
Chapter 3  Experimental Setup and Procedures

In this research, the surface of Nitinol was modified through electrochemical processes and two methods were employed, namely electrochemical etching and anodisation. This chapter describes the procedure of how to modify the Nitinol surface in detail and how the modified Nitinol surfaces were characterized.

3.1 Preparation of Modified Nitinol Surfaces

3.1.1 Surface pretreatment of Nitinol

The starting material in the present research was commercially available Nitinol plate, with a thickness of 2 mm. The Ni content in the bulk material is 55.6 wt.%, determined by chemical analysis. The Nitinol plate was cut into small square coupons with dimensions of 20 mm × 20 mm. These Nitinol coupons were pretreated using three different methods, viz. grinding, mechanical polishing and chemical etching, in order to vary the surface roughness and surface chemistry. The pretreated Nitinol coupons were then ultrasonically cleaned in acetone and ethanol, followed by rinsing in distilled water for 15 min before the surface modification process.

3.1.2 Surface modification of Nitinol

The pretreated Nitinol coupons were subjected to further surface modification using two different electrochemical processes: electrochemical etching and anodisation. In both processes, the Nitinol coupons were used as anode, while a platinum foil was used as cathode. The Nitinol anode and platinum cathode were placed face to face with a distance of 20 mm apart. A direct current (DC) power supply, or two DC power supplies connected in series, was used to apply voltage. Electrochemical etching and anodisation processes were both carried out at room temperature with continuous magnetic stirring at 300 rpm. Figure 3.1 shows a schematic diagram of the electrochemical modification process.
3.2 Characterization of Modified Nitinol Surfaces

3.2.1 Surface morphology

Surface morphologies of different Nitinol surfaces were observed with an Olympus BX60 optical microscope. More detailed surface morphologies of Nitinol surfaces were
observed with a scanning electron microscope (SEM, Philips XL 30s-FEG), with energy dispersive spectroscopy (EDS), operating at 5 kV acceleration voltage. Surface roughness was measured with a surface profiler (Taylor Hobson Surtronic 3).

### 3.2.2 Composition determination of Nitinol surfaces

Elemental compositions of the modified Nitinol surfaces were semi-quantitatively determined by X-ray photoelectron spectroscopy (XPS, kratos Axis Ultra DLD). XPS is a surface sensitive semi-quantitative spectroscopic technique that measures the elemental compositions and particularly the chemical and electronic state of the elements on the surface. The material within 10 nm from the top of a surface can be analysed. The principles of XPS are described below.

When a surface is irradiated by X-ray, electrons are emitted and measured with their number and energy (Fig. 3.2). The binding energy (BE) of each electron is so calculated according to the equation below:

\[
BE = h\nu - KE - \phi_{\text{spec}}
\]

where \(KE\) is the kinetic energy of the electron as measured by the instrument; \(\phi_{\text{spec}}\) is the work function; and the energy of a photon is given by \(h\nu\) (\(h\) – Planck constant and \(\nu\) - frequency of the radiation).

Figure 3.2 – Schematic example of (a) the photoelectron process and (b) the basic components of an XPS instrument [136].
For each Nitinol surface, a wide or survey scan and a high resolution scan of Ti 2p range were conducted. The XPS spectra were analysed using CasaXPS software. All the XPS peaks were charge corrected with C 1s binding energy of 284.2 eV before further data processing.

The elements present in the surface are identified from the survey scan. In a typical XPS spectrum, i.e. a plot of the number of electron detected per unit time vs. the binding energy, each element produces a characteristic set of XPS peaks at characteristic binding energies. One element could be confirmed only if all the peaks for that element are present. An example of characteristic oxygen peaks is shown in Fig. 3.3. The most intense photoelectron peak for an element is used to define a quantification region, so that the atomic composition can be obtained (Fig. 3.4).

Figure 3.3 – A typical XPS survey scan that shows peaks for oxygen [137].
Figure 3.4 – An XPS survey scan with defined area of each element [137].

The chemical state information of an element present in the surface is extracted from the high resolution scan spectrum by peak de-convolution. The peak of the elements of interest is then fitted with several component peaks, each representing a different chemical environment, whilst the total error is minimized. As p orbitals are split into doublets, pairs of doublet (Ti 2p\text{3/2} and Ti 2p\text{1/2}) represent a single chemical state. Constrains are applied when fitting Ti 2p peak, including an area ratio of 2:1 (Ti 2p\text{3/2} : Ti 2p\text{1/2}) and binding energy difference between each Ti 2p orbit splitting (Table 3.1). The relative amount of each chemical state is evaluated by calculating the areas under each peak.

Table 3.1 – Spectral fitting parameters for Ti 2p [138].

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ti(0)</th>
<th>Ti(II) Oxide</th>
<th>Ti(III) Oxide</th>
<th>Ti(IV) Oxide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ti 2p\text{3/2} (eV)</td>
<td>453.9</td>
<td>455.5</td>
<td>457.3</td>
<td>458.7</td>
</tr>
<tr>
<td>2p\text{1/2} - 2p\text{3/2} splitting (eV)</td>
<td>6.1</td>
<td>5.6</td>
<td>5.2</td>
<td>5.7</td>
</tr>
</tbody>
</table>

3.2.3 Contact angle measurement

Interfaces between biomaterials and body fluids play an important role in determining the nature of the interaction between biomaterials and the living organism. When considering the surface behavior in the development of biocompatible implant, its relationship to its
environment is a key factor. The measurement of the contact angle between the simulated body fluid and the modified Nitinol surfaces is used to define wettability for that particular environment.

The surface energy may be defined as the excess energy at the surface of a material compared to the bulk and is determined according to Fowkes’ theory. In Fowkes’ theory, the surface energy is divided into two components: surface energy due to the dispersive interactions and surface energy due to polar interactions. Incorporated into Young’s relation, the following principle equation is derived:

$$\frac{\sigma_L(\cos \theta + 1)}{2} = \sqrt{\sigma_S^p \sigma_L^p} + \sqrt{\sigma_S^D \sigma_L^D}$$  \hspace{1cm} (3.2)

where $\sigma$ represents the surface tension and $\theta$ represents the contact angle. The subscript S represents the solid surface and L represents the liquid. The superscript P represents the polar component and D represents the dispersive component.

By performing standard sessile drop contact angle measurements with two diagnostic liquids: one with both a dispersive and a polar component to its surface energy and one with only dispersive component to its surface energy (providing $\sigma_L^p = 0$ and $\sigma_L = \sigma_L^D$), the surface free energy of the solid surface of interest could be calculated by solving the above equation algebraically.

Contact angle measurements were conducted with an optical contact angle and surface tension meter (KSV instrument CAM 101). Contact angles were measured by placing droplets of the selected diagnostic liquids and SBF on different Nitinol surfaces. When a droplet of diagnostic liquid was placed on the Nitinol surface (Fig. 3.5), contact angles were obtained by fitting tangents (red lines) to the droplet (inside blue rectangle) using the Young-Laplace method. The fitted left and right angles were highlighted in blue in Fig. 3.5.
Deionized water and diiodomethane were used as the diagnostic liquids. The surface tension parameters of the diagnostic liquids are summarized in Table 3.2.

Table 3.2 – Diagnostic liquids and their surface tension parameters [139].

<table>
<thead>
<tr>
<th>Diagnostic Liquid</th>
<th>Surface Tension (mN/m)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
</tr>
<tr>
<td>Water</td>
<td>72.8</td>
</tr>
<tr>
<td>Di-iiodomethane</td>
<td>50.8</td>
</tr>
</tbody>
</table>

3.2.4 Electrochemical characterization of modified Nitinol surfaces

The electrochemical behavior of the modified Nitinol surfaces was evaluated in 3.5 wt.% NaCl solution, using an electrochemical workstation (CHI 604D). Open circuit potential
(OCP) was firstly recorded and then the potentiodynamic polarization curves, so that the corrosion resistance of modified Nitinol surfaces could be deduced. A standard three-electrode system was used in the electrochemical cell, which includes the modified Nitinol surface with 1 cm² exposed area to electrolyte as the working electrode, a platinum plate as the counter electrode and standard Ag/AgCl as the reference electrode. The polarization curves were measured at a constant scan speed of 0.2 mV/s.

The corrosion behavior was evaluated by Tafel extrapolation method. In the Tafel region, linear behavior exhibits in the overpotential versus current density (log scale) plot [140].

\[ \Delta V = A \times ln\left(\frac{i}{i_0}\right) \]  \hspace{1cm} (3.3)

where \( \Delta V \) is the overpotential; \( A \) is the Tafel slope; \( i \) is the current density; and \( i_0 \) is the exchange current density.

Extrapolation of anodic and cathodic Tafel regions back to the corrosion potential \( (E_{corr}) \), and the intersection point corresponds to corrosion current density \( (i_{corr}) \) or corrosion rate (Fig. 3.6). Tafel constants could be calculated from the anodic and cathodic slopes.

![Figure 3.6 – A typical Tafel plot.](image-url)
3.2.5 Antibacterial properties of modified Nitinol surfaces

Inhibition percentage test was performed to investigate the antibacterial property of the modified Nitinol samples [141]. *Escherichia coli* (*E. coli*), which have been extensively studied as a laboratory model organism, were used in this research to examine the antibacterial properties of Nitinol surfaces. A colony of *E. coli* ATCC25922 was first cultured in 100 mL liquid Tryptic Soy Broth (TSB) medium overnight at 37°C. Then 1 mL of *E. coli* containing TSB medium was inoculated into 100 mL fresh TSB medium in sterile conic flasks. Sterile conic flasks containing 100 mL diluted *E. coli* solution and Nitinol samples were incubated at 37°C for 18 hours. The control sample, which only contained 100 mL *E. coli* solution, was incubated without Nitinol coupon as well. After incubation, the optical density of *E. coli* solutions was measured with an UV-Vis spectrophotometer (Agilent 8453) at the wavelength of 600nm. All experiments were conducted in triplicate. The percentage inhibition was calculated according to the following equation [141].

\[
I\% = \left(\frac{\text{Con}_{18} - \text{Con}_0}{\text{Sample}_{18} - \text{Sample}_0}\right) \times 100
\]  

where the term, \(\text{Con}_{18} - \text{Con}_0\), is the number increase of *E. coli* on the control sample while \((\text{Sample}_{18} - \text{Sample}_0)\) is the number increase of *E. coli* on the Nitinol samples.

3.3 Biocompatibility Assessment of Anodised Nitinol Surfaces

3.3.1 Ni ion release in simulated body fluid

The toxic Ni ion release from the modified Nitinol surfaces into a physiological environment was investigated by immersion test. The surface modified Nitinol coupon was sealed, so that only 10 mm × 10 mm square area of the modified Nitinol surface was exposed. The sealed Nitinol coupon was immersed in 10 mL of SBF, with the exposed surface face up. The tube containing Nitinol coupon and SBF was left in water bath kept at 37 °C. Fresh SBF was changed every 4 days and the used SBF solutions were kept refrigerated for subsequent tests. Fresh SBF was changed 7 times for each Nitinol sample, so that the total immersion time was 28 days.
Followed by the immersion test, the concentration of Ni ion released from the modified Nitinol surface into SBF solution was determined by flame atomic absorption spectrometry (FAAS, Varian SpectrAA 50). The instrument parameters for measuring the Ni ion concentration by FAAS are given below in Table 3.3.

Table 3.3 – instrumental parameters for the measurement by FAAS.

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>Lamp current (mA)</th>
<th>Flame stoichiometry</th>
<th>Bandwidth (nm)</th>
<th>Fuel</th>
<th>Support</th>
</tr>
</thead>
<tbody>
<tr>
<td>232.0</td>
<td>4 mA</td>
<td>oxidizing</td>
<td>0.2</td>
<td>acetylene</td>
<td>air</td>
</tr>
</tbody>
</table>

Before the FAAS test, the stored SBF solutions were first acidified by adding one drop of concentrated nitric acid into each tube, and then filtered to eliminate any precipitates. The filtered SBF solutions were tested by FAAS and three readings were recorded for each solution sample. For each Nitinol sample, the Ni ion concentration released into SBF during every 4 days was recorded and accumulated to find out the Ni ion released over a 28-day period in a simulated physiological environment.

### 3.3.2 Hydroxyapatite formation on modified Nitinol surfaces

Hydroxyapatite (HA) is a main component of human bones. The ability of an implant material to induce the deposition and formation of HA is an important property. In this study, surface modified Nitinol coupons were sealed so that the exposed modified area was 150 mm². The sealed Nitinol coupons were then immersed in SBF. The tubes containing Nitinol coupons and SBF were kept in water bath at 37 °C. After 3 and 4 weeks, Nitinol coupons were removed from SBF and gently dried with line free paper towels. The Nitinol coupons after immersion were then subject to X-ray diffraction (XRD, Bruker AXS D8 Advanced, with monochromatic Cu-Kα source). Nitinol samples were scanned in the 2θ range from 20° to 80° at a scanning rate of 0.02° per second. All the XRD patterns are presented with background subtracted and Kα2 line stripped.
3.3.3 Cell-material interaction

Nitinol coupons anodised under 20 V for 1 h were investigated for the cell biology and the ground Nitinol samples were used as control. Nitinol samples were immersed in 70% ethanol the night before the cell experiment. Immediately before the cell experiment, each Nitinol sample was dipped in absolute ethanol and passed through flame so that ethanol could burn out. Well sterilized and dried Nitinol samples were then transferred into 24-well tissue culture plates, with the modified surface faced up.

Mammalian cell culture, murine fibroblast cell line L929 (ATCC CCL-1), was used to evaluate the cell-material interaction. The medium used for L929 cell culture was Dulbecco’s Modified Eagle Medium (DMEM) containing 10% heat activated fetal calf serum (FCS) (both from Life Technologies). 1 mL aliquots of cell culture medium containing $1 \times 10^5$ cells/mL L929 cells were placed into half wells with Nitinol samples. 1 mL aliquots of medium solution were placed into the other half wells with Nitinol samples and these half Nitinol samples were used as control. The plate containing both cells and medium control were incubated at 37 °C in 5% CO$_2$ in air for 24 h, after which Nitinol samples were transferred into clean wells, so that only the cells attached to the Nitinol samples would be cultured for following tests.

The cell viability at different stages of proliferation was tested using resazurin fluorescence assay. The Nitinol samples were first cleaned with 1 mL of phosphate buffered saline (PBS) twice and then 1 mL of 500 µM resazurin solution were placed in each well and incubated for 4 h. The supernatant was collected and centrifuged at 2000 rpm for 5 min at room temperature to pellet any cells. 100 µL of the centrifuged supernatant was aliquoted in triplicate into 96-well black microtitre plate and the fluorescence value was recorded at 530 nm excitation and 590 nm emission using a Perkin Elmer Enspire 2300 Multibabel Reader.

After the cell viability test, the cell culture on Nitinol samples were washed twice with PBS and then fresh DMEM supplemented with 10% FCS was added and returned to the incubator. The above procedure was repeated daily over 4 days. After the fluorescence reading of the fourth day was taken, the cell culture on Nitinol samples were washed with PBS and fixed with 2.5% glutaraldehyde in PBS and stored at 4 °C. The cells on Nitinol samples after 1 day culture were also fixed. The fixed cells were imaged using an environmental electron...
scanning microscope (ESEM, FEI Quanta 200FE) at 2 °C. The entire week cell experiment was repeated three times.
Chapter 4 Surface Pretreatment and Electrochemical Etching of Nitinol

4.1 Introduction

Nitinol has attracted much attention since its discovery in 1960s and it has been widely applied, especially in biomedical areas. Nitinol is normally surface modified for biomedical applications. As described in Chapter 2, electrochemical etching has been widely used to modify the surface of Nitinol [57, 61, 142]. This technique could efficiently eliminate toxic Ni from Nitinol surface and enhance the formation of protective TiO$_2$. In electrochemical etching process, the commonly used electrolyte is H$_2$SO$_4$-methanol solution [143-145]. A smooth surface finish could be obtained in this electrolyte, due to the suppressed etching rate, and so methanolic H$_2$SO$_4$ solution is normally applied to electropolishing Ti and Ti alloys. However, in order to efficiently etch samples that are covered with hard oxide layer, hydrofluoric acid works better [47]. In this chapter, a new electrolyte, which contained H$_3$PO$_4$ and NH$_4$F, was used to electrochemically etch Nitinol. As HF was not directly used, this new electrolyte was less dangerous. The influences of etching parameters – applied voltage and etching time – were also investigated. Before electrochemical etching, the Nitinol surface was pretreated using three different methods of grinding, mechanical polishing and chemical etching, to find out the most effective surface pretreatment method on Nitinol.

4.2 Experimental

4.2.1 Surface pretreatment

The material studied in this chapter was commercially supplied Nitinol plate (about 2 mm thick). Chemical analysis shows a Ni content of 55.6 wt.% (i.e. 50.5 at.%). The Nitinol plate was cut into small square coupons with dimensions of 20 mm × 20 mm. The surfaces of these Nitinol coupons were pretreated by three different procedures, namely grinding (G), mechanical polishing (Mp) and chemical etching (Ce).
Grinding was carried out under running water on a series of SiC papers (Struers) and Nitinol coupons were ground up to a grit of #2400. On each grinding paper, Nitinol coupons were ground in one direction until the grinding lines from the previous stage were removed; and when changing grinding paper, Nitinol coupons were thoroughly cleaned with water. Followed by grinding, some Nitinol coupons were mechanically polished using diamond paste of 6 µm, 3 µm and finally down to 1 µm. After grinding and mechanical polishing, Nitinol coupons were ultrasonically cleaned in acetone, ethanol and distilled water for 15 min respectively. Some ground Nitinol coupons were subjected to chemical etching. The solution used for chemical etching had a composition of 40% HNO₃ and 10% HF and chemical etching was conducted at room temperature for 6 min. Immediately after the substrate pretreatment, electrochemical etching was performed as described below.

4.2.2 Electrochemical etching

Electrochemical etching was carried out on the pretreated Nitinol coupons. The electrochemical etching solution was 1M H₃PO₄ solution with 10 wt.% NH₄F. Electrochemical etching process was undertaken at room temperature with continuous magnetic stirring at 300 rpm. In the electrochemical etching set-up, the Nitinol coupon was used as anode, while a nickel electrode was used as cathode. Nitinol coupon and nickel electrode were placed face to face with a distance of 20 mm apart. A direct current (DC) power supply was used and the applied voltage varied from 1 V to 10 V. The electrochemical etching time ranged from 5 min to 20 min.

4.2.3 Characterization of modified Nitinol surfaces

Surface morphologies of the modified Nitinol were observed with both optical microscopy (OM) and scanning electron microscopy (SEM), equipped with energy dispersive spectroscopy (EDS). Chemical compositions of the Nitinol surface layer were investigated by X-ray photoelectron spectrometry (XPS). Survey scans were conducted over a binding energy from 0 eV to 1000 eV and a constant pass energy of 50 eV at 0.8 eV/step was used. High resolution scan of Ti was recorded at constant pass energy of 20 eV at 0.1 eV/stsp. The spectra from survey scans and high resolution scans were analysed using CasaXPS software.
To evaluate the influence of microstructure of the modified Nitinol surface on surface wettability, contact angles were measured using a face contact angle goniometer equipped with a microscope and a camera. Corrosion resistance of the modified Nitinol surfaces was investigated by an electrochemical workstation (CHI 604D). The potentiodynamical polarization curves (Tafel region) were obtained in 3.5% NaCl solution at room temperature. A standard three-electrode system was used in the electrochemical cell, which includes the Nitinol as the working electrode, a platinum plate as the counter electrode and standard Ag/AgCl as the reference electrode. The polarization curves were measured at a constant scan speed of 0.2 mV/s.

4.3 Results and Discussion

4.3.1 Effect of surface pretreatment

After pretreating using different methods (grinding, mechanically polishing and chemical etching), different Nitinol surface conditions were obtained. Fig. 4.1 shows morphologies of these Nitinol surfaces.

After grinding with 2400-grit SiC paper, a relatively smooth surface was obtained (Fig. 4.1 (a)), with some scratch lines shown on the surface. The average surface roughness of the mechanically ground Nitinol sample was $R_a = 0.02 \pm 0.004 \ \mu m$. Further mechanically polishing the ground Nitinol sample down to 1 \ \mu m resulted in a smoother surface (Fig. 4.1 (b)), with an average surface roughness of $0.01 \pm 0.002 \ \mu m$. This value was similar with other reported roughness [84]. As expected, there is not any obvious surface detail shown without etching. Chemical etching of Nitinol sample resulted in an un-even surface (Fig. 4.1 (c)) and the measured surface roughness was $0.03 \pm 0.005 \ \mu m$. With the use of the 40% HNO$_3$ + 10% HF solution, the grain structures were not revealed. Instead, the chemically etched Nitinol surface was covered by round nodules, which were formed arising from different dissolution rates of titanium and nickel, or of different phases in the bulk Nitinol sample. The various pretreatment methods led to different surface roughness in the order as: mechanically polishing < grinding < chemical etching.
Figure 4.1 – SEM images of Nitinol surfaces prepared by: (a) grinding, (b) mechanical polishing and (c) chemical etching.
Several particles were found randomly on Nitinol surfaces and Fig. 4.2 below shows an enlarged picture of those particles. EDS analysis (Fig. 4.2 (b)) indicated that those particles were titanium carbide or titanium-nickel carbide. The graphite crucible used for alloying process provides the source of carbon, so such inclusions are normally found on Nitinol surfaces [64, 73].

![Image](a) ![Image](b)

Figure 4.2 – SEM image of inclusions found on Nitinol surface and its EDS measurement.

The chemical compositions of these pretreated Nitinol surfaces were also investigated by XPS. Both survey scan and high resolution scan of Ti spectra were recorded. Fig. 4.3 shows the XPS survey scan spectra, from which the chemical compositions of different Nitinol surfaces were obtained and are summarized in Table 4.1. The predominant elements presented on the Nitinol surfaces are carbon, oxygen, titanium and nickel for all the three types of surface. The concentration of C is always the highest, which is the major contaminant for the XPS samples. Carbon may come from the cleaning procedure (with absolute ethanol), adsorption from the atmosphere and contacting with carbon containing materials, such as plastic sample bag. The second highest element detected from Nitinol surfaces treated by three different pretreatment methods is oxygen, which counts about a quarter of the total atomic percentage. The high concentration of oxygen could result from the formation of oxide and adsorption of oxygen containing species from the ambient. Other than carbon and oxygen, there are some minor contaminants including calcium, nitrogen fluorine and copper. Ca came from the grinding procedure and was found on Nitinol surfaces treated by grinding and chemical etching. After mechanical polishing, Ca was totally
removed from the Nitinol surface and no Ca was detected by XPS on mechanically polished Nitinol surface. N came from environmental contamination, and for chemical etching sample, it also came from the etching solution residue, which contained nitric acid. F was only found on chemical etched Nitinol surface and was from etching solution. Cu was found only on mechanically polished Nitinol surface and was from the polishing cloth which was used to polish copper samples.

Figure 4.3 – XPS survey scan spectra of Nitinol surface treated with different methods, from bottom to top are grinding, mechanically polishing and chemical etching samples.

The atomic concentration of Ti and Ni could be influenced by the contamination on the Nitinol surfaces. The more contaminant is adsorbed onto the Nitinol surfaces; the lower is the atomic concentration of Ti and Ni detected by XPS. Therefore, Ti/Ni molar ratio is a more accurate expression of how much Ti and Ni presented in the surface layer of Nitinol after surface pretreatments and is shown in Table 4.1. Theoretically, the Ti/Ni molar ratio should be 1.0 and the Nitinol material used in this research has a Ni content of 55.6 wt.%, i.e., a Ti/Ni molar ratio of 0.98. However, the Ti/Ni ratios of the different Nitinol surfaces are different from the value for the bulk material and they are all significantly greater than 1.0. The surface Ti/Ni molar ratio would be altered by different surface pretreatment methods. The ground Nitinol surface shows a Ti/Ni ratio of 1.53. Nitinol is readily oxidized when exposed to air, resulting in coverage of titanium oxide layer. As a consequence, the Ti/Ni ratio of ground Nitinol surface is higher than 1.0. Further mechanical polishing led to a decreased Ti/Ni ratio of 1.35, as compared to the ground samples. It seems that the
mechanical polishing procedure preferentially remove Ti or titanium oxides from the Nitinol surface, resulting in a slight decreasing of Ti/Ni ratio.

Table 4.1 – Atomic concentration of the elements detected by XPS survey scan.

<table>
<thead>
<tr>
<th>Elements</th>
<th>Atomic concentration (%)</th>
<th>G</th>
<th>Mp</th>
<th>Ce</th>
</tr>
</thead>
<tbody>
<tr>
<td>C 1s</td>
<td></td>
<td>54.00</td>
<td>68.44</td>
<td>38.96</td>
</tr>
<tr>
<td>O 1s</td>
<td></td>
<td>30.79</td>
<td>21.18</td>
<td>30.43</td>
</tr>
<tr>
<td>Ti 2p</td>
<td></td>
<td>6.83</td>
<td>4.19</td>
<td>15.50</td>
</tr>
<tr>
<td>Ni 2p</td>
<td></td>
<td>4.52</td>
<td>3.10</td>
<td>9.57</td>
</tr>
<tr>
<td>Ca 2p</td>
<td></td>
<td>2.36</td>
<td>-</td>
<td>0.50</td>
</tr>
<tr>
<td>N 1s</td>
<td></td>
<td>1.39</td>
<td>1.63</td>
<td>3.58</td>
</tr>
<tr>
<td>F 1s</td>
<td></td>
<td>-</td>
<td>-</td>
<td>1.45</td>
</tr>
<tr>
<td>Cu 2p</td>
<td></td>
<td>-</td>
<td>1.46</td>
<td>-</td>
</tr>
<tr>
<td>Ti/Ni ratio</td>
<td></td>
<td>1.53</td>
<td>1.35</td>
<td>1.62</td>
</tr>
</tbody>
</table>

When the Nitinol sample is subjected to chemical etching, chemical reactions take place at the interface of Nitinol surface and etching solution as follows [146]:

- Dissolution of TiO$_2$ layer

\[ \text{TiO}_2 + \text{HF}^- + 4\text{H}^+ \rightarrow [\text{TiF}_6]^{2-} + 2\text{H}_2\text{O} \]  \hspace{1cm} (4.1)

- Preferential oxidation of Ti back to TiO$_2$

\[ \text{Ti} + 4\text{NO}_3^- + 4\text{H}^+ \rightarrow \text{TiO}_2 + 4\text{NO}_2 + 2\text{H}_2\text{O} \]  \hspace{1cm} (4.2)

- Simultaneously, Ni dissolves

\[ \text{Ni} + 2\text{H}^+ \rightarrow \text{Ni}^{2+} + \text{H}_2 \]  \hspace{1cm} (4.3)
As a result of competing dissolution and formation reactions, an uneven Nitinol surface with round nodules are produced (Fig. 4.1 (c)). The Nitinol surface has been treated in an oxidizing environment and so the Ti/Ni ratio (1.62) is higher than that of just ground Nitinol surface. By all the three surface pretreatment methods, Ti enriched surfaces were produced on Nitinol samples. There are also reports showing similar results of enrichment of Ti by grinding [61], mechanical polishing [57, 142] and chemical etching [61, 142].

The XPS survey scan spectra give an indication of what elements are presented on the Nitinol surfaces. To further quantitatively investigate how the surface pretreatment methods affect the resulted Nitinol surfaces, high resolution spectra of the main component, titanium, were recorded. Fig. 4.4 shows the spectra of Ti and from which, the chemical state of Ti could be found out.

![Figure 4.4 – High resolution spectra of Ti 2p on (a) ground, (b) mechanically polished and (c) chemically etched Nitinol surfaces.](image)
The Ti 2p peak has Ti 2p\textsubscript{3/2} and Ti 2p\textsubscript{1/2} splitting, and so the Ti high resolution scan spectrum could be fitted by four pairs of peaks, representing the four chemical states of Ti: Ti\textsuperscript{4+}, Ti\textsuperscript{3+}, Ti\textsuperscript{2+} and Ti\textsuperscript{0}. The fitting constraints and parameters were described in Chapter 3. Ti(IV) represents TiO\textsubscript{2}, Ti(III) and Ti(II) represent sub-oxides of Ti, and Ti(0) could be elemental Ti or represents the intermetallic bond between Ti and Ni in the substrate. By calculating the area under the peaks, the concentration of each Ti chemical state could be determined and is summarized in Table 4.2.

### Table 4.2 – Concentration of each Ti chemical state on Nitinol surfaces treated with different methods.

<table>
<thead>
<tr>
<th>Ti components</th>
<th>Concentration of Ti components (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G</td>
</tr>
<tr>
<td>Ti(IV) oxide</td>
<td>76.29</td>
</tr>
<tr>
<td>Ti(III) oxide</td>
<td>17.63</td>
</tr>
<tr>
<td>Ti(II) oxide</td>
<td>3.78</td>
</tr>
<tr>
<td>Ti(0)</td>
<td>2.21</td>
</tr>
</tbody>
</table>

After surface pretreatment, the resulting Nitinol surfaces were covered by oxides layer with different titanium contents. On the ground Nitinol surface, the concentration of Ti(IV) was 76.29% – the highest among all the three Nitinol surfaces pretreated by different methods. This indicates that grinding could produce a surface which enhances the native titanium oxide layer to grow on it. With further mechanically polishing the ground Nitinol surface, the concentration of Ti(IV) dropped to 54.20%. It seemed that mechanical polishing removed the TiO\textsubscript{2} and TiO layers from the previous procedure. A very smooth surface finish was obtained on Nitinol by mechanical polishing, which is more corrosion/oxidation resistant. After chemical etching, the concentration of Ti(IV) further dropped to 40.77%, indicating that the TiO\textsubscript{2} layer was destroyed or dissolved during chemical etching. However, the concentration of the other titanium sub-oxides increased, and the total amount of all oxides (Ti(IV), Ti(III) and Ti(II)) is higher than that of the mechanically polished Nitinol surface. It is noted that on the chemical etched Nitinol surface, all the titanium oxides added up to a concentration of
83.8%, compared with 71.2% on the mechanically polished Nitinol surface. As mentioned before, chemical etching is a competing process of oxidation and dissolution. While the native TiO$_2$ layer is broken down by chemical etching process, the oxidation of Ti is still oxidized to some extent.

The effect of surface pretreatment on the corrosion behavior of different Nitinol surfaces was also investigated. The polarization curves of different Nitinol surfaces are shown in Fig. 4.5. The ground Nitinol surface exhibited the least negative corrosion potential and lowest corrosion current, indicating the best corrosion resistance in 3.5 % NaCl solution. The significantly different corrosion potentials observed in these Nitinol surfaces indicate the different nature of the oxides presented in the surface.

![Potentiodynamic polarization curves](image)

Figure 4.5 – Potentiodynamic polarization curves recorded for Nitinol after different surface pre-treatments in 3.5 wt.% NaCl solution.

In summary, three methods were used to pretreat Nitinol surface: grinding, mechanical polishing and chemical etching. The effect of different surface pretreatment methods on the morphology, chemical composition and corrosion behavior of Nitinol surfaces were discussed in this section. Grinding, as a simple procedure, could effectively produce a surface finish that is relatively smooth and corrosion resistant, and therefore was chosen to pretreat Nitinol surface for the following surface modification experiments.
4.3.2 Effect of electrochemical etching parameters

4.3.2.1 Surface morphologies

By electrochemical etching, the surface of Nitinol has been modified. The etching parameters, both applied voltage and etching time influenced the resultant Nitinol surface. Different Nitinol surfaces were firstly examined under an optical microscope and are shown in Fig. 4.6.

![Optical microscopic images of Nitinol surfaces treated under different conditions: etching time of 5 min, 10 min and 20 min; and applied voltage of 1 V, 3V and 6 V.](image)

Figure 4.6 - Optical microscopic images of Nitinol surfaces treated under different conditions: etching time of 5 min, 10 min and 20 min; and applied voltage of 1 V, 3V and 6 V.

Generally, Nitinol surfaces became rough by electrochemical etching and round nodules were produced on Nitinol surfaces. However, different Nitinol surfaces resulted from electrochemical etching under different conditions. In the case of etching at 1V, some round nodules appeared on the surface at random locations if they were etched for 5 min. Increasing the etching time to 10 min led to more nodules produced. When the etching time was further increased to 20 min, Nitinol surface was totally covered with round nodules. Similar phenomenon was observed on Nitinol surfaces that were electrochemically etched at 3 V,
where round nodules started to appear at 5 min and the number of nodules increased with increasing etching time. In the case of the applied voltage of 6 V, round nodules could be observed on Nitinol surface that was etched for 5 min. However, there were significantly more nodules produced at 5 min when electrochemically etched at 6 V than 1 V and 3 V. At 6 V, the Nitinol surface became very rough and clear images could not be obtained on the surfaces etched for 20 min by optical microscopy. Therefore, SEM was used to get a closer examination of the electrochemically etched Nitinol surfaces.

From the optical microscope images, it is noticed that both the applied voltage and etching duration time affect the electrochemically etched Nitinol surfaces. Fig. 4.7 shows the different surface morphologies of Nitinol which were electrochemically etched for 5 min, but under different voltages ranging from 1 V to 10 V. On the Nitinol surface etched at 1 V, there are some small nodules appearing, but the grinding lines from the pretreatment process could be clearly observed, Fig. 4.7 (a). In the samples etched at 3 V, more and larger nodules could be seen on the Nitinol surface, with most grinding lines disappearing (Fig 4.7 (b)). With further increasing etching voltage yet remaining the same time of 5 min, both the number and size of the nodules on Nitinol surface increased. The Nitinol surface had be fully covered with round nodules when the voltage increased to 6 V, Fig. 4.7 (c). The size of those nodules increased from about 10 µm (at 1V) to about 30 µm at 10V.

Etching time also influences the surface morphology of Nitinol. Fig. 4.8 above shows the Nitinol surfaces etched at 3 V, but for different times. Extending the etching time from 5 min to 10 min led to more nodules produced on the Nitinol surface, and the nodules were bigger after etching for 10 min. After being etched for 20 min, the Nitinol surface was fully covered by round nodules; the size of these nodules did not significantly increase yet the number increased significantly.

Increasing etching time had similar effect on the Nitinol surfaces which were etched at 6 V (Fig. 4.9). The size and number of round nodules increased with increasing etching time from 5 min to 10 min. However, the Nitinol surface was fully with nodules when etched for 10 min and extending etching time to 20 min, the surface morphology of Nitinol did not change obviously. In other words, a higher voltage would accelerate the etching process.
Figure 4.7 – SEM micrographs showing the surface morphology of Nitinol being electrochemically etched for 5 min at different applied voltage (a) 1V, (b) 3 V, (c) 6 V, (d) 8 V and (e) 10V.
Figure 4.8 – Nitinol surface etched at 3 V for (a) 5 min, (b) 10 min and (c) 20 min.
Figure 4.9 – Nitinol surface etched at 6 V for (a) 5 min, (b) 10 min and (c) 20 min.
4.3.2.2 Chemical compositions of Nitinol surfaces

After electrochemical etching, the chemical compositions of Nitinol surfaces were also altered. Table 4.3 below lists the XPS results of the Nitinol surface electrochemically etched at 3 V, but for different etching times. When the Nitinol surface was electrochemically etched for 5 min, the Ti/Ni molar ratio was 6.30, much higher than that of the ground Nitinol surface (1.35). This is due to the oxidation of Ti and dissolution of Ni during the electrochemical etching process. After 10 min of electrochemical etching, the Ti/Ni ratio on the Nitinol surface decreased to 4.45, and after further etching of 20 min, the Ti/Ni ratio decreased further to 2.30. It seems that at the start of the electrochemical etching process, oxidation of Ti is much enhanced, but the oxide layer dissolves with a longer etching time.

Also as shown in Table 4.3, the surface oxides mainly consist of approximately 70% of TiO₂ and 10% of metallic Ti. The remaining oxides were made up with titanium sub-oxides, which largely depend on the etching time.

Table 4.3 – XPS results of Nitinol surface electrochemically etched at 3 V.

<table>
<thead>
<tr>
<th>Component</th>
<th>5 min</th>
<th>10 min</th>
<th>20 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ti(IV) oxide</td>
<td>65.6</td>
<td>73.8</td>
<td>66.2</td>
</tr>
<tr>
<td>Ti(III) oxide</td>
<td>6.19</td>
<td>11.8</td>
<td>17.7</td>
</tr>
<tr>
<td>Ti(II) oxide</td>
<td>18.1</td>
<td>5.94</td>
<td>6.89</td>
</tr>
<tr>
<td>Ti(0)</td>
<td>10.1</td>
<td>8.48</td>
<td>15.0</td>
</tr>
<tr>
<td>Ti/Ni ratio</td>
<td>6.30</td>
<td>4.45</td>
<td>2.30</td>
</tr>
</tbody>
</table>

Similar findings were observed on the Nitinol surfaces electrochemically etched at 6 V (shown in Table 4.4). When the Nitinol surface was etched for 5 min, the Ti/Ni ratio was 6.59, slightly higher than that of the Nitinol etched at 3 V. The titanium oxide layer formed on the Nitinol surface etched at 6 V contained about 80% of TiO₂ and only about 4% of metallic Ti. This is due to the higher applied voltage and therefore enhanced oxidation. When the Nitinol surface was electrochemically etched for 10 min, the Ti/Ni ratio dropped to 2.46. The decrease in the Ti/Ni ratio with longer etching time is again due to the dissolution of titanium.
oxide layer. However, further increasing the etching time to 20 min did not change the Ti/Ni ratio significantly (2.33 for 20 min). The titanium oxide layers on the Nitinol surfaces etched for 10 and 20 min also had similar compositions of about 70% of TiO₂ and 13% of metallic Ti. As described before, the two Nitinol surfaces had similar morphologies. It seems that 10 min was the point when the electrochemical etching process at 6 V reaches equilibrium, so that the surface morphology and chemical compositions did not change with a longer etching time.

Table 4.4 – XPS results of Nitinol surface electrochemically etched at 6 V.

<table>
<thead>
<tr>
<th>Component</th>
<th>5 min</th>
<th>10 min</th>
<th>20 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ti(IV) oxide</td>
<td>81.0</td>
<td>64.2</td>
<td>71.4</td>
</tr>
<tr>
<td>Ti(III) oxide</td>
<td>3.88</td>
<td>14.6</td>
<td>7.1</td>
</tr>
<tr>
<td>Ti(II) oxide</td>
<td>11.3</td>
<td>7.64</td>
<td>9.00</td>
</tr>
<tr>
<td>Ti(0)</td>
<td>3.79</td>
<td>13.6</td>
<td>12.5</td>
</tr>
<tr>
<td>Ti/Ni ratio</td>
<td>6.59</td>
<td>2.46</td>
<td>2.33</td>
</tr>
</tbody>
</table>

Generally, the Ni content in the surface layer of Nitinol was reduced and the oxidation of Ti was enhanced by electrochemically etching. Even though the Ti/Ni ratio decreases with increasing etching time, the lowest value obtained (2.30 for 3V and 20 min) is still higher than that of the ground Nitinol surface.

4.3.2.3 Discussion on the electrode reactions

To analyse how the above surface morphologies and chemical compositions are generated on the electrochemically etched Nitinol surface, the reactions occurring during the electrochemical etching process will be described in this sub-section. The ground Nitinol surface is covered with naturally formed titanium oxide layer. With the presence of H⁺ and F⁻ ions in the electrolyte, HF formed and attacked the TiO₂ layer and so dissolves into electrolyte through the following reaction:

\[
\text{TiO}_2 + 4\text{HF} + 2\text{F}^- \rightarrow \text{TiF}_6^{2-} + 2\text{H}_2\text{O} \quad (4.4)
\]
When a voltage is applied, current flows from the Nitinol surface into the electrolyte, so that the following oxidation reactions start at the Nitinol anode:

\[
\text{Ti} + 2\text{H}_2\text{O} \rightarrow \text{TiO}_2 + 4\text{H}^+ + 4\text{e}^-(4.5)
\]

\[
\text{Ni} \rightarrow \text{Ni}^{2+} + 2\text{e}^-(4.6)
\]

If the applied voltage is sufficiently high, the oxidation of water to form oxygen gas will accompany as well:

\[
2\text{H}_2\text{O} \rightarrow \text{O}_2 + 4\text{H}^+ + 4\text{e}^-(4.7)
\]

At counter Ni cathode, water reduction is observed through the reaction (4.8) and the OH\(^-\) produced overcompensates the H\(^+\) from the Nitinol anode.

\[
2\text{H}_2\text{O} + 2\text{e}^- \rightarrow \text{H}_2 + 2\text{OH}^- (4.8)
\]

An electrolytic process is controlled by many parameters, and there is not one theory that is able to explain all the phenomena observed during the electrolytic process of Nitinol. The most plausible theory was proposed by Jacquet [147], whose model was modified or incorporated in other models. According to this theory, a viscous film exists between the anode and electrolyte and is presumed flat on the outer side, which is the side close to the electrolyte (Fig. 4.10). Cations, such as Ni\(^{2+}\) and H\(^+\), produced at the anode surface have to diffuse through this viscous film before they could move towards cathode.

As the surface of anode is not perfectly flat, the thickness of that viscous film is not constant. A close up view of the viscous film on top of the Nitinol anode surface is shown in Fig. 4.11. At protruding peak, the viscous film is thinnest (d\(_1\)), providing the lowest electrical resistance. In the valley, the viscous film has the greatest thickness (d\(_2\)), providing the highest resistivity. The Nitinol surface is covered by naturally formed TiO\(_2\) layer, when voltage is applied, more TiO\(_2\) formed through reaction (4.5). Even though this passive layer dissolves while created, this solid TiO\(_2\) layer always exits during the electrolytic process, as the electrochemically etched Nitinol surface had higher Ti/Ni ratio than ground Nitinol surface.
As mentioned above, three reactions associated with Nitinol occur at anode: dissolution of Ni into Ni\(^{2+}\) (Eq. 4.6), oxidation of Ti into TiO\(_2\) (Eq. 4.5) and dissolution of TiO\(_2\) by HF (Eq. 4.4). At the protruding peak, the first two reactions are promoted, due to lower electrical resistance. In the valley area, protons produced via reactions (4.5) and (4.7) cause a local pH value decrease. As the diffusion path of H\(^+\) is longer from valley than from protruding peak, protons accumulate in the valley area. The following speciation equilibria exist:
\[
\text{HF} \leftrightarrow \text{H}^+ + \text{F}^- 
\] (4.9)

\[
\text{HF}_2^- \leftrightarrow \text{HF} + \text{F}^- 
\] (4.10)

An increase in the concentration of \(\text{H}^+\) makes reactions (4.9) and (4.10) moving to the left, resulting in an increased concentration of HF and HF\(_2^-\). Therefore, the dissolution rate of TiO\(_2\) is higher in the valleys than at the protruding peak.

As a result of competing oxidation and dissolution reactions on the Nitinol surface, as well as the combination of different reaction rate in different areas, the surface morphologies are generated as shown before. At the point when equilibrium is reached, the surface morphology and chemical compositions remain unchanged. With increasing the etching voltage, the reaction rate of electrochemical etching process is increased, so that it takes less time for the entire process to reach equilibrium. Further extending the etching time will only reduce the thickness of the Nitinol coupon, but will not influence the resulting surface morphology and chemical compositions. As for the Nitinol surfaces electrochemically etched at 3 V, the surface morphology (Fig. 4.8) and chemical compositions (Table 4.3) varied during the 20-min etching. However, for the Nitinol surfaces electrochemically etched at 6 V, equilibrium is reached when etched for 10 min, as the morphology (Fig. 4.9) and chemical compositions (Table 4.4) did not change significantly if etched for 20 min.

4.3.2.4 Surface wettability

The wettability of electrochemically etched Nitinol surfaces were measured with simulated body fluid (SBF) and the contact angles with SBF are shown in Fig. 4.12.
Figure 4.12 – Contact angle with SBF measured on Nitinol surfaces electrochemically etched at (a) 3 V and (b) 6 V for different etching time.

The ground Nitinol surface had a contact angle with SBF of 84°. After electrochemically etching at 3 V for 5 min, the contact angle on Nitinol surface showed a lower value of 78°. With increasing etching time, the contact angles decreased to 73° for 10 min and 70° for 20 min.

The decreasing trend of contact angle with increasing etching time was also found on the electrochemically etched Nitinol surfaces at 6 V. After etching for 5 min, the Nitinol surface had a contact angle of 72°. When etched for 10 min, the contact angle decreased to 56° and decreased to slightly lower value of 55° for 20 min.
For the same etching time, the Nitinol surface electrochemically etched at a higher voltage showed a lower contact angle with SBF. The decreased contact angle or increased wettability is an indication of increase in surface energy, and is deemed to result from different Nitinol surface morphologies associated with different etching parameters. A rougher surface is obtained by a longer etching time or a higher applied voltage and such Nitinol surface showed better wettability with SBF (lower contact angle). The enhanced wettability is beneficial for cell attachment and implies better biocompatibility.

4.3.2.5 Corrosion behavior in NaCl solution

The electrochemical characteristics of the electrochemically etched Nitinol surfaces were investigated and polarization curves recorded in 3.5 % NaCl solution are shown in Fig. 4.13 and Fig. 4.14 for Nitinol surfaces electrochemically etched at 3 V and 6 V respectively.

Figure 4.13 – Potentiodynamic polarization curves recorded for Nitinol electrochemically etched at 3 V, for different etching time, in 3.5 wt.% NaCl solution.
Figure 4.14 – Potentiodynamic polarization curves recorded for Nitinol electrochemically etched at 6 V, for different etching time, in 3.5 wt.% NaCl solution.

Table 4.5 – Corrosion parameters of different Nitinol surfaces deduced from polarization curves.

<table>
<thead>
<tr>
<th>Surface</th>
<th>$E_{\text{corr}}$ (V)</th>
<th>$I_{\text{corr}}$ (µA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td>-0.35</td>
<td>0.76</td>
</tr>
<tr>
<td>3V, 5 min</td>
<td>-0.32</td>
<td>1.4</td>
</tr>
<tr>
<td>3V, 20 min</td>
<td>-0.29</td>
<td>2.3</td>
</tr>
<tr>
<td>6V, 5 min</td>
<td>-0.29</td>
<td>2.9</td>
</tr>
<tr>
<td>6V, 20 min</td>
<td>-0.26</td>
<td>2.3</td>
</tr>
</tbody>
</table>

From the Tafel regions, the corrosion potential $E_{\text{corr}}$ and corrosion current $I_{\text{corr}}$ were determined and are listed in Table 4.5. Generally, the corrosion potentials became less negative for electrochemically etched Nitinol surfaces, compared with the ground Nitinol surface. This is a confirmation that the surface layer contains less amount of Ni. However, the corrosion current increased by electrochemical etching. This might be due to the rougher
Nitinol surface generated by electrochemical etching, as the contact area with NaCl solution increased. Therefore, the corrosion current increased with increasing etching time, regardless of etching voltages (3 V or 6 V).

4.4 Summary

After electrochemical etching, rough Nitinol surfaces with round nodules were generated. The surface morphologies and chemical compositions of Nitinol were controlled by the electrochemical etching process parameters, including voltage and etching time. With a longer etching time, the Nitinol surface would be covered by more round nodules. With a higher voltage, larger round nodules were produced and equilibrium would be reached in a shorter time. The resulting surface morphology and chemical compositions are due to the competing dissolution and oxidation reactions and the combination of different reaction rate at different locations on the Nitinol surface. The electrochemically etched Nitinol surface showed a better wettability than the ground Nitinol surface, indicating better biocompatibility. Moreover, the electrochemically etched Nitinol surface exhibited better corrosion resistance than the ground Nitinol surface. Therefore, electrochemical etching is proved to be an effective method to modify Nitinol surface for biomedical applications.
Chapter 5  
Anodisation of Nitinol – Influence of Electrolyte Compositions

5.1 Introduction

In biomedical areas, the surface condition of implant is important for its effect on metal ion release, biocompatibility and corrosion behaviour. The biocompatibility of Nitinol implant generally depends on the naturally formed corrosion-resistant titanium oxide layer, which provides a barrier of allergic and toxic Ni ion release [34]. However, this naturally formed TiO$_2$ layer is too thin to act as a good barrier of Ni ion diffusion from corrosion and leaching, surface treatments are necessary for biomedical applications of Nitinol.

Anodisation is a common, low temperature method to modify the surface of Nitinol. Anodisation of Nitinol could result in a rough and porous surface structure of Nitinol [100, 148]. In this Chapter, an ethylene glycol based electrolyte containing fluorine ions was studied. This electrolyte is able to produce very thick TiO$_2$ nanotube layer on pure Ti and Ti alloys [149]. The aim of this study is to evaluate the feasibility of anodisation, using fluorine ions containing organic electrolyte and to produce biocompatible oxide layers on the surface of Nitinol. It is generally accepted that the cell-material interaction is influenced by the surface characteristics of an implant material, including chemical composition, surface morphology, roughness, wettability and surface energy [150]. Thickening the TiO$_2$ layer, reduction in the toxic Ni content in the surface layer and generation of rough surface would improve its biocompatibility. Furthermore, a surface with antibacterial properties would add extra benefits to the biological implants. The antibacterial performance of Nitinol is rarely reported [151, 152].

In this chapter, anodisation of Nitinol was carried out in a less aggressive, fluorine ion containing ethylene glycol based electrolyte. The effect of electrolyte compositions, including the fluorine ion concentration and water content, on the anodised Nitinol surface morphology and relating properties were investigated.
5.2 Experimental

Anodisation was carried out in ethylene glycol (EG) based electrolytes containing fluorine ion and water. The compositions of electrolytes varied. The concentration of NH₄F in the electrolyte varied from 0.05 M to 0.2 M and the water content varied from 2% to 10% by volume. Nitinol coupons and platinum foil were used as anode and cathode respectively. The electrodes were placed face to face with a distance of 20 mm apart. A direct current (DC) power supply was used and anodising processes were carried out at 20V at room temperature. The anodisation duration time was 1 h for all samples.

The surface morphologies of different Nitinol surfaces were observed by scanning electron microscopy (SEM). The chemical compositions of the anodised Nitinol surfaces were investigated by X-ray photoelectron spectroscopy (XPS).

Contact angle measurement was conducted using a face contact angle goniometer equipped with a camera (KSV Instruments CAM101). Simulated body fluid (SBF) was used to characterize the potential application of Nitinol in biomedical fields. The pH of SBF was 7.4 (at 36.4°C) and the chemical compositions of the SBF were: Na⁺ 142 mM, K⁺ 5.0 mM, Mg²⁺ 1.0 mM, Ca²⁺ 2.5 mM, Cl⁻ 147.8 mM, HCO₃⁻ 4.2 mM, HPO₄²⁻ 1.0 mM, SO₄²⁻ 0.5 mM [124]. Contact angles between Nitinol surfaces and two diagnostic liquids, water and diiodomethane, were also measured to calculate surface free energy.

Inhibition percentage test of *E. coli* ATCC25922 was performed to investigate the antibacterial property of modified Nitinol samples in physiological environment (in SBF solution) [141]. Fig. 5.1 below shows the procedure of antibacterial activity experiment.
5.3 Results and Discussion

5.3.1 Surface morphologies

The surface morphology of Nitinol was altered by anodisation. Electrolyte compositions, fluorine ion concentration and water content influenced the resultant surface morphologies. As discussed in Chapter 4, the ground Nitinol surface was smooth and no obvious texture could be observed. After anodisation, Nitinol surface became rough and porous.

Fig. 5.2 shows the morphologies of Nitinol surfaces anodised in the electrolyte containing 2 vol.% water but different concentrations of fluorine ion. The images in the left column have lower magnification and more detailed images are shown in the right column.

Figure 5.1 – Procedures of inhibition percentage experiment.
With an increase in the fluorine ion concentration from 0.05 mol/L to 0.2 mol/L, the Nitinol surface became rougher and more porous. The pore size of Nitinol surface anodised in electrolyte containing 0.05 mol/L fluorine ions was about 40 nm (Fig. 5.2 (b)). The surface morphology of Nitinol did not significantly change when the fluorine ion concentration doubled to 0.1 mol/L, with the pore size only slightly increased to about 50 nm. When the fluorine ion concentration was further increased to 0.2 mol/L, the Nitinol surface became much more porous with a pore size of about 100 nm.

The water content in electrolyte also influences the anodised Nitinol surface morphology and the corresponding SEM images are shown in Fig. 5.3, with lower magnification images in the left column and higher magnification images in the right column.

Compared with the influence of fluorine ion concentration, change of water content in the electrolyte greatly altered the anodised Nitinol surfaces. When the water content increased from 2 vol.% to 5 vol.%, the Nitinol surface became much more rougher and porous. The pore size increased to about 200 nm in the case of 5 vol.% water. With further increasing the water content to 10 vol.%, the pore size on the Nitinol surface increased to about 500 nm. It is noted that the cellular wall in the pores developed with the water content; the pore edges became smooth and sharp for the 10 vol.% water content.

Other than surface morphology, changing in fluorine ion concentration also affected the thickness of the oxide layer formed on Nitinol surface. The SEM images of different Nitinol cross-sections are shown in Fig. 5.4. The oxide thickness became larger with increasing fluorine ion concentration from ~ 300 nm, ~ 350 nm to ~ 400 nm for 0.05 mol/L (Fig. 5.4 (a)), 0.1 mol/L (Fig. 5.4 (b)) and 0.2 mol/L (Fig. 5.4 (c)) respectively. However, changing the concentration of water in the electrolyte did not influence the thickness of the oxide layer on Nitinol surface. The Nitinol samples anodised in electrolyte containing varying water content all had an oxide layer of about 350 nm thick. The oxide layers were uniform in thickness, and the oxide layer texture or morphology appeared very similar in both top view and cross-sectional images, indicating that the pores were uniformly distributed in the oxide layer.
Figure 5.2 – Surface morphologies of Nitinol anodised in electrolyte containing 2 vol.% water but different F⁻ concentrations: (a) and (b) 0.05 mol/L; (c) and (d) 0.1 mol/L; (e) and (f) 0.2 mol/L.
Figure 5.3 – Surface morphologies of Nitinol anodised in electrolyte containing 0.1 mol/L fluorine ion but different water contents: (a) and (b) 2 vol.%; (c) and (d) 5 vol.%; (e) and (f) 10 vol.%. 
Figure 5.4 – SEM image of cross-section of Nitinol surface anodised in electrolyte containing 2 vol.% water and (a) 0.05 mol/L, (b) 0.01 mol/L and (c) 0.2 mol/L fluorine ion.
5.3.2 Chemical compositions

The chemical compositions of the oxide layers produced by anodisation were analysed by XPS. The Ti/Ni molar ratios on different anodised Nitinol surface were extracted from XPS survey scans and are shown in Fig. 5.5.

![Diagram](image)

Figure 5.5 – Ti/Ni molar ratio of Nitinol surfaces anodised in different electrolyte.

The ground Nitinol surface had a Ti/Ni ratio of 1.5 (discussed in Chapter 4) and after anodisation, the Nitinol surfaces contained more Ti with higher Ti/Ni molar ratios. With the same water content of 2 vol.%, when the fluorine ion concentration increased from 0.05
mol/L to 0.1 mol/L (Fig. 5.5 (a)), the Ti/Ni ratio increased from 2.3 to 4.4. This indicates an enhanced oxidation of Ti and a selective dissolution of Ni in the surface layer. However, if the fluorine ion concentration was further increased to 0.2 mol/L, the Ti/Ni dropped to 2.5. It seemed that if the fluorine ion concentration is too high, the formed titanium oxide layer would dissolve.

The water content in the electrolyte also affected the surface chemical compositions of anodised Nitinol. With fixed fluorine ion concentration of 0.1 mol/L, increasing water content from 2 vol.% to 5 vol.% (Fig. 5.5 (b)) resulted in a decrease in Ti/Ni ratio from 4.4 to 3.0. With a further increasing the water content to 10 vol.%, the value of Ti/Ni ratio increased to 4.4 again.

In addition to Ti/Ni ratio, the oxidation of Ti on Nitinol surface was also influenced by electrolyte compositions and the results are presented in Table 5.1.

Table 5.1 – The concentration of different Ti states on different Nitinol surfaces.

<table>
<thead>
<tr>
<th>Nitinol surfaces</th>
<th>Ti(IV)</th>
<th>Ti(III)</th>
<th>Ti(II)</th>
<th>Ti(0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05M NH₄F, 2% H₂O</td>
<td>94.9</td>
<td>0.0</td>
<td>5.1</td>
<td>0.0</td>
</tr>
<tr>
<td>0.1M NH₄F, 2% H₂O</td>
<td>94.0</td>
<td>1.1</td>
<td>4.9</td>
<td>0.0</td>
</tr>
<tr>
<td>0.2M NH₄F, 2% H₂O</td>
<td>78.4</td>
<td>0.8</td>
<td>19.0</td>
<td>1.9</td>
</tr>
<tr>
<td>0.1M NH₄F, 5% H₂O</td>
<td>94.4</td>
<td>0.0</td>
<td>5.6</td>
<td>0.0</td>
</tr>
<tr>
<td>0.1M NH₄F, 10% H₂O</td>
<td>92.9</td>
<td>0.0</td>
<td>6.8</td>
<td>0.0</td>
</tr>
</tbody>
</table>

The oxide layer on the ground Nitinol surface contained 76.3% of TiO₂ with the rest consisting of titanium sub-oxides and metallic Ti. Anodisation greatly enhanced the oxidation of Ti. Increasing fluorine ion concentration from 0.05 mol/L to 0.1 mol/L, but with 2 vol.% water content for example, the concentration of TiO₂ remained unchanged as 94%. However, less amount of TiO₂ (78%) was found on the Nitinol surface if the electrolyte contained 0.2 mol/L of fluorine ion. On the other hand, change in the water content did not influence the oxidation of Ti, with all the Nitinol surfaces contained about 94% to TiO₂.
5.3.3 Anodisation reactions

The surface morphologies and chemical compositions of anodised Nitinol are a result of the reactions taking place during the anodising process. In this section, the anodic reactions and how each component in the electrolyte affects those reactions will be discussed.

Cattarin, et al. [63] proposed the following reactions occurring at Nitinol anode:

\[
\text{Ti} + 2\text{H}_2\text{O} \rightarrow \text{TiO}_2 + 4\text{H}^+ + 4\text{e}^- \quad (5.1)
\]

\[
\text{Ni} \rightarrow \text{Ni}^{2+} + 2\text{e}^- \quad (5.2)
\]

Ti is hydrolysed to form TiO\(_2\) (Eq. 5.1), while Ni dissolves into the electrolyte (Eq. 5.2). In an electrolyte without fluorine ion, a passive layer of TiO\(_2\) would be formed and consequently the reactions cease. However, in an electrolyte containing fluorine ion, TiO\(_2\) would be attacked and chemical dissolution of TiO\(_2\) occurs at the interface between the Nitinol surface and electrolyte, according to the following reaction:

\[
\text{TiO}_2 + 4\text{HF} + 2\text{F}^- \rightarrow [\text{TiF}_6]^{2-} + 2\text{H}_2\text{O} \quad (5.3)
\]

Therefore, rough and porous surface layer structure is produced as a result of the above two competing field-assisted processes of reactions (Eq. 5.1) and (Eq. 5.3).

With the same water content in the electrolyte, a higher fluorine ion concentration causes a higher concentration of HF. Therefore, the dissolution rate of TiO\(_2\) increases, according to reaction 5.3. This results in an increasing number and size of pores with increasing concentration of fluorine ion (Fig. 5.2), thereby a rougher and more porous Nitinol surface is generated with a higher fluorine ion concentration in the electrolyte. Due to the higher dissolution rate of TiO\(_2\), the oxide layer could grow thicker with constant applied voltage and time, and this explains why the oxide layer had greater thickness if anodised in electrolyte containing more fluorine ions. As listed in Table 5.1, when the fluorine ion concentration increased to 0.2 mol/L, the TiO\(_2\) content in the oxide layer reduced from 94% (for 0.05 mol/L and 0.1 mol/L) to 78%. This is because of the higher dissolution rate of TiO\(_2\) as well.

Ni dissolves in an acidic solution simply via Eq. 5.2. With the presence of HF in the electrolyte, the dissolution rate of Ni is also increased with the concentration of fluorine ion.
However the dissolution rates of TiO$_2$ and Ni are different with different fluorine ion concentrations and therefore different Ti/Ni molar ratios (Fig. 5.5) result.

The other component in the electrolyte, water, plays three roles during the anodising process. Firstly, water acts as oxidant, which changes Ti from metallic state into various oxidation states, including Ti$^{4+}$, Ti$^{3+}$ and Ti$^{2+}$ states. As such, a titanium oxide passivation layer is formed on Nitinol surface. It seemed that 2 vol.% of water in the electrolyte provided enough oxidizing agent and therefore further increasing water content does not further enhance the oxidation of Ti, resulting in a constant Ti(IV) concentration (about 94%) in the surface layer. Concomitant with titanium oxide layer formation, hydrogen ions are generated, according to reaction (5.1). At the presence of fluorine ion, this will increase the local concentration of HF, which in turn leads to a higher rate of TiO$_2$ dissolution and Ni dissolution. Therefore, different Ti/Ni ratios are obtained with different water contents (Fig. 5.5). The third role water plays is to provide a medium for charge transferring. The electrolyte becomes less viscous and more aggressive by increasing the water content, which is reflected by an increased current density under the same applied voltage. Therefore, by increasing the water content, the pore texture which could be observed on the Nitinol surface anodised in electrolyte containing 2 vol.% water disappeared. The pores became larger and the pore edge became smoother with higher water content.

As a result of the above discussed reactions, especially the competing oxidation of Ti and dissolution of TiO$_2$, different surface morphologies and chemical compositions were obtained in the electrolyte with different concentration of fluorine ion and water.

5.3.4 Surface wettability and surface free energy

The change in the surface morphology and surface chemistry will influence the wettability of Nitinol. Contact angles between Nitinol surfaces and SBF were measured and shown in Fig. 5.6.
Figure 5.6 – Contact angles with SBF on different Nitinol surfaces.

The ground Nitinol surface before anodisation showed the highest contact angle of 83.6° with SBF. The contact angle with SBF greatly decreased on the anodised Nitinol surfaces, indicating a significant enhancement of the wettability of Nitinol surfaces.

When anodisation was carried out in electrolyte containing 2 vol.% water but different concentrations of fluorine ion, increasing fluorine ion concentration from 0.05 mol/L to 0.1 mol/L, the contact angle with SBF did not change significantly. For example, the contact angle was 14.9° for 0.05 mol/L and 14.8° for 0.1 mol/L respectively. Further increase of the fluorine ion concentration to 0.2 mol/L caused the contact angle to be further reduced to
10.2°. Water content in the electrolyte also affects the surface morphology and so the contact angle. For instance, in the electrolyte containing 0.1 mol/L fluorine ion, the contact angle gradually decreased with water content from 14.8° for 2 vol.%, 9.2° for 5 vol.% and 8.5° for 10 vol.% water content.

The contact angle with SBF on Nitinol surface is significantly lowered by anodisation, suggesting that the Nitinol surface becomes more hydrophilic. This is due to the formation of titanium oxide layer by anodisation, which changes the Nitinol surface chemistry [153-155]. The creation of pores and enhanced surface roughness also cause a decrease of contact angle, thereby improving surface wettability [97].

To evaluate the surface energy of Nitinol, contact angles with two diagnostic liquids, deionised water and diiodomethane, were also measured and summarised in Table 5.2. Based on the contact angle values, the surface energies of different Nitinol surfaces were calculated as per the following equation 5.4 and shown in Fig. 5.7.

\[
\frac{\sqrt{\sigma_L (\cos \theta + 1)}}{2} = \sqrt{\sigma_S^P} \sqrt{\sigma_L^P} + \sqrt{\sigma_S^D} \sqrt{\sigma_L^D}
\]  

(5.4)

where \(\sigma\) represents the surface tension and \(\theta\) represents the contact angle. The subscript S represents the solid surface and L represents the liquid. The superscript P represents the polar component and D represents the dispersive component.

Table 5.2 – Contact angles of different Nitinol surfaces with two different diagnostic liquids.

<table>
<thead>
<tr>
<th>Nitinol surfaces</th>
<th>DI water (°)</th>
<th>Diiodomethane (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground</td>
<td>79.5</td>
<td>47.5</td>
</tr>
<tr>
<td>0.05M NH₄F, 2% H₂O</td>
<td>13.0</td>
<td>25.3</td>
</tr>
<tr>
<td>0.1M NH₄F, 2% H₂O</td>
<td>12.9</td>
<td>18.2</td>
</tr>
<tr>
<td>0.2M NH₄F, 2% H₂O</td>
<td>9.9</td>
<td>20.6</td>
</tr>
<tr>
<td>0.1M NH₄F, 5% H₂O</td>
<td>8.4</td>
<td>19.6</td>
</tr>
<tr>
<td>0.1M NH₄F, 10% H₂O</td>
<td>8.3</td>
<td>19.0</td>
</tr>
</tbody>
</table>
The ground Nitinol surface had a total surface energy of 39 mJ/m². After anodisation, the surface energy significantly increased. All the anodised Nitinol surfaces showed similar surface energy of approximately 76 mJ/m². Both the dispersive and polar parts contributed to the increase in the total surface energy and are listed in Table 5.3.
Table 5.3 – Surface energy, including both dispersive and polar part, of different Nitinol surfaces.

<table>
<thead>
<tr>
<th>Nitinol surfaces</th>
<th>Surface energy (mJ/m²)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Dispersive</td>
<td>Polar</td>
</tr>
<tr>
<td>Ground</td>
<td>39.0</td>
<td>35.7</td>
<td>3.29</td>
</tr>
<tr>
<td>0.05M NH₄F, 2% H₂O</td>
<td>75.5</td>
<td>46.0</td>
<td>29.5</td>
</tr>
<tr>
<td>0.1M NH₄F, 2% H₂O</td>
<td>76.4</td>
<td>48.3</td>
<td>28.2</td>
</tr>
<tr>
<td>0.2M NH₄F, 2% H₂O</td>
<td>76.7</td>
<td>47.6</td>
<td>29.2</td>
</tr>
<tr>
<td>0.1M NH₄F, 5% H₂O</td>
<td>77.2</td>
<td>47.9</td>
<td>29.3</td>
</tr>
<tr>
<td>0.1M NH₄F, 10% H₂O</td>
<td>77.3</td>
<td>48.1</td>
<td>29.2</td>
</tr>
</tbody>
</table>

Higher surface energy is normally required for implant material, as cell-material interaction would be promoted due to the readily available adhesion energy [57]. Nitinol surface becomes more hydrophilic with enhanced wettability with SBF, which is beneficial in biomedical applications, as it could improve osseointegration and avoid protein denaturation [97].

5.3.5 Evaluation of antibacterial activity

The effect of different Nitinol surfaces on the inhibition of *E. coli* growth in a physiologic environment was studied and the results are shown Fig. 5.8. Generally, anodised Nitinol surface showed better antibacterial effect than ground Nitinol surface. The ground Nitinol surface in *E. coli* solution with SBF showed a growth inhibition of 20.8%. With the same water content of 2 vol.%, the Nitinol surface anodised in electrolyte containing 0.05 mol/L fluorine ion inhibited the growth of *E. coli* by 37%. Increasing the fluorine ion concentration to 0.1 mol/L, the anodised Nitinol surface showed lower inhibition of 26% and then the inhibition value increased to 38% with higher fluorine ion concentration of 0.2 mol/L.
Figure 5.8 – Inhibition percentage of *E. coli* growth on different Nitinol surfaces in SBF.

When anodising in the electrolyte containing the same fluorine ion concentration of 0.1 mol/L, the Nitinol surface showed a slightly lower *E. coli* growth inhibition with higher water content of 5 vol.%. However, further increasing water content to 10 vol.%, the *E. coli* growth was inhibited by 58%. The Nitinol surface anodised in the electrolyte containing 0.1 mol/L fluorine ion and 10 vol.% water exhibited the best antibacterial property.

The antibacterial experiments were performed with exposure to light. As reported, titanium dioxide could damage microorganism cells due to its photocatalytic properties [156-158]. This might be one reason why Nitinol inhibited *E. coli* growth. Moreover, it is generally
accepted that the antibacterial effect of Nitinol is due to the release of small quantity of nickel ion from the bulk Nitinol [151, 159] and how much nickel ion released from different Nitinol surfaces will be evaluated in Chapter 7. Nevertheless, the interaction between different Nitinol surfaces and E. coli still needs further investigation.

5.4 Summary

In this Chapter, the morphology, chemical composition, wettability and antibacterial properties of anodised Nitinol surfaces were studied, to investigate the influences of electrolyte compositions. The electrolyte used for anodisation was ethylene glycol based and contained fluorine ion and water. Both the fluorine ion concentration and water content affected the anodised Nitinol surfaces.

A rougher and more porous Nitinol surface was produced by anodisation and the Nitinol surface became rougher by increasing both the concentration of fluorine ion and water, as the electrolyte became more aggressive. The Ti/Ni molar ratio on Nitinol surface was also enhanced by anodisation, indicating that the anodised Nitinol surface was covered with mainly titanium oxide with depleted Ni content. The anodised Nitinol surface also showed better wettability with physiological fluids, which would be favoured in the cell-material interactions. Moreover, the inhibition of E. coli growth with contact of Nitinol surface was improved by anodisation. This study suggests that anodisation in fluorine ion containing ethylene glycol electrolyte is an efficient method to modify Nitinol surface and to produce biocompatible surfaces for biomedical applications.
Chapter 6  Anodisation of Nitinol – Influence of Anodising Conditions

6.1 Introduction

In Chapter 5, the effect of the electrolyte compositions was reported. This chapter investigates the effect of anodisation processing parameters. Although extensive investigations have been devoted to anodisation of titanium alloys, it has to point out that the applicability of some anodisation procedures originally developed for pure Ti to Nitinol is not always straightforward. Indeed in many cases, direct adoption of such procedures to Nitinol may be not only unable to bring any improvement, rather cause surface damage because of the Ni involvement in the surface treatment processes [33]. The electrochemical methods, which have been extensively investigated on Ti and Ti alloys, have not been explored on Nitinol until in 2007 when a few studies were reported [72, 102]. In the first evaluations of anodising Nitinol, a 5 μm thick oxide film was produced on Nitinol, when anodised in acetate and borate buffer solutions [102]. Unfortunately the anodising did not reduce the Ni content; the surface obtained, even under an optimized condition, were severely cracked [102]. In the other attempted electrolytes such as acetic acid, 0.1 M sulphuric acid and alkaline solution, no thick oxide films resulted from anodisation. In the alkaline solutions, a continuous exfoliation of the anodisation products indeed occurred from the specimen surface [102]. When electrochemical methods are used, the appearance of anodised Nitinol surface greatly depends on the electrolyte compositions, in addition to anodising parameters. In an attempt of obtaining longer ridge-free TiO₂ nanotubes, Macak et al [160] and Sobieszczyk [91] employed highly viscous and less aggressive organic solutions, such as ethylene glycol. To the best of our knowledge, such viscous organic electrolytes have not been reported for anodising Nitinol.

This chapter further investigates the ethylene glycol based electrolyte containing fluorine ions, which was reported in chapter 5. The aim was to find out proper anodisation processing conditions to produce a thick TiO₂ layer with much reduced Ni content on the Nitinol surfaces. It is accepted that biocompatibility of Nitinol can be significantly improved if the Nitinol surface is dominated by Ti oxides instead of Ni or Ni oxides [38]. In addition, a rough surface texture is beneficial to the cell attachment, adhesion and proliferation on the
implanted materials [150, 161]. Thickening the TiO₂ layer, reduction in the toxic Ni content in the surface layer and generation of rough surface would improve its biocompatibility. Furthermore, a surface with antibacterial properties would add extra benefits to the biological implants. The antibacterial performance of Nitinol is rarely reported [151, 152]. It is noted that most of the results presented in this chapter have been published [8].

6.2 Experimental

6.2.1 Nitinol sample preparation

Ground and cleaned Nitinol coupons were anodised in an ethylene glycol (EG) based electrolyte, which contained 0.1 mol/L NH₄F and 2 vol.% water. Anodisation process was carried out at room temperature with continuous magnetic stirring at 300 rpm. A direct current (DC) power supply was used and the applied voltage varied from 20 V to 60 V. The anodisation duration time ranged from 0.5 to 3 h.

6.2.2 Characterization of anodised Nitinol surfaces

Surface morphologies of the different Nitinol surfaces were observed by scanning electron microscopy (SEM). Surface roughness was measured by Taylor Hobson Surtronic 3 surface profiler. Chemical compositions of Nitinol surfaces were semi-quantitatively determined by X-ray photoelectron spectroscopy (XPS). Both survey scan and high resolution scan of Ti were conducted. The XPS spectra were analyzed using CasaXPS software.

Contact angles between different Nitinol surfaces and simulated body fluid (SBF) were measured to evaluate the influence of the surface microstructure on the surface wettability. Contact angle measurements were conducted using an optical contact angle and surface tension meter (KSV Instruments CAM101). Surface free energy is calculated as per the most widely used Owens-Wendt method [162].

Inhibition percentage test of E. coli was performed according the procedures described in Chapter 5. In this chapter, E. coli was cultured in the water based medium instead of SBF, in order to investigate the antibacterial property of surface modified Nitinol samples.
6.3 Results and Discussion

6.3.1 Surface morphology

Surface morphologies of anodised Nitinol were examined with SEM and were influenced by both the anodisation voltage and duration time. The ground Nitinol surface before anodisation was smooth and featureless (as shown in Chapter 4). In contrast, rough surfaces were formed after anodisation in the viscous ethylene glycol based electrolyte. A close-up observation revealed many nano-sized pores on the surface layer (Fig. 6.1 and Fig. 6.2 right column images). Compared with Nitinol surfaces anodised in aqueous electrolytes [95, 96], more regular surface with evenly distributed pores was obtained on the Nitinol surface when anodised in the ethylene glycol electrolyte. The anodised surface microstructure and surface chemistry depend on the anodising time and voltage used, as described below.

Fig. 6.1 shows the SEM images of different Nitinol surfaces that were anodised for different duration times at 20 V. At this voltage, increasing anodising time resulted in rough and porous surface. The pore size apparently increased with time (Fig. 6.1). The shape of the pores on the Nitinol surfaces also appeared to be associated with anodising time. During the first 0.5 h of anodisation, uniformly distributed pores formed (Fig. 6.1 (b)). This is similar to the second step of the process during the growth of TiO\(_2\) nanotubes on pure Ti [94]. When the anodising time was further increased to 1h or 3h (Fig. 6.1 (d) and (f)), the surface pores became more irregular and interconnected.

Unlike the effect of anodising time, the anodising voltage seems to have less significant effect on the surface morphology, as illustrated in Fig. 6.2. For instance, when the applied voltage increased from 20 V (Fig. 6.2 (b)) to 40 V (Fig. 6.2 (d)) or 60 V (Fig. 6.2 (f)), the pore size did not significantly change. Nevertheless, the pore contour appeared slightly coarser at the higher voltages of 40 V and 60 V.
Figure 6.1 – Surface morphologies of Nitinol anodised at 20 V but for different duration time of 0.5 h ((a) and (b)), 1 h ((c) and (d)) and 3 h ((e) and (f)).
Figure 6.2 – Surface morphologies of Nitinol anodised for 1 h but at different applied voltage of 20 V ((a) and (b)), 40 V ((c) and (d)) and 60 V ((e) and (f)).

Increasing the anodising time under the constant voltage of 20 V also thickened the oxide layer. Fig. 6.3 shows the cross-sections of Nitinol anodised for different duration times. When the anodising time was extended from 0.5 h to 3 h, the thickness of the formed oxide layer increased from ~ 150 nm (Fig. 6.3 (a)) to ~ 350 nm (Fig. 6.3 (b)) and ~ 1 µm (Fig. 6.3 (c)). Increasing the anodising voltage from 20 V to 60 V, the thickness of oxide layer increased slightly from ~ 350 nm to ~ 400 nm for the Nitinol coupons anodised for 1 h.
Figure 6.3 – SEM images of cross-sectional Nitinol surface anodised at 20 V for (a) 0.5 h, (b) 1 h and (c) 3 h.
6.3.2 Surface roughness

The surface roughness of Nitinol is an important factor that influences biological cell behavior when Nitinol is in intimate contact with human tissue. It has been reported that improving surface roughness would result in better cell attachment and proliferation [161]. The degree of surface roughness would influence and control the cell-implant interactions [163]. The roughness of anodised Nitinol surfaces was measured using surface profiler and shown in Fig. 6.4.

Figure 6.4 – The effects of anodisation time and voltage on surface roughness of anodised Nitinol in 0.1M NH₄F + 2 vol.% H₂O in EG. (a) varying anodisation time with the same anodisation voltage of 20 V and (b) varying anodisation voltage with the same anodisation time of 1 h.
As compared with the ground Nitinol surface, the roughness of Nitinol surfaces significantly increased after anodisation. The ground Nitinol surface showed a smooth surface with a roughness value of 0.02 µm. On the other hand, the roughness value of the anodised surface varied from 0.03 µm up to 0.1 µm, depending on the anodisation parameters (Fig. 6.4). The measured roughness data was in good agreement with the surface morphological observation, as shown in Fig. 6.1 and Fig. 6.2, where rougher and more porous surface morphology was observed on all anodised Nitinol surfaces. Increasing anodisation time (Fig. 6.4 (a)) or increasing voltage (Fig. 6.4 (b)) both resulted in an increase in the roughness, but probably in a different way. Increasing anodisation time under a constant potential renders the pores growing larger and deeper, resulting in a significantly increased surface roughness. Anodisation voltage affects the roughness differently in that it coarsens the pore contour, while the pore size and shape remain unchanged. Therefore the surface roughness varies slightly with anodisation voltage.

6.3.3 Chemical compositions

Both survey scan and narrow scan of Nitinol were carried out. As the XPS spectra obtained from different Nitinol surfaces had similar patterns, only the XPS spectrum of the anodised Nitinol surface at 20 V for 1 h is shown here as an example (Fig. 6.5).

Figure 6.5 – XPS spectrum of anodised Nitinol surface at 20 V for 1 h (a) survey scan and (b) high resolution scan of Ti.
The elements presented in the surface layer and their concentrations were analyzed from survey scans. The peaks of C, Cu, F and Ca as shown in the survey scan (Fig. 6.5 (a)) are due to the contamination from the ambient, copper clips, electrolyte and grinding process. The molar ratio of Ti to Ni in the surface layer is an important indicator of Ni toxicity and is given in Fig. 6.6.

Figure 6.6 – Ti/Ni molar ratio of Nitinol surfaces anodised under different conditions.

After anodisation, the Ti/Ni ratio was greatly increased from 1.4 for ground Nitinol surface. For instance, after anodisation for 0.5 h, the Ti/Ni ratio greatly increased to 8.3, as a barrier layer of TiO$_2$ was formed, which was dense and compact. Further anodisation of Nitinol for another 0.5 h (1 h in total), dissolution of this barrier TiO$_2$ layer started, so that a porous surface morphology appeared, causing the Ti/Ni ratio reduced to 4.8. At this stage,
equilibrium of TiO$_2$ formation and dissolution may well be established. Extending anodising time would result in the growth of porous TiO$_2$ layer. After 3 h of anodisation of Nitinol under 20 V, thick TiO$_2$ layer was generated on the surface, with Ti/Ni ratio of 9.8.

Anodisation voltage, on the other hand, affects the surface chemistry only insignificantly (Fig. 6.6 (b)). For example, variation of anodisation voltage from 20 V to 60 V slightly changed the surface Ti/Ni ratio from 4.8 to 5.3 for the Nitinol sample when anodised for 1 hour. Nonetheless, the increased Ti/Ni ratio indicates an enhanced Ti oxide and a reduced Ni content in the surface layer. This results from the much higher affinity of Ti with O, indicated by significantly different Gibbs free energies of formation of TiO$_2$ ($\Delta G_{TiO_2} = -889.5$ kJ/mol) and NiO ($\Delta G_{NiO} = -221.8$ kJ/mol) [75].

The degree of oxidation of Ti was analysed from the narrow scan of the Ti2p peak. The Ti narrow scan spectrum was fitted by four pairs of peaks, representing four chemical states of Ti: Ti(IV) represents TiO$_2$, Ti(III) and Ti(II) represent sub-oxides of Ti, and Ti(0) represents the intermetallic bond between Ti and Ni in the substrate. The concentration of each Ti chemical state is summarized in Table 6.1. The content of Ti(IV) after anodisation significantly increased from 76.3 (for ground Nitinol surface) to ~ 90, and the content of Ti(0) was reduced to approximately zero. This indicates that anodisation not only enhance the oxidation of Ti but also the degree of oxidation.

Table 6.1 – The concentration of Ti with different chemical states on anodised Nitnol surfaces.

<table>
<thead>
<tr>
<th>Nitinol surfaces</th>
<th>Ti(IV)</th>
<th>Ti(III)</th>
<th>Ti(II)</th>
<th>Ti(0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anodised 20 V / 0.5 h</td>
<td>90.1</td>
<td>1.6</td>
<td>6.7</td>
<td>1.6</td>
</tr>
<tr>
<td>Anodised 20 V / 1 h</td>
<td>94.0</td>
<td>1.1</td>
<td>4.9</td>
<td>0.0</td>
</tr>
<tr>
<td>Anodised 20 V / 3 h</td>
<td>83.1</td>
<td>15.3</td>
<td>0.0</td>
<td>1.6</td>
</tr>
<tr>
<td>Anodised 40 V / 1 h</td>
<td>91.9</td>
<td>2.4</td>
<td>5.7</td>
<td>0.0</td>
</tr>
<tr>
<td>Anodised 60V / 1 h</td>
<td>77.7</td>
<td>5.7</td>
<td>11.7</td>
<td>5.0</td>
</tr>
</tbody>
</table>
6.3.4 Formation mechanism of anodised Nitinol surface texture

The rough and porous structure obtained with anodisation on Nitinol surfaces is different from that on pure Ti and Ti alloys substrates, where self-organized nanotube layers were observed in many cases [91]. As nickel is consisted and contributed half of Nitinol, the dissolution of Ni influenced the formation of surface structure.

As discussed in Chapter 5, the following reactions taking place at the interface between Nitinol surface and electrolyte during the anodising process [63]:

\[ \text{Ti} + 2\text{H}_2\text{O} \rightarrow \text{TiO}_2 + 4\text{H}^+ + 4\text{e}^- \quad (6.1) \]

\[ \text{TiO}_2 + 2\text{F}^- + 4\text{HF} \rightarrow [\text{TiF}_6]^{2-} + 2\text{H}_2\text{O} \quad (6.1) \]

\[ \text{Ni} \rightarrow \text{Ni}^{2+} + 2\text{e}^- \quad (6.2) \]

The porous structure is a result of competing oxidation of Ti (Eq. 6.1) and dissolution of TiO\(_2\) (Eq. 6.1) and Ni (Eq. 6.2). The proposed mechanism is described below.

When voltage was applied, an initial barrier layer formed (Fig. 6.7 (a)) and this was reflected by an exponential decrease in the current at the very start of anodising process. This barrier titanium oxide layer was dense and compact and so provided a resistance to reduced current [164]. The current ceases dropping when a steady state is reached.

It is noted that in the absence of F\(^-\) ions in the electrolyte, the formation of TiO\(_2\) oxide layer on the surface will reduce the anodising current and eventually terminate the process. With the presence of fluorine ion in the electrolyte, dissolution of the barrier oxide layer starts (Fig. 6.7 (b)). It is suggested that the chemical dissolution takes place at random locations on the surface [165-168]. Because of the dissolution of oxide layer, nanopores are produced on the Nitinol surface (Fig. 6.7 (c)).
In a single pore on the Nitinol surface (Fig. 6.8 (a)), dissolution and formation of titanium oxide take place simultaneously. Therefore, the pore becomes deeper and larger. Accompanied with the oxidation of Ti, protons are produced according to reaction (6.1). This causes an increase in proton concentration and so the concentration of HF. The concentration of HF near the surface can be lowered by continuous agitation. However, in the bottom of the pore, protons accumulate; and together with the movement of negatively charged fluorine ions towards anode, due to electric field, this causes an increased concentration of HF in the bottom of the pore. With the diffusion of HF towards the bulk electrolyte, there is a gradient in HF concentration along the depth of the pore. Difference in the concentration of HF results in different dissolution rate of titanium oxide layer on the wall of pores. The dissolution rate is higher at the bottom of a pore than that near the opening of a pore, so that the pore became deeper. This explains that the oxide layer became thicker with longer anodisation time.

The chemical dissolution of Ni also takes place with reactions associated with Ti. Dissolution of Ni occurs on the wall of pores, so that smaller pores appear in the bigger pores (Fig. 6.8 (b)). Competing oxidation and dissolution reactions also take place in such small pores and so disturb the formation of nanotube. As a result, a 3-D interconnected pore structure is established on the Nitinol surface (Fig. 6.8 (c)).
Figure 6.8 – Schematic representation of porous surface layer formation on Nitinol surface.

6.3.5 Wettability and surface free energy

The surface anodisation of Nitinol would influence the wettability. Contact angles between different Nitinol surfaces and SBF solution were measured and the results are presented in Fig. 6.9. The ground Nitinol showed the highest contact angle of 83.6° with respect to SBF. Contact angles decreased greatly after anodisation, and the lowest contact angle value was found on the Nitinol surface anodised at 20 V for 3 h (8.8°). This is in agreement with its highest surface roughness value on this sample (Fig. 6.4). Increasing anodisation time gradually decreased contact angle. For example, at 0.5 h anodisation time the contact angle with SBF was 15.6°. It decreased to 14.8° at 1 h and further to 8.8° at 3 h. Similar to the effect on roughness, anodisation voltage has less significant effect on wettability. The contact angles varied from 14.8° at 20 V, 12.0° at 40 V and 12.9° at 60 V. These data are in agreement with surface roughness, as shown in Fig. 6.4, since an increased surface roughness would result in a decrease in contact angle value [169]. The decreased contact angle on the
anodised Nitinol surface indicates that the surface becomes highly hydrophilic, which favours cell attachment and spreading [96].

![Graph](image1)

**Figure 6.9** – Contact angles measured on the ground and anodised Nitinol surfaces, against SBF solution.

Surface free energy, to some extent, reflects the cell-material interaction. The surface free energies of the various Nitinol surfaces are presented in Table 6.2. Generally, minimal cell adhesion would be expected on materials with a surface energy value of 20 to 30 mJ/m². Surface free energy higher than 30 mJ/m² would greatly enhance the cell-material interaction [170]. The lower contact angle of anodised Nitinol surfaces corresponds to a higher surface free energy than the ground Nitinol surface. The ground Nitinol surface showed a surface free energy of 39.0 mJ/m². After anodisation, the surface free energy was much increased and the highest surface energy of 77.6 mJ/m² was found on the Nitinol surface anodised under 20 V
for 3 h. Surface free energy of a solid is also related to surface roughness [171]. The surface roughness of Nitinol was greatly increased by anodisation, which resulted in decreased contact angle with SBF and higher surface free energy.

Table 6.2 – Surface free energy of different Nitinol surfaces, including dispersive and polar components.

<table>
<thead>
<tr>
<th>Nitinol surfaces</th>
<th>Surface energy (mJ/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dispersive part</td>
</tr>
<tr>
<td>Ground</td>
<td>35.7</td>
</tr>
<tr>
<td>Anodised at 20V for 0.5h</td>
<td>46.1</td>
</tr>
<tr>
<td>Anodised at 20V for 1h</td>
<td>48.3</td>
</tr>
<tr>
<td>Anodised at 20V for 3h</td>
<td>48.9</td>
</tr>
<tr>
<td>Anodised at 40V for 1h</td>
<td>47.0</td>
</tr>
<tr>
<td>Anodised at 60V for 1h</td>
<td>46.4</td>
</tr>
</tbody>
</table>

6.3.6 Evaluation of antibacterial activity

The antimicrobial activity of ground and anodised Nitinol surfaces were investigated using *E. coli* ATCC 25922, and the percentage inhibition of *E. coli* growth of different Nitinol surfaces is shown in Fig. 6.10. Compared to the ground Nitinol surface, the anodised Nitinol surfaces showed an enhanced inhibition on *E. coli* growth. The ground Nitinol surface only inhibited the *E. coli* growth by 4.2%, while the anodised Nitinol surfaces demonstrated an inhibition of ~ 13% after a contact time of 18 h. The highest inhibition of *E. coli* growth was 18.2% for the surface of Nitinol that was anodised at 60 V for 1 h. It is suggested that the antibacterial effect of Nitinol is due to the release of small quantity of nickel ion from the alloy [151, 159]. However, the interaction between different Nitinol surfaces and *E. coli* needs further investigation.
Figure 6.10 – The percentage inhibition of E. coli growth for different Nitinol surfaces.

6.4 Summary

Nitinol surface was anodised in a viscous fluorine ion containing ethylene glycol electrolyte. The influence of anodisation conditions on the surface properties of Nitinol was investigated. The surface microstructure and surface chemistry were significantly changed by anodisation. The surface roughness increased by a factor of ten. The molar ratio of Ti to Ni in the surface layer was significantly altered due to the oxidation of Ti and dissolution of Ni. The anodised Nitinol surfaces had a higher Ti/Ni ratio and therefore are much more favourable for implant applications. Compared with the ground Nitinol surface, the anodised Nitinol surfaces showed an improved wettability. Together with the increased surface roughness, anodised
Nitinol surfaces would be better for the cell-implant interaction. Anodised Nitinol surfaces also showed an improved bacterial inhibition of *E. coli* growth. Moreover, even a porous oxide layer was produced on Nitinol surface, the tensile properties of the bulk material were not influenced. This study suggests that anodisation in fluorine ion containing ethylene glycol electrolyte is an efficient method to modify Nitinol surface and to produce biocompatible surfaces for biomedical applications.
Chapter 7  Biocompatibility of Anodised Nitinol Surfaces

7.1 Introduction

As mentioned in Chapter 2, the biocompatibility of a metallic alloy is largely reliant on its corrosion resistance, and therefore on the by-products coming from the corrosion reactions. For Nitinol, the by-product from corrosion is Ni ion, which is toxic and would induce allergic response to human body. Therefore, Ni ion release remains the major concern in terms of the biocompatibility of Nitinol. Various surface modification methods have been used to change Nitinol surfaces, aiming to reduce the Ni ion release and improve its biocompatibility. Another factor describing the biocompatibility of Nitinol is the ability to form apatite layer in a simulated physiologic environment. Researchers have found that the in vivo apatite formation could be reproduced in a solution with ion concentrations nearly equal to those of human blood plasma and this solution is so called simulated body fluid (SBF). Immersion test in SBF provided a way to predict and evaluate the in vivo bone bioactivity of Nitinol as an implant material. A more direct approach to evaluation of the biocompatibility is to analyse the cell-material interaction. The responses from the cells in contact with a material indicate the cytotoxicity and so how biocompatible that material is.

In this chapter, the biocompatibility of anodised Nitinol surfaces is evaluated. Immersion tests in simulated body fluid were used to calculate the amount of and releasing rate of toxic Ni ion released from different Nitinol surfaces. Immersion of Nitinol samples in SBF was also utilized to evaluate the ability to form hydroxyapatite on Nitinol surfaces, which indicates the degree of bioactivity. Finally, the cell-material interaction was also analysed with cell line L929.
7.2 Experimental

The experimental procedures were presented in Section 3.3. The following section briefly summarises the procedures.

7.2.1 Ni ion release test

The anodised Nitinol surfaces were wrapped with tape so that that exposed surface area was 100 mm$^2$. The back side and edges of the Nitinol coupons were well sealed with silicone. The immersion test was carried out in simulated body fluid (SBF) which has a chemical composition listed in Table 2.5. The sealed Nitinol coupons were immersed in 10 mL of SBF in tubes with caps on, and these tubes were immersed in water bath kept at 37 °C (as shown in Fig. 7.1). 10 mL of fresh SBF was changed every 4 day, and the total immersion duration was 28 days. The concentration of Ni ion released into SBF solution in 4 days duration was determined by flame atomic absorption spectrometry (FAAS).

Figure 7.1 – Immersion test layout.

7.2.2 Hydroxyapatite formation test

The Nitinol samples with different surface conditions were prepared as above, but the exposed anodised surface area was 150 mm$^2$. The sealed Nitinol samples were immersed in 15 mL of SBF and were kept in water bath as shown in Fig. 7.1. After soaking in SBF solution for 3 weeks and 4 weeks, the Nitinol samples were taken out and gently washed with
distilled water and dried. The dried Nitinol samples were then tested by X-ray diffraction (XRD) for any hydroxyapatite (HA) formation.

### 7.2.3 Cell-material interaction

Murine fibroblast cell line L929 (ATCC CCL-1), was used to evaluate the cell-material interaction. The medium was Dulbecco’s Modified Eagle Medium (DMEM) supplemented with 10% heat activated fetal calf serum (FCS) (both from Life Technologies). 1 mL aliquots of cell culture medium containing $1 \times 10^5$ cells/mL L929 cell line were seeded onto anodised and ground Nitinol samples. Wells containing Nitinol samples and medium solution without any cells were also set up as controls. The cells were incubated at 37 °C in 5% CO$_2$ in air.

The cell viability at different stage of proliferation was measured with resazurin fluorescence assay, which was recorded at 530 nm excitation and 590 nm emission using a Perkin Elmer Enspire 2300 Multibabel Reader. The cells on Nitinol samples after 1 day and four days culture were fixed for imaging using an environmental electron scanning microscope (ESEM, FEI Quanta 200FE). The procedures of cell experiment are shown in Fig. 7.2 below.

![Figure 7.2 – Cell viability experiment procedures.](image-url)
7.3 Results and Discussion

7.3.1 Ni ion release

As discussed in the previous chapters, the Nitinol surfaces were modified by anodisation resulting in different surface morphologies and chemical compositions. Ni ion release from the bulk Nitinol substrate was influenced by the surface conditions. As the surface conditions were controlled by anodisation parameters, how these parameters affect Ni ion release over 28 days will be discussed in this section.

7.3.1.1 Influence of anodisation duration time

Fig. 7.3 below shows the Ni ion release from different Nitinol surfaces that were anodised at 20 V, but for different duration times.

![Graph showing Ni ion release over time for different anodisation times](image)

Figure 7.3 – Ni ion release from Nitinol surfaces anodised at 20 V for different anodising times.

Each data point on the cures shown in Fig. 7.3 represents the amount of Ni ion released up to a specific period of immersion time. On Day 28, the samples anodised at 20 V for 0.5 h and 1.0 h have released similar amounts of Ni ions (1.7 ppm). In remarkable contrast, the sample anodised for 3 h has released significantly more Ni ions (3.4 ppm) – doubled amount of Ni
ions in this sample. From Day 1 to Day 24, the amount of Ni release was least in the 1 h-anodised sample while highest in the 3 h-anodised sample. As reported in Chapter 6, uniformly distributed pores were formed on the Nitinol surface when the sample was anodised at 20 V for 0.5 h. When the anodising voltage is applied, electric current passes though the surface layer of Nitinol, so that the naturally formed TiO$_2$ surface layer is removed from point to point, leaving behind pores on the Nitinol surface. The anodised Nitinol surface morphology is a result of competing dissolution of surface layer and oxidation of Ti. Further increasing anodisation duration time to 1 h, even though the surface layer is further dissolved, the oxidation of Ti is enhanced, resulting in a protective surface layer containing mainly TiO$_2$. Thus the Nitinol surface anodised for 1 h gave a better protection of the underneath Nitinol substrate than the one anodised for 0.5 h. As a consequence, less Ni ion is released into the SBF solution.

However, further increasing the anodisation duration time to 3 h actually caused more Ni ion release (Fig. 7.3). In this case, a thicker and more porous TiO$_2$ layer was formed (Fig. 7.4). This more porous microstructure results in an increased area exposed to the immersion solution, so that more Ni ions is released from the Nitinol surface.

![SEM image of Nitinol surface anodised in 0.1M NH$_4$F + 2 vol.% H$_2$O in EG at 20V for 3 h.](image)

Figure 7.4 – SEM image of Nitinol surface anodised in 0.1M NH$_4$F + 2 vol.% H$_2$O in EG at 20V for 3 h.

The surface conditions of the anodised Nitinol also influence the Ni ion release rate (kinetics). Fig. 7.5 shows the amount of Ni ion released in every 4 days from the Nitinol surfaces that were anodised at 20 V, but for different anodisation duration times. In other words, Fig. 7.5 presents the Ni ion release rate of the Nitinol surfaces anodised for different duration time.
The blue bars show the Ni ion released from the 0.5 h-anodised Nitinol surface. The height of blue bars gradually decreases with increasing immersion time, from 0.55 ppm/4 days to 0.1 ppm/4 days, indicating that the Ni ion release rate gradually decreased over immersion time. The red bars (anodised for 1 h) show a similar decreasing trend, but not as obvious as the 0.5 h sample. However, the green bars (anodised for 3 h) do not show any trend and the amount of Ni ion released in every 4 days was around 0.5 ppm. The difference in the Ni ion release rate over 28 days period is due to the difference in the surface morphology and surface chemistry. In general, the more porous the surface, the more is the Ni ion release. The more protective TiO$_2$ on the surface, the less the ion release.

![Graph showing Ni ion release rate for different anodisation times](image)

Figure 7.5 – Ni ion release rate calculated for every 4 days for various Nitinol surfaces anodised at 20 V.

7.3.1.2 Influence of anodisation voltage

The Ni ion released from Nitinol surfaces that were anodised for 1 h, but under different voltages are presented in Fig. 7.6. More Ni ions were released from the Nitinol surface anodised at 60 V. After 28 days of immersion in SBF, 2.2 ppm of accumulated Ni ion was released from the Nitinol surface anodised at 60 V, compared with that from the Nitinol surface anodised at 20 V (1.7 ppm). Again the increased Ni ion release is due to a rougher Nitinol surface, which was obtained at 60 V. As reported in Chapter 6, an increase in the
anodisation voltage from 20 V to 60 V led to an increased Nitinol surface roughness from 0.05 µm to 0.08 µm.

Figure 7.6 – Ni ion release from Nitinol surfaces which were anodised for 1 h, at 20 V and 60 V.

Fig. 7.7 shows the Ni ion release rate of the Nitinol surfaces anodised at different voltages. The initial Ni ion release rate (in the first 4 days period) from Nitinol surface anodised at 60 V was 0.6 ppm, higher than that from Nitinol surface anodised at 20 V (0.4 ppm). This is again due to the rougher Nitinol surface when anodised at 60 V. Afterwards, the Ni ion release rate from 60 V-anodised Nitinol surface gradually decreased over time and at the end of 28 days of immersion, the Ni ion release rate dropped to 0.07 ppm. This value was much lower than that from Nitinol surface anodised at 20 V (0.18 ppm). At a higher anodisation voltage, the dissolution rate of Ni is increased and the oxidation of Ti is enhanced. As a result of the above competing reactions, the Ti/Ni ratio on the Nitinol surface increased from 4.8 to 5.3 (Fig. 6.6 (b)). The Nitinol surface anodised at 60 V has fewer Ni content. Therefore, even though the initial Ni ion release rate was high, it decreased to a low level when the surface was stabilized.
7.3.1.3 Influence of water content in the anodisation electrolyte

As the morphology and chemical compositions of Nitinol surface are also influenced by the compositions of anodisation electrolyte, the resulting Nitinol surfaces would behave differently. Fig. 7.8 shows the Ni ion release from Nitinol surfaces which were anodised in ethylene glycol based electrolytes containing 0.1M fluorine ion but with different water contents, i.e., 2% and 10% by volume.

The amount of Ni ion released from Nitinol surface was greatly increased with higher water content in the anodisation electrolyte. At the end of 28 days of immersion, the accumulated Ni ion released from Nitinol surface anodised in electrolyte with 10 vol.% water content was 3.1 ppm, which was higher than that from Nitinol surface anodised in electrolyte with 2 vol.% water content (1.7 ppm). The higher Ni ion release is due to the rougher and more porous Nitinol surface. As shown in Chapter 5, with an increase of water content in the anodisation electrolyte, the resulting Nitinol surface became rougher with enlarged pores. An example is shown below in Fig. 7.9. This leads to a larger exposure area, and thus a higher Ni ion release from the Nitinol surface.

Figure 7.7 – Ni ion release rate calculated for every 4 days for Nitinol surfaces anodised for 1 h, at 20 V and 60 V.
Figure 7.8 – Ni ion release from Nitinol surfaces which were anodised in different electrolytes containing 2 vol.% and 10 vol.% water content.

Figure 7.9 – SEM images of Nitinol surfaces anodised in electrolyte containing 2 vol.% (a) and 10 vol.% (b) water.

The Ni ion release rates are presented in Fig.7.10. In the first 4 days, Ni ion released from the Nitinol surface anodised in electrolyte with 10 vol.% water content was 1.1 ppm, much higher than that from the Nitinol surface anodised in electrolyte with 2 vol.% water content (0.4 ppm). This is again explained by the much porous Nitinol surface as mentioned above. Then Ni ion release dropped to about half (0.5 ppm) in the second 4 days and gradually decreased over immersion time, reaching a steady state. The Ni ion release from both Nitinol surfaces reached a steady release rate of around 0.2 ppm in the end of 28 days of immersion,
because both Nitinol surfaces had very similar chemical compositions (Ti/Ni ratio about 4.4) and surface layer thickness (~ 350 nm).

Figure 7.10 – Ni ion released in every 4 days from Nitinol surfaces anodised in electrolytes containing 2 vol.% and 10 vol.% water content.

### 7.3.1.4 Summary of Ni ion release

The accumulated Ni ion released from different Nitinol surfaces over 28 days in SBF is summarized in Table 7.1. In general, more Ni ion is released from the anodised Nitinol surfaces, compared to the ground control sample. Ni ion is hardly released from the ground Nitinol surface over 28 days of immersion. The ground Nitinol surface is protectively covered with continuous naturally formed oxide layer. Therefore the Nitinol substrate is not a source of Ni release [36, 117, 121, 172]. The highest accumulated Ni ion release over 28 days of immersion was found from the Nitinol surface that was anodised under 20 V for 3 h (3.37 ppm), as shown in Fig. 7.11.
Table 7.1 – Accumulated Ni ion released from different Nitinol surfaces in SBF.

<table>
<thead>
<tr>
<th>Nitinol surfaces</th>
<th>Anodisation condition variables</th>
<th>Ni ion release in 28 days (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time (h)</td>
<td>Voltage (V)</td>
</tr>
<tr>
<td>1</td>
<td>0.5</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>20</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>60</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>6</td>
<td>Ground Nitinol sample</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Figure 7.11 – Ni ion released from ground and anodised Nitinol surfaces.

If a real stent is considered, which has dimensions as shown in Fig. 7.12 and with an estimated surface area of 40 cm², the highest accumulated Ni ion released in to 10 mL of SBF solution from 1 cm² anodised Nitinol surface over 28 days immersion was 3.4 ppm, which gave a daily release of 1.2 µg/cm². If the stent has the same surface condition, it will release 48 µg of Ni ion per day, which is much below the estimated daily Ni dietary intake of
200~300 mg [173]. Therefore, the Ni ion release from Nitinol would not be a concern, if the patients are not strongly allergic to Ni.

![Stent of Nitinol wire](image)

Figure 7.12 – A stent made of Nitinol wire: stent length = 10 cm and Nitinol wire diameter = 0.2 mm.

The amount of Ni ion released in to SBF solution depends on the surface morphology of Nitinol. More Ni ion would release from a rougher and more porous Nitinol surface. The initial Ni ion release rate also depends on the surface roughness, leading to a higher Ni ion release rate with a rougher surface.

For all of the anodised Nitinol samples, except the one anodised for 3 h, the Ni ion release rate tends to stabilize at the end of 28 days of immersion. This indicates that the source of Ni ion release is the outermost atomic layers of the oxide layer, i.e. the Ni content in the surface of the oxide layer. For the Nitinol sample anodised for 3 h, the random Ni ion release rate during 28 days of immersion might be caused by the much thicker surface layer.

### 7.3.2 Hydroxyapatite formation ability

Fig. 7.13 shows the XRD patterns of the ground Nitinol immersed in SBF solution for different period of time. The XRD pattern of the ground Nitinol before immersion confirms the B2 phase (austenite), with the most prominent peak around 42.2° [174]. After 3 weeks of immersion in SBF, one small peak was found. The peak at 2θ of around 25.8° corresponds to hydroxyapatite [175], indicating that a small amount of HA formed. The peak is broad,
indicating a low crystallinity of the formed hydroxyapatite [176]. However, extending the immersion time to 4 weeks, there was not any visible change in the XRD patterns. This implies that no more hydroxyapatite formed and/or the crystallinity of HA does not change over the immersion time.

Figure 7.13 – XRD patterns of ground Nitinol (top) and that were immersed in SBF solution for 3 weeks (middle) and 4 weeks (bottom).

Fig. 7.14 shows the XRD patterns of the Nitinol surfaces anodised under 20 V for 1 h and were subsequently immersed in SBF solution for different period of time. Again, only the peaks belonging to the B2 Nitinol phase were identified from the XRD pattern of these anodised samples. After 3 weeks of immersion in SBF, two small peaks appeared. The two peaks at 2θ of around 25.8° and 31.8° correspond to hydroxyapatite (002) and (211) planes [175]. Increasing immersion time in SBF to 4 weeks, the relative intensity of hydroxyapatite (211) peak increased, suggesting the continuous formation of hydroxyapatite on the anodised Nitinol surfaces.
Figure 7.14 – XRD patterns of anodised Nitinol (top) and that were immersed in SBF solution for 3 weeks (middle) and 4 weeks (bottom).

After immersion in SBF for 4 weeks, it is obvious that the HA peaks from the anodised Nitinol surface (Fig. 7.14 bottom) have much higher intensity than that from the ground bare Nitinol surface (Fig. 7.13 bottom). This indicates that anodised Nitinol surface has better apatite forming ability and a bioactive Nitinol surface could be generated by anodisation.

SBF is a supersaturated solution and is metastable. With formation of apatite crystals, the SBF system becomes thermodynamically stable [177]. Generally, apatite crystals could form on any material if the immersion time of that material in SBF is long enough [178, 179]. The induction time of apatite indicates the bioactivity of a material. The induction time of apatite is related to the formation time of thermodynamically stable crystal nuclei. A stable crystal could form if it overcomes the activation energy for crystallization and so it has to reach a critical particle radius \( r_c \) in the solution as shown below [179]:

\[
r_c = 2\beta_a \sigma V/(3\beta_v RT \ln S)
\]

(7.1)

where, \( \beta_a \) is the area related geometric factor of nuclei; \( \beta_v \) is the volume related geometric factor of nuclei; \( \sigma \) is the free energy of nucleus-solution interface; \( V \) is the molar volume of the precipitate; \( T \) is the temperature; \( R \) is the gas constant and \( S \) is the saturation ratio.
The induction time also depends on the rate of forming stable crystal nuclei, $J(t)$ and is expressed as [179]:

$$J(t) = J_0 \exp(-t/\tau)$$

(7.2)

where, $J_0$ is the steady state nucleation rate; $t$ is the time and $\tau$ is the induction time, which could be determined as:

$$t = 6d^2 n^*/(D \ln S)$$

(7.3)

where, $d$ is the molecular diameter; $n^*$ is the critical number of atoms/molecules/ions in the critical size nucleus; and $D$ is the diffusion coefficient.

The saturation ratio ($S$) is the ratio of the actual concentration of dissolved precipitate to its thermodynamically equilibrium concentration. Therefore, it would be easier (Eq. 7.1) and faster (Eq. 7.2 and 7.3) to induce the formation of apatite with a higher actual concentration of dissolved apatite. Fig. 7.15 shows the O 1s high resolution scans of ground and anodised Nitinol surfaces. The O 1s peak for both surfaces could be fitted with three component peaks located at about 530 eV, 531 eV and 532.5 eV, which represented metal oxides, hydroxyl group (-OH) and carbon single bond (C-O) respectively [180]. The area percentage of each component peak is summarized in Table 7.2.

Figure 7.15 – High resolution spectrum of O 1s of (a) ground and (b) anodised Nitinol surfaces.
Table 7.2– Area percentages of O 1s spectra fitting peaks.

<table>
<thead>
<tr>
<th>Nitinol surfaces</th>
<th>Area percentage of (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Metal oxides</td>
</tr>
<tr>
<td>Ground</td>
<td>46.03</td>
</tr>
<tr>
<td>Anodised</td>
<td>54.75</td>
</tr>
</tbody>
</table>

The anodised Nitinol surface has a higher area percentage of -OH peak than that of ground Nitinol surface (42.61% compared with 41.05%), and much lower area percentage of C-O peak than that of ground Nitinol surface (2.64% compared with 12.92%). This implies that most of the -OH group on ground Nitinol surface associated with absorbed organic species. However, on anodised Nitinol surface, most of -OH group could associate with Ti-OH group, as the anodised Nitinol surface was treated under aqueous condition. With the presence of Ti-OH on the anodised Nitinol surface, the local pH value of SBF increased, leading to a decrease of hydroxyapatite solubility and consequently accelerated the nucleation of apatite [181].

7.3.3 L929 cell behavior

To ensure that the Nitinol samples, both ground and anodised, would not react with resazurin solution and hence influence the fluorescence reading, controls were set up. Fig. 7.16 shows the fluorescence intensity of controls for ground and anodised Nitinol surfaces over 4 day period. Fluorescence intensity is presented relative fluorescence unit (RFU). All the reading values are very close to zero within error, indicating none of the chemicals presented on, or released from the ground and anodised Nitinol surfaces when contacted with resazurin solution. Therefore, resazurin solution is proper to be used to test cell viability on Nitinol samples.
Figure 7.16 – Fluorescence value of controls of ground (G) and anodised Nitinol (A) surfaces.

The growth of L929 cells on ground and anodised Nitinol surfaces were measured daily over 4 day period and are presented in Fig. 7.17. One day after L929 cells were seeded onto Nitinol surfaces, the ground Nitinol showed slight better ability of cell attachment, as its fluorescence reading was slightly higher than the cells on anodised Nitinol surface. It might be due to the turbulence when handling the samples, as the Nitinol samples were moved into new wells after 24 h incubation. After 2 days of incubation, the L929 cells on the anodised Nitinol surface showed a growth with an increased fluorescence reading. The L929 cells on anodised Nitinol surface remained proliferating and reached the highest cell number on the fourth day of incubation. The L929 cells on the ground Nitinol surface, on the other hand, did not show such good growth. The cell number dropped after 2 days of incubation and remained unchanged. A porous Nitinol surface was obtained by anodisation and this surface was rougher and more hydrophilic, which favors the cell-material interaction.
The L929 cell growth on ground and anodised Nitinol surfaces was also demonstrated by ESEM images. After 1 day of incubation, L929 cells attached to both ground and anodised Nitinol surfaces and the cell densities were similar (Fig. 7.18 (a) and Fig. 7.19 (a)). Fig. 7.18 (b) and Fig. 7.19 (b) show a close-up image of the L929 cells on the ground and anodised Nitinol surfaces respectively. The cells are in round or oval shape, implying that the cells have attached to the Nitinol surfaces, but not spread out. These cells could be easily shaken away from the attached surface, if they come across turbulence.

At the end of 4 days incubation, only a few L929 cells were found on the ground Nitinol surface (Fig. 7.18 (c)). A close up image of L929 cell (Fig. 7.18 (d)) shows that the cell is still in round shape and proliferation of cell could not be detected. On the other hand, the growth of L929 cells on the anodised Nitinol surface was significant, as the surface was fully covered by L929 cells (Fig. 7.19 (c)). A close up image (Fig. 7.19 (d)) clearly shows the excellent proliferation of L929 cells on the anodised Nitinol surface.
Figure 7.18 – SEM images of L929 cells on ground Nitinol surface after 1 day ((a) and (b)) and 4 days ((c) and (d)) of incubation.
Figure 7.19 – SEM images of L929 cells on anodised Nitinol surface after 1 day ((a) and (b)) and 4 days ((c) and (d)) of incubation.

If the fluorescence values of each day (as shown in Fig. 7.17) are connected into a curve, the area under the curve (AUC) expresses the total number of L929 cells grown in 4 days of incubation. The L929 cell viability experiment was carried out in three independent experiments and the AUC measurement of each experiment are presented in Fig. 7.20. Even though the cell numbers are different from experiment to experiment, the number of L929 cells grown on anodised Nitinol surface is always larger (about twice larger) than that on the ground Nitinol surface. This confirms that anodised Nitinol surface has better ability for L929 cells to attach, spread and proliferate than the ground Nitinol surface.
7.4 Summary

In this chapter, the biocompatibility of Nitinol was evaluated by analysing Ni ion release in SBF, hydroxyapatite formation ability and L929 cell-material interaction. Generally, the anodised Nitinol surface showed an improved biocompatibility. Ni ion release from Nitinol surface depended on the surface morphology and surface layer thickness. A rough and porous Nitinol surface would result in a higher Ni ion release from it. Even though the Ni ion release from anodised Nitinol was higher than that from the ground Nitinol surface, the amount of Ni ion released is still below the tolerated concentration. Despite Ni ion release, anodised Nitinol surface showed better hydroxyapatite formation ability and cell-material interaction. After 3 weeks of immersion in SBF, HA formed on anodised Nitinol and continuously formed with longer immersion time, while only a small peak of HA was found on the ground Nitinol surface after immersion in SBF. Moreover, L929 mammalian cells could attach and proliferate well on anodised Nitinol surface, but such behavior of L929 cells was not found on ground Nitinol surface. Therefore, the anodised Nitinol is more bio-active and more biocompatible than the ground bare Nitinol surface. Anodisation has been proved to be an effective way to improve the biocompatibility of Nitinol.
Chapter 8  Conclusions and Future Work

This chapter summarizes the main findings from the previous chapters and makes some recommendations for future work.

8.1 Conclusions

In this thesis, the Nitinol was surface modified by several methods. The morphologies and chemistry of modified Nitinol surfaces were evaluated. Firstly, Nitinol surfaces were pre-treated by three different procedures to find out the most efficient surface pre-treatment of Nitinol. Pre-treated Nitinol surfaces were then modified by two electrochemical methods, namely electrochemical etching and anodisation. Modified Nitinol was then evaluated to investigate the influences of processing parameters on the resulting surface conditions. The biocompatibility of anodised Nitinol surface was also investigated. The following conclusions can be summarised.

Three different processes were used to pretreat Nitinol, including grinding with SiC paper up to grit of P2400, mechanical polishing with diamond paste down to 1 µm and chemical etching in 40% HNO₃ and 10% HF solution for 6 min. The ground Nitinol surface was relatively smooth and corrosion resistant. Therefore, grinding, as a simple procedure, was chosen to pre-treat Nitinol surface.

Electrochemical etching of Nitinol was carried out in 1M H₃PO₄ solution with 10 wt.% NH₄F, with varying applied voltage and duration time. Rough Nitinol surfaces with round nodules were generated by electrochemical etching. The morphologies and chemical compositions of electrochemically etched Nitinol surfaces were controlled by the electrochemical etching process parameters. The Nitinol surface was covered by more round nodules with longer etching time and larger round nodules were produced with higher voltage. The surface morphology and chemical compositions of electrochemically etched Nitinol surfaces were due to the competing dissolution (of TiO₂ and Ni) and oxidation (of Ti) reactions and the combination of different reaction rates at different locations on the Nitinol surface. Better wettability was also resulted on electrochemically etched Nitinol surfaces, implying better biocompatibility. Moreover, the electrochemically etched Nitinol surfaces exhibited better
corrosion resistance. Therefore, electrochemical etching was proved to be an effective method to modify Nitinol surface for biomedical applications.

Anodisation of Nitinol was conducted in an ethylene glycol based electrolyte with varying concentrations of fluorine ion and water. Rough and porous Nitinol surface was generated by anodisation and the Nitinol surface became rougher by increasing both the concentration of fluorine ion and water. The anodised Nitinol surface was covered by titanium oxide layer with depleted Ni content. Compared with ground Nitinol surface, the anodised Nitinol surface was more hydrophilic with physiological fluids, thereby offering better cell-material interactions. Moreover, the inhibition of *E. coli* growth with contact of Nitinol surface was also improved by anodisation.

The influence of anodising parameters (voltage and time) on the surface properties of Nitinol was also investigated. The surface roughness greatly increased with increasing voltage and time. The Ti/Ni ratio on Nitinol surface was significantly altered, due to the oxidation of Ti and dissolution of Ni. Nitinol surfaces showed an improved wettability compared with the ground Nitinol surface. Moreover, even a porous oxide layer was produced on Nitinol surface, but the tensile properties of the bulk material were not influenced. This study suggests that anodisation in fluorine ion containing ethylene glycol electrolyte is an efficient method to modify Nitinol surface and to produce biocompatible surfaces for biomedical applications.

The biocompatibility of anodised Nitinol surfaces was evaluated by analyzing Ni ion release in SBF, hydroxyapatite formation ability and L929 cell-material interaction. Ni ion release from anodised Nitinol surface depended on the surface morphology and the thickness of surface layer. More Ni ion would be released from rougher and more porous Nitinol surface. However, the amount of Ni ion released from anodised Nitinol surface is still below the tolerated concentration. Despite Ni ion release, anodised Nitinol surfaces showed better hydroxyapatite (HA) formation ability and L929 cell-material interaction. HA formed on anodised Nitinol surface after 3 weeks of immersion in SBF and continuously formed with longer immersion time. On the other hand, only a small peak of HA (detected by XRD) was found on the ground Nitinol surface after immersion in SBF. L929 mammalian cells could attach and proliferate better on anodised Nitinol surface than the ground Nitinol surface. Therefore, anodisation has been proved to be an effective way to improve the biocompatibility of Nitinol.
8.2 Future work

Even beyond the scope of this research, the tensile properties of ground and anodised Nitinol samples were tested, which were very similar, including ultimate tensile strength, fracture strain and Young’s modulus. This indicates that anodisation had little influence on the mechanical properties of Nitinol. Even though the anodised Nitinol surface was porous, the oxide layer was still too thin to affect the property of bulk material. Nevertheless, the anodised surface would affect the dynamic properties such as fatigue and fracture toughness. In order to gain better understanding of modified Nitinol surfaces, the following works are recommended for future investigations.

1. The electrochemical behaviour of electrochemically etched Nitinol surfaces needs further investigation, especially by using electrochemical impedance spectroscopy (EIS), so that how electrochemically etched Nitinol surface behaves in a corrosive solution would be better understood.

2. Detailed study concerning the initial stage of anodising process is required, which helps to gain a better understanding of how the porous surface structure formed on Nitinol. Electrochemical tests and transmission electron microscopy (TEM) are needed.

3. Immersion test of anodised Nitinol in simulated body fluid (SBF) should be carried out for longer time to evaluate the long term Ni ion release from anodised Nitinol surfaces.

4. The cell-material interaction of anodised Nitinol surface with other cell lines, for example MC3T3 mouse calvaria osteoblast cell line or CRL-11372 human fetal osteoblast cell line, could be conducted, in order to confirm the biocompatibility of Nitinol.

5. The change of bacterial cell membrane integrity and DNA damages with contact of Nitinol need to be studied in details, in order to better understand the antibacterial property of Nitinol.
References


