科技部補助專題研究計畫成果報告
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應用電化學陰極沉積鈣磷處理提升具超低彈性係數新型Ti-Nb-Zr-Sn鈦合金表面之骨細胞相容性質

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中 文 摘 要 ： 在生醫植入材應用研究中，Ti-24Nb-4Zr-8Sn（Ti2448）是一種新型β-Ti合金，由無毒元素組成且具有與骨組織相近的低彈性係數(約45 GPa)。然而，Ti2448合金的耐蝕性和生物相容性仍需要進一步提升以達成臨床長期使用成功的需求。在本研究中，將Ti2448合金浸泡在含有鈣磷成分的溶液中進行簡單且快速的電化學陽極氧化處理，改變其表面特性以提高其耐蝕性和生物活性。陽極氧化處理於Ti2448合金表面產生具混合形貌的氧化層（厚度50-120 nm），外層為含有鈣磷成分的奈米多孔形貌(鈣磷比約1.5)，而內層靠近基材底部則為較緻密的內層氧化層。實驗結果顯示，此內部緻密氧化層能有效提高Ti2448合金的耐蝕性。此外，此外層含有鈣磷的奈米多孔形貌表現與各種生物物質相近的尺寸形貌，進而有效提升Ti2448合金表面潤濕性和蛋白質吸附的能力。根據上述結果，具有低彈性係數的陽極氧化Ti2448合金具有應用於臨床骨科的潛力。

中 文 關 鍵 詞 ： Ti-Nb-Zr-Sn合金、低彈性係數、電化學處理、鈣/磷、腐蝕、骨細胞反應

英 文 摘 要 ： Ti-24Nb-4Zr-8Sn (Ti2448) is a new β-type Ti alloy which consists of nontoxic elements and exhibits exceptional low elastic modulus around 45 GPa for biomedical implant applications. Nevertheless, the corrosion resistance and biocompatibility of Ti2448 alloys need to be improved for long-term clinical use. In this study, a simple and fast electrochemical anodization treatment in Ca/P-containing solution was used on Ti2448 alloys to enhance bio-corrosion resistance and biological responses of Ti2448 alloys via altering the surface characteristics of it. The anodization process produced a thicker oxide layer (50-120 nm) composing of two different sections, one was outer section with Ca/P-containing nanoporous topography (Ca/P ratio 1.5) and the other was inner dense section near the substrate. The results showed that the inner dense section of oxide layer obviously enhanced the bio-corrosion resistance of Ti2448 alloys. Moreover, the Ca/P-containing nanoporous topography showing the similar scale of various biological species significantly improved the wettability and protein adsorption. In terms of above results, the anodized Ti2448 alloys with low elastic modulus have a potential for orthopedic applications.

英 文 關 鍵 詞 ： Ti-Nb-Zr-Sn alloy, low elastic modulus, electrochemical treatment, Ca/P, corrosion, bone cell response.

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摘要

在生醫植入材應用研究中，Ti-24Nb-4Zr-8Sn（Ti2448）是一種新型β-Ti 合金，由無毒元素組成且具有與骨組織相近的低彈性係數(約 45 GPa)。然而，Ti2448 合金的耐蝕性和生物相容性仍需要進一步提升以達成臨床長期使用成功的需求。在本研究中，將 Ti2448 合金浸泡在含有鈣磷成分的溶液中進行簡單且快速的電化學陽極氧化處理，改變其表面特性以提高其耐蝕性和生物活性。陽極氧化處理於 Ti2448 合金表面產生具混合形貌的氧化層（厚度 50-120 nm），外層為含有鈣磷成分的奈米多孔形貌(鈣磷比約 1.5)，而內層靠近基材底部則為較緻密的內層氧化層。實驗結果顯示，此內部緻密氧化層能有效提高 Ti2448 合金的耐蝕性。此外，此外層含有鈣磷的奈米多孔形貌表現與各種生物物質相近的尺寸形貌，進而有效提升 Ti2448 合金表面潤濕性和蛋白質吸附的能力。根據上述結果，具有低彈性係數的陽極氧化 Ti2448 合金具有應用於臨床骨科的潛力。

關鍵字：Ti-Nb-Zr-Sn 合金、低彈性係數、電化學處理、鈣/磷、腐蝕、骨細胞反應。

Abstract

Ti-24Nb-4Zr-8Sn (Ti2448) is a new β-type Ti alloy which consists of nontoxic elements and exhibits exceptional low elastic modulus around 45 GPa for biomedical implant applications. Nevertheless, the corrosion resistance and biocompatibility of Ti2448 alloys need to be improved for long-term clinical use. In this study, a simple and fast electrochemical anodization treatment in Ca/P-containing solution was used on Ti2448 alloys to enhance bio-corrosion resistance and biological responses of Ti2448 alloys via altering the surface characteristics of it. The anodization process produced a thicker oxide layer (50-120 nm) composing of two different sections, one was outer section with Ca/P-containing nanoporous topography (Ca/P ratio ~ 1.5) and the other was inner dense section near the substrate. The results showed that the inner dense section of oxide layer obviously enhanced the bio-corrosion resistance of Ti2448 alloys. Moreover, the Ca/P-containing nanoporous topography showing the similar scale of various biological species significantly improved the wettability and protein adsorption. In terms of above results, the anodized Ti2448 alloys with low elastic modulus have a potential for orthopedic applications.

Key words：Ti-Nb-Zr-Sn alloy, low elastic modulus, electrochemical treatment, Ca/P, corrosion, bone cell response.

1. Introduction (including literature survey and study purpose)

More than millions of bone graft procedures are expected to be performed yearly to fill bone defects or improve fracture healing and repair. This number is expected to continue to increase with the rapid growth of the elderly population. Therefore, developing a suitable material for repairing and regenerating bones that have been fractured due to disease, trauma and aging is a significant clinical challenge[1]. Titanium (Ti) and Ti-based alloys are widely used as biomaterials for orthopedic and dental implant applications due to their suitable mechanical properties, exceptional corrosion resistance and the highest biocompatibility with bone among all metallic biomaterials[2,3]. Although Ti and Ti-based alloys have specific and excellent properties for biomedical applications, some problems must be addressed for the long-term implantation of these materials. One of the most important issues is biomechanical incompatibility, especially in the elastic modulus of the commercially used Ti and Ti-based alloys, with human bone because both bone overload and excessive stress protection can result in bone resorption[4,5].

Compared with conventional metallic materials, such as stainless steel and Co-Cr-Mo alloys, Ti and Ti-based alloys show a relatively lower elastic modulus than those of stainless steel (200 GPa) and Co-Cr-Mo alloys (200-230 GPa). However, the elastic modulus of most of the commonly used commercial Ti and Ti-based alloys, including commercially pure Ti (CP-Ti), Ti-6Al-4V and Ti-6Al-7Nb (100-110 GPa)[6], is still an order of magnitude higher than that of human cortical bone (10-30 GPa)[7,8]. This mismatch between the elastic modulus of bone and that of implants will cause an insufficient load transfer from the implant to the adjacent bone and thus induce a stress-shielding effect during long-term implantation at the sites of load-bearing bones. This type of stress-shielding effect will lead to bone resorption at the bone-implant interface and eventually result in implant failure[9,10].

β-type Ti alloys have been the most attractive materials, for overcoming the incompatibility between the elastic modulus of Ti-based implants and that of bone, for orthopedic applications due to their non-toxic components, high mechanical strength and low elastic modulus[3,11,12]. Of all β-type Ti alloys, Ti-24Nb-4Zr-8Sn (wt%, hereafter designated Ti2448) is a recently developed β-type Ti alloy for biomedical applications. This novel alloy consists of non-toxic and non-allergic elements and possesses a low elastic modulus of approximately 45 GPa[13,14], close to that of human cortical bone. Such a low elastic modulus may prevent the stress-shielding effect caused by the inhomogeneous stress transfer between metal implants and the adjacent bone. Furthermore, this alloy not only has a low elastic modulus that is close to that of human bone, but it also has high mechanical strength - an ideal combination/balance of properties that is not available in the other β-type Ti alloys that have been developed so

far.

For long-term clinical use, the corrosion resistance of metallic materials is a great concern, especially when a metallic substitute is implanted in the virulent electrolytic environment of the human body, because corrosion is a gradually degradation of materials by electrochemical attack. Therefore, the corrosion performance of Ti2448 is extremely important for orthopedic and dental implant applications. Cheng *et al*. reported that for dental implant applications, the Ti2448 alloy showed a corrosion resistance similar to that of CP-Ti and the Ti6Al4V alloy in simulated oral environments, such as modified Fusayama artificial saliva and a lactic acid solution[15]. Moreover, Bai *et al*. showed that the corrosion resistance of the Ti2448 alloy was better than that of Ti6Al4V but comparable to that of CP-Ti in different simulated physiological solutions[16,17]. It has been commonly accepted that Ti and Ti-based alloys show exceptional corrosion resistance due to the natural formation of a passive oxide film. However, this protective passive film, which inhibits the release of metal ions, may become unstable in the human body due to deterioration during long-term implantation. Once the passive surface film is disrupted, corrosion proceeds, and metal ions are released continuously, which leads to ion accumulation and thus results in biological side effects and eventually implant failure[18,19]. Although the corrosion behavior of the Ti2448 alloy is similar to that of Ti, there is still a potential risk of metal ion release from the Ti2448 alloy due to the corrosion process. Therefore, determining how to further improve the bio-corrosion resistance of the Ti2448 alloy for future long-term implantation is one of the important purposes of this study.

Moreover, the passive oxide film on metallic materials plays an influential role not only in corrosion resistance but also in the biocompatibility of materials. It is well known that surface properties, including surface topography, roughness, chemical composition and wettability, are important factors in biocompatibility because they affect the biological responses at the bone-implant interface[20,22]. Surface topography is a key surface property because it is able to regulate the responses of cells that are located on material surfaces. Some studies have reported the design and creation of surface geometries with a suitable nanoscale topography that were able to improve cell responses, such as cell adhesion[23], migration[24], proliferation[25] and differentiation[26]. There are various surface modification treatments that can create a nanoscale topography on a metallic material surface to provide the desired biological responses. In our previous studies, a fast and simple electrochemical anodization treatment was used to produce a nano-networked oxide layer on the surfaces of CP-Ti and Ti-based alloys to improve the hemocompatibility and cell responses. Moreover, the layer was also able to enhance the corrosion resistance of biomedical Ti alloys[27-29].

Our hypothesis is that a Ca/P-containing oxide layer with a nanoscale porous topography could be produced and thickened on the surface of the Ti2448 alloy using a simple and fast electrochemical anodization treatment in Ca/P-containing solution to improve the bio-corrosion resistance and biocompatibility of this new β-type Ti alloy with low elastic modulus. To analyze this hypothesis, the polished Ti2448 specimens were created, as well as polished Ti control specimens, and Ti2448 specimens treated with electrochemical anodization treatment in Ca/P-containing solution to produce a nanoscale porous topography containing Ca/P composition. Within this research, the surface characteristics, including the surface morphology, oxide layer thickness, crystal structure and surface wettability, were investigated after the anodization treatment. Furthermore, the corrosion behaviors of the anodized Ti2448 alloys in simulated body environments were evaluated. To provide helpful indicators for further *in vivo* studies and clinical applications, the protein adsorption of the anodized Ti2448 alloy was studied as well.

2. Materials and Methods

2.1 Specimen preparation

Discs (diameter of 15 mm; thickness of 1 mm) of Ti2448, the new β-type of Ti alloys, which were polished with SiC papers from #120 up to #1200 and then cleaned in ethyl alcohol were used as substrates. Pure commercial Ti disks (diameter of 15 mm; thickness of 1 mm) that were polished and cleaned according to the above-mentioned procedure were used as a reference control.

A potentiostat was used for the electrochemical anodization treatment to apply two different anodic currents, A1 and A2 (A1< A2 < 0.5 ampere), to the polished Ti2448 substrate for a few tens of min in Ca/P-containing alkaline solution (Ca^{+2} < 0.5M; PO_4^{-3} < 0.5M). The polished Ti and Ti2448 specimens that were not subjected to the electrochemical anodization treatment were termed Ti-M and Ti2448-M, respectively. The polished Ti2448 specimen treated by electrochemical anodization with the applied currents A1 and A2 were termed Ti2448-A1 and Ti2448-A2, respectively.

2.2 Surface characteristics analysis

The surface morphology and average roughness of the test specimens were evaluated using field emission scanning electron microscopy (FE-SEM) and atomic force microscopy (AFM), respectively. The crystallographic structure and thickness of the outermost surface layer of the anodized Ti2448 specimens were evaluated using transmission electron microscopy (TEM). Prior to the TEM evaluation, the cross-sectional test specimens were prepared using a focused ion beam milling process. The

chemical composition of the outer surface layer of the specimens was analyzed by X-ray photoelectron spectroscopy (XPS). The surface wettability of the test specimens was analyzed using a contact angle goniometer, and the surface free energy of the specimens was calculated with two different solutions: polar double-distilled H_2O and non-polar diiodomethane.

2.3 Bio-corrosion resistance analysis

The surface corrosion resistance of the polished Ti-M and Ti2448-M and the electrochemical anodization-treated Ti2448-A1 and Ti2448-A2 was evaluated using a potentiostat. A saturated calomel electrode (SCE) and a platinum sheet were used as the reference and counter electrode, respectively. The test specimens with and without the electrochemical anodization treatment were used as the working electrodes. Neutral simulated blood plasma (SBP; pH 7.4)[30] and modified Fusayama artificial saliva (AS; pH 5.2)[31] were used as the corrosion test electrolyte and were maintained at 37°C during the experiment. The SBP was used to simulate the environment inside human body, and the AS was used to simulate that of the human mouth. All test specimens were placed into two different electrolytes, and the polarization curves of all specimens were measured from -1 V to + 1.5 V (with respect to the SCE).

2.4 Protein adsorption analysis

Two different types of protein were used as model proteins to evaluate the difference in protein adsorption on the test specimens. One was bovine serum albumin (BSA, Sigma), the most abundant protein in human body plasma[32]; the other was fibronectin (Sigma), an important protein involved in cell adhesion, migration, proliferation and differentiation[33]. The test specimens were immersed in two different phosphate buffered saline (PBS) solutions, one containing 5 mg/ml BSA and the other containing 50 μg/ml fibronectin. After 5 min of incubation at 37°C, the test specimens were washed with double-distilled H_2O and then dried at room temperature. XPS was used to analyze the nitrogen spectra (in terms of N1s) of the test specimens to estimate the ability for proteins to adsorb on the various treated test specimens.

2.5 Statistical analysis

The number of samples was 3 for all measurements, and the results were expressed as the means ± standard deviation (SD). Student's two-tailed t-test was used to determine the level of the significance, and $p<0.05$ was considered statistically significant.

3. Results

3.1 Surface characteristics

The surface morphologies of Ti2448 before and after the electrochemical anodization treatment are shown in Fig. 1. The FE-SEM images of the polished Ti (Ti-M) and Ti2448 (Ti2448-M) only showed oriented polished grooves (Fig. 1a and 1b). After the electrochemical anodization treatment, a nanoscale porous oxide layer was formed on the anodized Ti2448 (Ti2448-A1 and Ti2448-A2) surface (Fig. 1c and 1d). The pore size of the porous oxide layer on the Ti2448-A1 and Ti2448-A2 ranged from a few nm to 50 nm; however, Ti2448-A2, which was treated with a larger anodic current for a longer time, showed a bigger pore size than that of Ti2448-A1. XPS analysis results showed that the chemical compositions of Ti-M, Ti2448-A1, and Ti2448-A2 were the same, and mainly contained TiO_2 and Nb_2O_5 with small amounts of ZrO_2 and SnO_2. Moreover, the XPS depth profiles, in terms of the atomic concentrations of O, Ti, Nb, Zr, Sn in the surface of the test specimens indicated that the electrochemical anodization treatment significantly increased the thickness of the surface oxide layer. The ratio of Ca/P on the electrochemically treated Ti 2448-A1 and Ti2448-A2 surfaces was close to 1.5.

Furthermore, the thickness and crystal structure of the nanoporous oxide layer on Ti2448-A1 and Ti2448-A2 were analyzed by TEM and are shown in Fig. 2. The TEM images indicate that the cross-sectional thickness of the nanoscale porous oxide layer on Ti2448-A1 and Ti2448-A2 was approximately 50 to 80 nm and 80 to 120 nm, respectively (Fig. 2a and 2b). Compared with the untreated Ti-M and Ti2448-M, the treated Ti2448-A1 and Ti2448-A2 exhibited significant thickening of the surface oxide layer. The crystal structure of the oxide layer was amorphous. The oxide layer could be separated into two different sections: the outer porous section close to the surface and the inner nonporous section between the substrate and the outer porous section. Compared with the outer section, the inner section had a structure that was tighter with higher density (Fig. 2c).

AFM was used to evaluate the effect of the anodization treatment on the surface roughness of Ti2448-A1 and Ti2448-A2. The evaluation showed that there were no significant differences in surface roughness between the treated and untreated test samples (Fig. 3). For all test samples, the average surface roughness (R_a) was approximately 0.1 μm.

Finally, a contact angle goniometer was used to image and calculate the contact angle and surface free energy to evaluate the wettability of the test samples. The result shown in Fig. 4 indicates that the polar double-distilled H_2O on the anodized Ti2448-A1 and Ti2448-A2 showed a smaller contact angle (11-16°) than that on the untreated Ti-M and Ti2448-M ($>56°$). In contrast, the surface free energies of Ti2448-A1 and Ti2448-A2 were much higher than those of the untreated groups, suggesting that

Ti2448-A1 and Ti2448-A2 exhibited better surface wettability than that of Ti-M and Ti2448-M.

The above-mentioned results indicate that the electrochemical anodization treatment did not change the surface roughness on the μm scale, but it successfully created a hydrophilic double oxide layer which consists of outer nanoporous section and inner dense section.

3.2 Bio-corrosion resistance

The polarization curves for Ti-M, Ti2448-M, Ti2448-A1 and Ti2448-A2 are shown in Fig. 5. Compared to the corrosion potential (E_{corr}) of the untreated Ti-M and Ti2448-M, the E_{corr} of Ti2448-A1 and Ti2448-A2 was shifted to much nobler values. In addition, in the passive region, when the same anodic voltage was applied to the specimens, the anodic current (I_{pass}) of Ti2448-A1 and Ti2448-A2 was approximately ten times smaller than that of Ti-M and Ti 2448-M. In the SBP solution, the values of the corrosion rate (I_{corr}) for Ti-M, Ti2448-M, Ti2448-A1 and Ti2448-A2 were 0.041, 0.102, 0.019 and 0.023 $\mu A/cm^2$, respectively. In the AS solution, the I_{corr} values of Ti-M, Ti2448-M, Ti2448-A1 and Ti2448-A2 were 0.028, 0.093, 0.040 and 0.066 $\mu A/cm^2$, respectively. In both the SBP and AS solutions, the I_{corr} of the anodized Ti2448 group was smaller than that of the untreated Ti2448. The above-mentioned results suggest that the amount of total ions released by Ti2448-A1 and Ti2448-A2 was lower than that from either Ti-M or Ti2448-M, indicating that the thickened oxide layer produced by the electrochemical anodization treatment provided better protection than that of the untreated group.

3.3 Protein adsorption ability

Protein adsorbed on the surface of material is the very beginning step before cells attachment. Moreover, it might affect the following cell responses especially cell adhesion. Therefore, the protein adsorption, in terms of nitrogen (N1s) on all test specimens was measured using XPS and was shown in Fig. 6. The result indicated that the treated Ti2448-A1 and Ti2448-A2 showed higher N1s intensities than the untreated Ti-M and Ti2448-M in both the albumin (Fig. 6(a)) and fibronectin (Fig. 6(b)) solutions. The amount of albumin and fibronectin adsorbed on the Ti2448-A1 and Ti2448-A2 was more than 1-3 times then that on the untreated Ti-M and Ti2448-M, indicating that the electrochemical anodized treatment could improve the protein adsorption ability of Ti2448 alloy.

4. Discussion

For long-term clinical use, the corrosion resistance of the Ti2448 alloy is a great concern, especially when the Ti2448 alloy is implanted in the virulent electrolytic environment of the human body. The results of this study showed that the corrosion resistance of the Ti2448 alloy could be significantly enhanced through an electrochemical anodization treatment due to the formation of dense inner oxide layer on the substrate (Fig. 5).

It has been commonly accepted that the passive oxide film formed on the surface of metallic materials plays an important role in corrosion resistance. In previous studies, Cheng *et al.* reported that the Ti2448 alloy showed the lower total metal ions release than that of CP-Ti and Ti6Al4V alloy in static immersion test in AS, lactic acid solution, fluoridated saliva and fluoridated acidified saliva. This indicated that Ti2448 alloy had better performance of corrosion resistance than that of CP-Ti and Ti6Al4V alloy for dental implant applications[15]. In contrast, Bai *et al*. indicated that the Ti2448 alloy exhibited a wider passive region than that of CP-Ti and Ti6Al4V alloy and its corrosion current density was comparable to that of CP-Ti in PBS, Hank's solution and AS solution[16,17].

As shown in Fig. 5, Ti2448-M showed a corrosion resistance comparable to that of Ti-M in both SBP and AS solutions. Furthermore, the anodized Ti2448 alloys showed much better corrosion resistance than that of Ti-M and Ti2448-M (Fig. 5). This exceptional bio-corrosion resistance of the anodized Ti2448 alloys may be due to the protective oxide film formed on the surface of it. The passivity and corrosion resistance of many pure metals used for biomedical applications have been studied. The results showed that Ti, Nb, Zr and Sn metals exhibited great corrosion resistance and in the following order: Nb>Ti>Zr>Sn[36,37]. Moreover, several studies also concluded that the addition of Nb and Zr improves the corrosion resistance of Ti alloys because of the formation of Nb_2O_5 and ZrO_2, which can strengthen the TiO_2 oxide film on Ti alloys[38-40]. Based on the above-mentioned studies, the surface oxide film of Ti2448-M, Ti2448-A1 and Ti2448-A2, which contains corrosion-resistant components (e.g., mainly TiO_2 and Nb_2O_5 with small amounts of ZrO_2 and SnO), can provide Ti2448 alloy with good corrosion resistance.

The corrosion resistance of Ti and Ti alloys depends not only on the composition of the surface oxide film but also on their thickness and crystal structure. It is well known that the surface oxide film which spontaneously forms on Ti and Ti alloys is thin (<10 nm), uniform and dense[41]. Therefore, any surface treatments that can thicken this protective oxide film were expected to improve the corrosion resistance of Ti and Ti alloys. Cigada *et al.* used anodic oxidation to improve the corrosion resistance of Ti6Al4V alloy in buffered physiological solution by thickening the oxide film on the Ti6Al4V alloys[42]. Other research from Birch *et al.* and Velten *et al.* also showed that

oxide films thickened by thermal oxidation, anodic oxidation or sol-gel coating exhibits better corrosion resistance than that of polished Ti and Ti alloys[41, 43].

As shown in Fig. 2, the surface oxide film on Ti2448-A1 and Ti2448-A2 was approximately 50-80 nm and 80-120 nm thick, respectively. The anodization treatment significantly thickened the passive oxide film on Ti2448-A1 and Ti2448-A2 compared with that spontaneously formed on Ti-M and Ti2448-M. In contrast with the naturally forming dense oxide film on Ti and Ti alloys surface, the protective oxide film formed on the anodized Ti2448 alloys exhibited a nanoscale porous surface topography with a pore size that ranged from a few to 50 nm (Fig. 1c and 1d). Although the oxide layer on the anodized Ti2448 alloys showed a porous surface topography, the TEM analysis demonstrated that this oxide film could be separated into two sections. One was the outer porous section close to the outer most surface, and the other was the inner nonporous dense section between the substrate and the outer porous section. Compared with the outer section, the inner section of the anodized Ti2448 alloys had denser and tighter structure which contributed to the improvement of the corrosion resistance of Ti2448 alloy. Therefore, the existence of this thicker, dense, inner section of oxide film improves the corrosion resistance of Ti2448 alloys in both SBP and AS solutions.

Furthermore, the crystal structure of this inner section was amorphous. Amorphous oxide structures lack grain boundaries make it difficult for electrochemical corrosion[44]. Therefore, many studies have demonstrated that an amorphous oxide film could improve the corrosion resistance of metal materials[44-46]. The above-mentioned results are consistent with the results of this study: the potential dynamic polarization curves of the anodized Ti2448 alloys with the thicker and inner dense amorphous oxide layer displayed higher E_{corr}, lower values of anodic current density and lower I_{corr} than those of the untreated Ti2448-M (Fig. 5).

According to previous study, the Ti2448 alloy, which consists of non-toxic, non-allergic elements and possesses a low elastic modulus of approximately 45 GPa[13], has properties that are beneficial for biomedical applications. The low elastic modulus of the Ti2448 alloy may balance the inhomogeneous transfer of stress between the metal implant and the adjacent bone, thus further preventing the stress-shielding effect. For future clinical application, bio-corrosion resistance of the Ti2448 alloy and good bio-interaction between tissue and the Ti2448 alloy will both be a great concern. In the previous studies, many surface modified treatments have been used to enhance the biocompatibility of biomaterials. In studies of Ti2448 alloy, many previous researches used micro-arc oxidation (MAO) treatment to modify the surface topography and chemical composition of the Ti2448 alloy to improve its biological responses[47-49]. However, the MAO treatment combines electrochemical oxidation with a high-voltage spark treatment in an electrolyte solution. The high voltages used in the above-

mentioned studies all exceeded 200 V, and the high energy made the process relatively dangerous and expensive. In contrast with the MAO treatment, the electrochemical anodization treatment used in this study is safer and cheaper. Moreover, the bio-corrosion resistance and biocompatibility of the Ti2448 alloy was significantly improved through the simple and fast electrochemical anodization treatment in this study.

It is commonly accepted that surface properties, such as topography, roughness, chemical composition and wettability, affect the biological responses at the bone-implant interface. Surface topography is a key property because it is able to regulate the responses of cells on the materials surface. In previous studies, nanoscale topographies, such as nanotubes, nanopits and nanonetworks, have influenced cell adhesion, spreading, migration, proliferation and differentiation *in vitro*, as well as bonding strength and bone formation *in vivo*[50-53]. In the present study, a disordered but homogeneous nanoscale porous oxide layer could be created on the surface of Ti2448 alloys through the electrochemical anodization treatment and the chemistry of the oxide layer was similar to that of previously used materials. The pore size of the porous oxide layer on the anodized Ti2448 alloys ranged from a few nm to 50 nm (Fig. 1c and 1d). Ti2448-A2 showed a bigger pore size than that of Ti2448-A1 due to the difference in the applied anodic current. Moreover, Ti2448-A1 and Ti2448-A2 showed little difference in their nanofeatures. According to the previous report by Rani *et al.*, the nanofeature of Ti2448-A1 was more similar to a nanoleaf, and that of Ti2448-A2 was more similar to a nanoscaffold[54]. The surface free energy of the anodized Ti2448 alloys was enhanced, regardless of the difference in their nanofeatures. The results of contact angle and surface free energy analysis suggested that Ti2448-A1 and Ti2448-A2 showed better surface wettability than Ti-M and Ti2448-M. Bico *et al.* concluded that surface wettability could be affected by the surface chemistry and topography[55]. In our research, the Ti2448-M, Ti2448-A1 and Ti2448-A2 alloys exhibited similar surface chemistries but different surface topographies. Therefore, we suggest that the improved surface wettability caused by the electrochemical anodization treatment was mainly due to formation of the nanoscale porous topography surface oxide layer. In contrast, it has been reported that the cell attachment and spreading were significantly greater on hydrophilic surfaces than on hydrophobic surfaces[56]. Therefore, we expected that this hydrophilic surface of Ti2448-A1 and Ti2448-A2 could result in easy penetration by body fluids, thereby enhancing the protein adsorption to trigger the subsequent cell responses.

Protein adsorption is the first step that occurs when a biomaterial is implanted into a biological environment, and it is a key determinant of the responses of cells to the material surface. As shown in Fig. 6, the amount of albumin and fibronectin adsorbed

on Ti2448-A1 and Ti2448-A2 was more than 1- to 3 fold higher than the amount adsorbed on the untreated Ti-M and Ti2448-M. In previous studies, one mechanism of protein adsorption was the electrostatic interaction between proteins and the material surface[57]. The isoelectric point of TiO_2 and Nb_2O_5, the two main component of the surface oxide layer of the anodized Ti2448 alloys, is approximately 4.1 to 4.5, which is less than the pH of the physiological environment (~pH 7.0) and makes the oxide surface negatively charged[58,59]. Therefore, the negatively charged surface could attract positively charged ions, such as Ca^{2+}. When Ca^{2+} ions bind to the material surface, they could act as a bridge between the negatively charged metal oxide surface and proteins to induce protein adsorption onto the material surface.

In addition, surface topographical characteristics are also important factors and influence the protein adsorption. Sela *et al.* reported that the preferential adsorption of plasma protein may be explained by the increase in the 3D surface area of the modified Ti surface[60]. Richert *et al.* also reported that the greater surface area resulting from the creation of the nanoporous structure provides more binding sites for protein adsorption[61]. In this study, the nanoscale porous oxide layer produced by the electrochemical anodization treatment was negatively charged and hydrophilic, and its surface area was expected to be much larger than that of untreated groups. These changes significantly enhanced the protein adsorption ability of Ti2448 alloys. This finding is consistent with the above-mentioned researches. This behavior may trigger the subsequent cell adhesion, proliferation, migration and differentiation[62-64].

5. Conclusions and Suggestions

In the one-year project, using a simple and fast electrochemical anodization treatment, a nanoporous oxide multilayer could be successfully produced and thickened on the surface of the newly developed β-type Ti-24Nb-4Zr-8Sn (Ti2448) alloy with a low elastic modulus of 45 GPa. The dense inner section of the nanoporous oxide multilay er resulted in enhanced resistance to bio-corrosion. This resistance was demonstrated by an increase in the corrosion potential and a decrease in the anodic current in both the SBP and AS conditions. We suggest that the presence of the nanoporous oxide multilayer provides excellent protection for the substrate. This layer prevents ion release from the substrate and decreases the risk of cytotoxicity caused by metal ions accumulation during clinical implantations.

Moreover, the Ca/P-containing (ratio of Ca/P: 1.5) nanoporous outer section of the oxide multilayer also improved the surface wettability of the Ti2448 alloy. In addition to the improved hydrophilicity, this unique nanoporous topography has a range of pore sizes from a few nm up to approximately 50 nm; this range covers the scale of various

types of proteins in the human body. From the results of this study, it is thus reasonable to conclude that the above-mentioned characteristics may enhance the protein adsorption ability of the Ti2448 alloy. Because of the improved bio-corrosion resistance and biological responses of the anodized Ti2448 alloys, we suggest that the surface-modified Ti2448 alloys with low elastic modulus have a great potential for biomedical implant applications. Further in vivo study on the osseointegration of the developed materials is suggested

6. References

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7. Figures

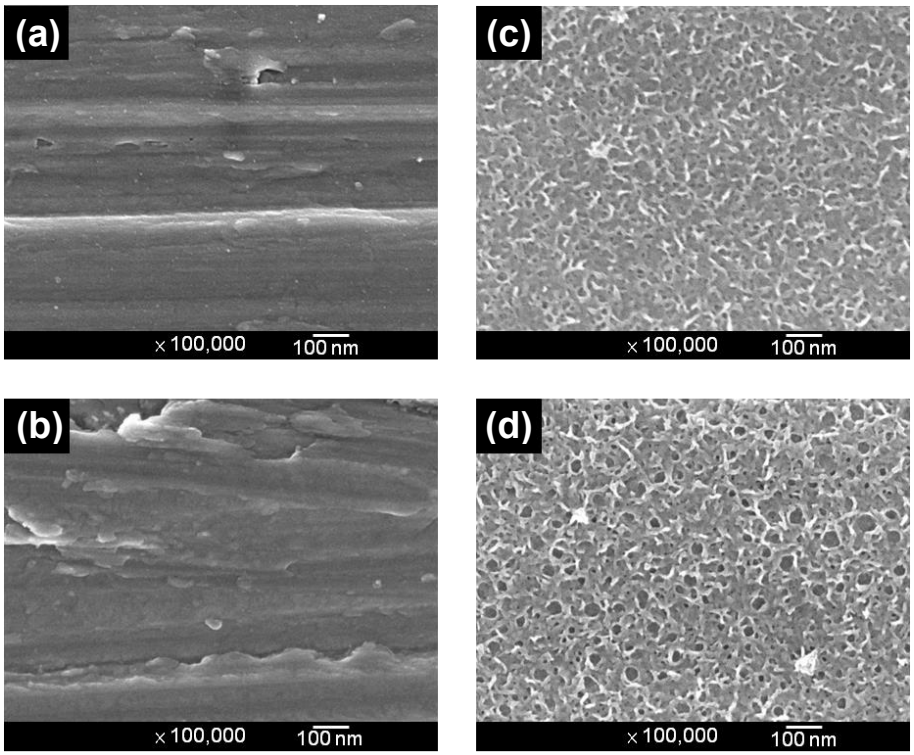


Fig. 1. Surface FE-SEM morphologies of test Ti and Ti2448 specimens: (a) Ti-M: Ti specimen polished with SiC paper upto #1200; (b) Ti2448-M: Ti2448 specimen polished with SiC paper upto #1200; (c) Ti2448-A1: Ti2448-M treated through electrochemical anodization with current A1; (d) Ti2448-A2: Ti2448-M treated with current A2.

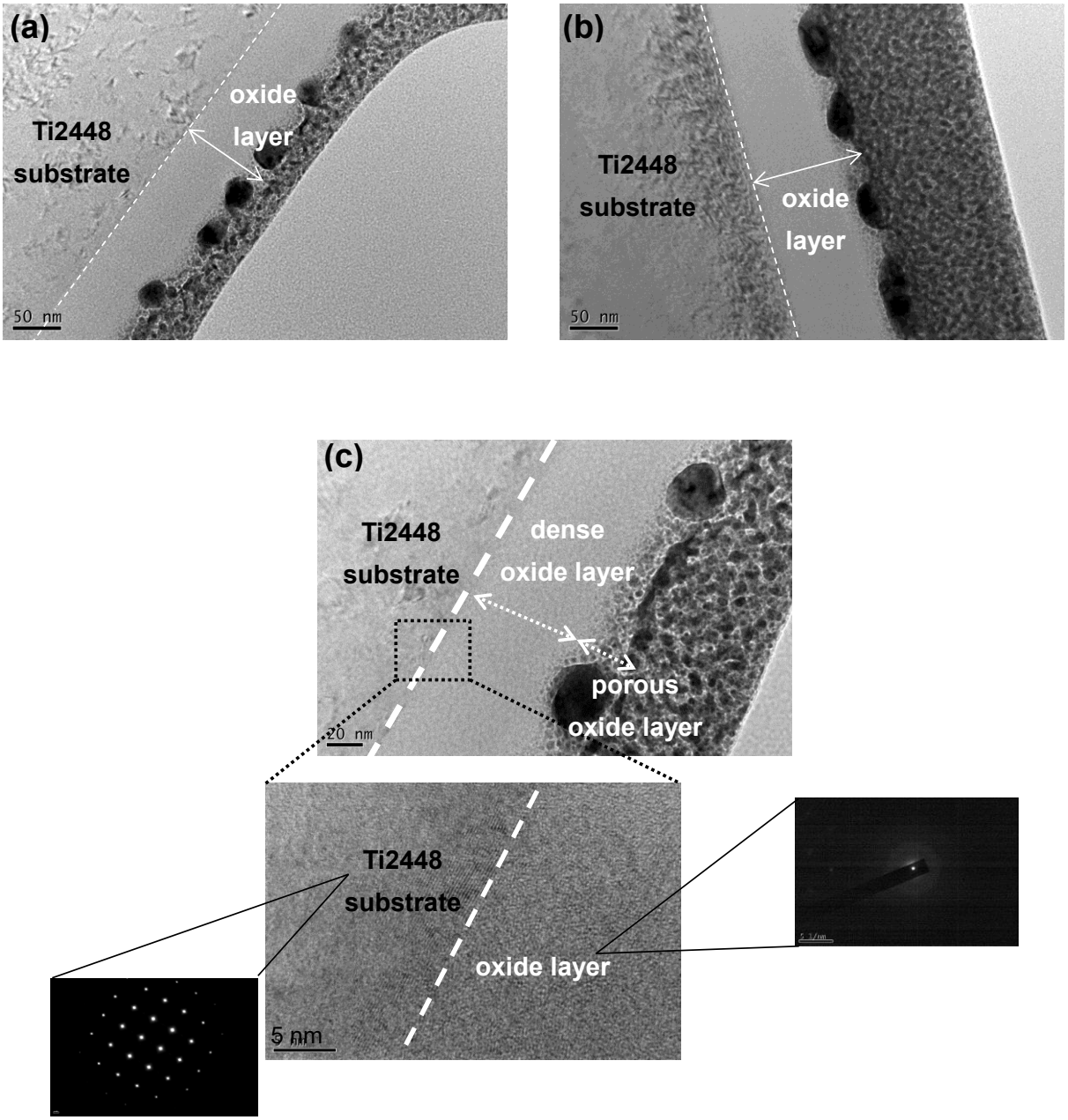


Fig. 2. Cross-sectional TEM images of anodized Ti2448 specimens: (a) Ti2448-A1; (b) Ti2448-A2; (c) Higher magnification of (a) with selected area diffraction patterns.

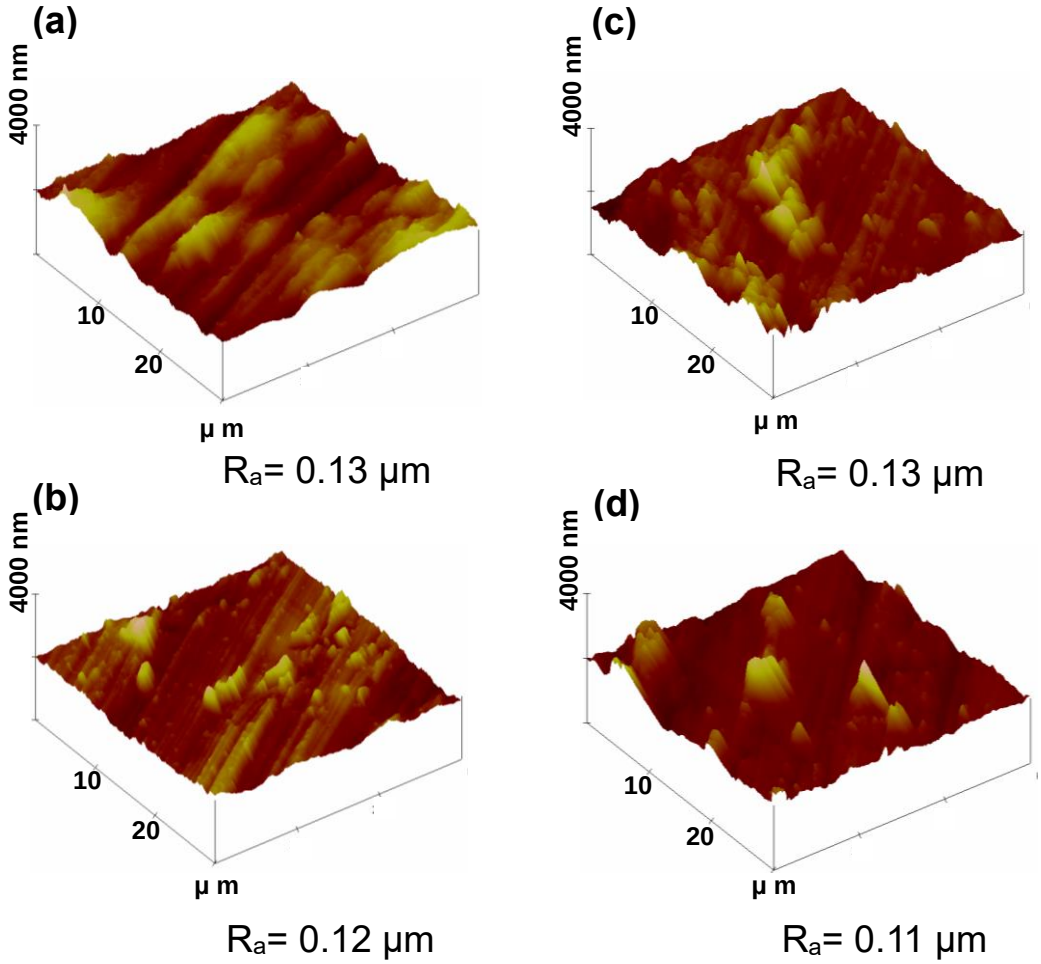


Fig. 3. Surface AFM topography and roughness of test Ti and Ti2448 specimens: (a) Ti-M; (b) Ti2448-M; (c) Ti2448-A1; (d) Ti2448-A2.

Materials	Ti-M	Ti2448-M	Ti2448-A1	Ti2448-A2
ddH_2O (polar)				
Contact angle (θ)	56.5 ± 4.7 ˚	64.3 ± 1.7 ˚	15.9 ± 4.3 ˚	11.0 ± 0.7 ˚
Diiodo-Methane (non-polar)				
Contact angle (θ)	33.8 ± 3.2 ˚	35.8 ± 4.4 ˚	40.8 ± 2.7 ˚	46.0 ± 1.6 ˚
Surface free energy (mN/m)	56	51	74	74

Fig. 4. Contact angle and surface free energy of test Ti and Ti2448 specimens.

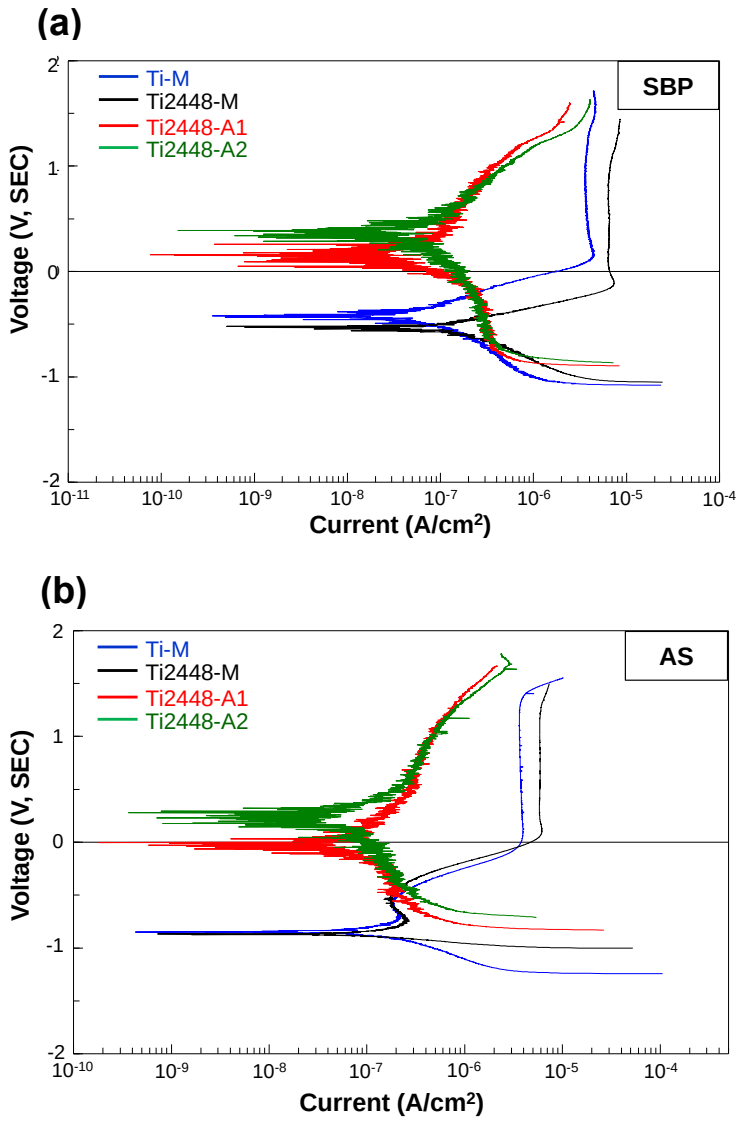


Fig. 5. Polarization curves of test Ti and Ti2448 specimens in different electrolytes: (a) SBP; (b) AS.

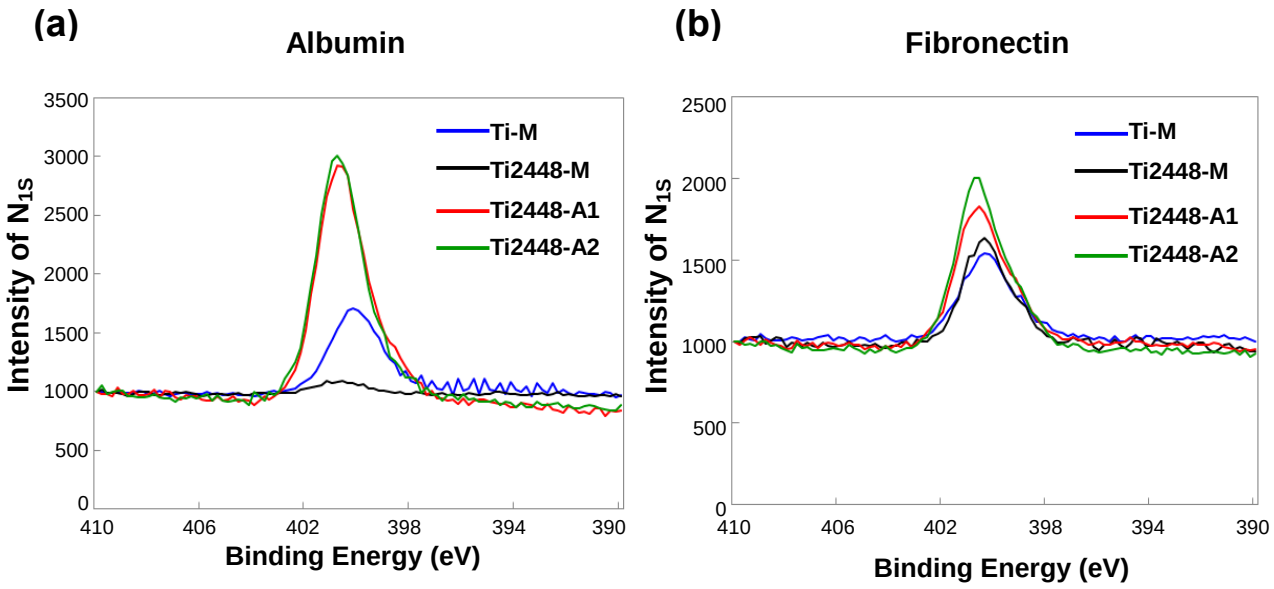


Fig. 6. Protein adsorption analysis, in terms of N1s spectra, obtained using XPS of test Ti and Ti2448 specimens: (a) Albumin; (b) Fibronectin.

附件

科技部補助專題研究計畫出席國際學術會議心得報告

日期：2016 年 10 月 31 日

計畫編號	MOST 104-2221-E-040 -003		
計畫名稱	應用電化學陰極沉積鈣磷處理提升具超低彈性係數新型Ti-Nb-Zr-Sn鈦合金表面之骨細胞相容性質		
出國人員姓名	黃何雄 (共同主持人)	服務機構及職稱	國立陽明大學牙醫系/教授
會議時間	2015年 8月 30日 至 2015年 9月 03日	會議地點	波蘭、克拉科夫
會議名稱	(中文) 2015年第27屆歐洲生醫材料會議 (英文) 27th European Conference on Biomaterials (ESB2015)		
發表題目	(中文) 探討天然交聯劑京尼平接枝第一型膠原蛋白於鈦表面之人類骨髓間葉幹細胞反應 (英文) Human Bone Marrow Mesenchymal Stem Cells Responses to Titanium Surface Coated with Type I Collagen Using Natural Cross-linker Genipin		

一、 參加會議經過

筆者於2015年08月底至波蘭克拉科夫參加27th European Conference on Biomaterials (ESB 2015)，發表科技部研究成果，會議期間為2015年08月30~09月03日。

二、 與會心得

本次「2015年第27屆歐洲生醫材料會議」會場位於波蘭克拉科夫中心 (圖一)，附近交通相當便捷，非常適合舉行國際性會議。該會議會場的設施完善，令筆者印象非常深刻。大會於08月31日舉行開幕式(圖二)。並邀請許多世界上知名的學者進行大會特邀報告，其中加拿大學者Michael V. SEFTON教授演講的"Vascularization in Tissue Engineering: Alternative Foreign Body Responses"、波蘭學者Małgorzata LEWANDOWSKA-SZUMIEŁ教授演講的"Cell-made or Man-made Materials for Bone Reconstruction"及四川大學顧忠偉教授演講的" Bioinspired Design of Dynamic Macromolecular for Gene Delivery"(圖三)皆讓筆者大有所獲。

會議進行的同時，會場外面亦同時舉行了廠商產品展示會及貼示報告交流，此為一般國際會議中常見到的安排，其目的是增加與會者更多的交流管道。會議中也舉辦許多比賽，促進大家在研究領域中的認真與積極，讓年輕學者有許多實質的交流機會。大體而言，會場的布置及展示會的內容而言，主辦單位非常用心的投入。筆者於09月01日參加poster session (圖四)，會場交流相當熱絡，筆者也與相關領域學者們進行學術交流(圖五)。筆者所發表的oral報告題目為"Human Bone Marrow Mesenchymal Stem Cells Responses to Titanium Surface Coated with Type I Collagen

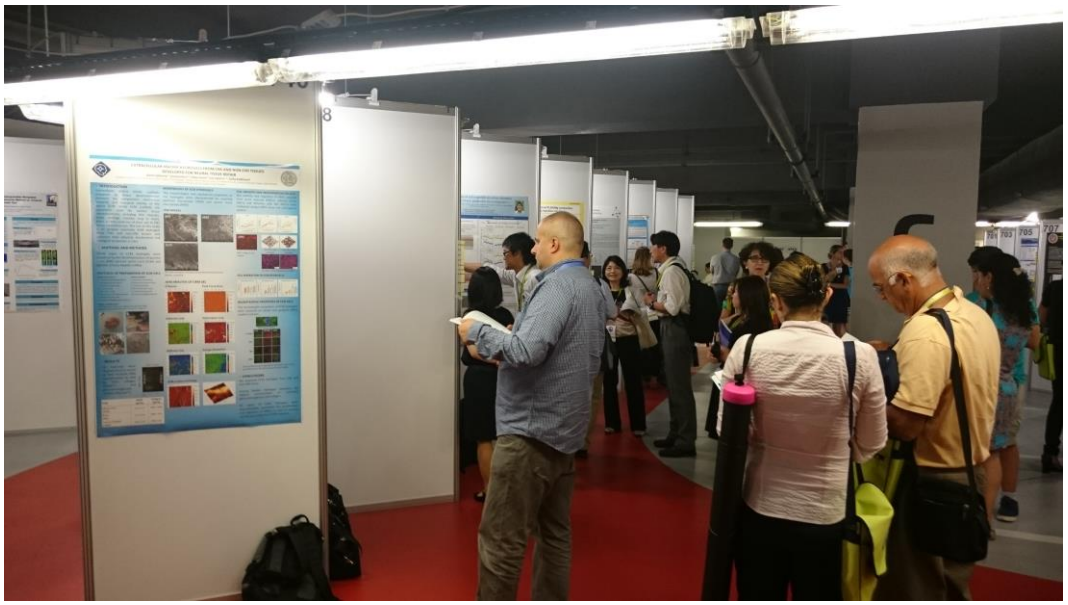
Using Natural Cross-linker Genipin”(圖六)，為科技部計畫之研究成果。

本次會議的主題眾多，包括Bone Tissue Engineering、Cartilage Tissue Engineering、Drug Delivery、Surface Modification、Cell Instructive Materials、Advanced Manufacturing、Osteointegration、Neural Regeneration等數專業領域。筆者主要參加Synthesis and Fabrication of Biomaterials and Devices及Interactions of Biomaterials and cells等主題的論文發表。

圖一、ESB 2015 會場外觀。

圖二、ESB 2015 大會主席於 08 月 31 日會議開幕式中致詞。

圖三、筆者聆聽四川大學顧忠偉教授演講。



圖四、會議poster session會場一角。

(a)

(b)

圖五、筆者在會場與中國科學院金屬研究所徐堅教授 ((a)圖中) 及 (b) 四川大學顧忠偉教授((b)圖中)、浙江大學高長有教授((b)圖右)進行學術交流與合影。

圖六、筆者於 08 月 31 日進行的口頭報告。

三、 發表論文摘要

Human Bone Marrow Mesenchymal Stem Cells Responses to Titanium Surface Coated with Type I Collagen Using Natural Cross-linker Genipin

Ying-Sui Sun[1], Her-Hsiung Huang[1], Kai-Chun Chang[2]

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INTRODUCTION

Various surface treatments have been focused on coating bioinert metallic implants with biomolecular in order to regulate the functions of cells on implant surfaces[1]. Type I collagen, the most abundant protein in the bone matrix, has been used to improve the biological activity on implant surfaces[2]. This study sought to immobilize the type I collagen to biologically inert titanium surface through the use of the nontoxic, natural cross-linker genipin. Surface characterizations and cell responses of the treated titanium surfaces for dental implant application were investigated.

EXPERIMENTAL METHODS

We selected type I collagen for the biomolecular coating. For the sake of simplicity and low cost, we used the nontoxic natural cross-linker genipin as the medium with which to immobilize type I collagen to the machined titanium surface designated TiGC. The untreated machined titanium surface was used as control designated Ti. Surface characterizations, including the morphology, chemistry, hydrophilicity, and roughness, of the test materials were analyzed. Human bone marrow mesenchymal stem cells (hBMSCs) responses, including the adhesion, migration, proliferation, mineralization, and differentiation, to the test materials were performed. At least five samples were used for each test group. Statistical analysis was performed using Student's t-test with alpha of 0.05.

RESULTS AND DISCUSSION

X-ray photoelectron spectroscopy analysis results demonstrated that the genipin could successfully immobilize the type I collagen to titanium surface. Scanning electron spectroscopy (SEM) analysis results showed that the thickness of the type I collagen coated on titanium surface could be controlled at a nanoscale. The resulting surface of TiGC included a mixture of reticular submicro- and nanostructures (Figure 1(b)) and displayed high hydrophilicity (water contact angle < 22°). Following few months of storage at 4°C, the type I collagen on titanium surface still presented good bonding and functionality, which promoted the mineralization of hBMSCs. Cell responses analysis revealed that the type I collagen coating promoted the adhesion (Figure 2(b)), migration, and osteogenesis of hBMSCs as well as the mineralization of the extracellular matrix (ECM). Compared to the untreated Ti, the type I collagen-coated TiGC had higher expression levels of osteogenic markers, in terms of osteopontin (Figure 3(a)), bone sialoprotein, and osteocalcin (Figure 3(b)).

CONCLUSION

The use of the natural cross-linker genipin as the medium to immobilize type I collagen to titanium surface promoted the adhesion, migration, and osteogenesis of hBMSCs as well as the mineralization of the ECM. The proposed surface treatment technique is a simple low-cost process that can be potentially employed for titanium dental implant.

REFERENCES

1. Park J.W. *et al*., Acta Biomater. 7:3222-9, 2011.
2. Schliephake H. *et al.*, Clin. Oral Implants Res. 20:31-7, 2009.

ACKNOWLEDGMENTS

The authors would like to thank the financial support from Ministry of Science and Technology, Taiwan (MOST 101-2314-B-010-052-MY3). Thanks are also due to Dr. S.H. Chiou from Taipei Veterans General Hospital, Taiwan, for kindly providing us with the cells.

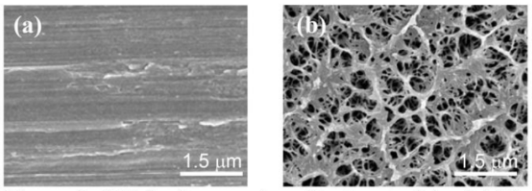


Figure 1 SEM micrographs of test materials: (a) Ti; (b) TiGC.

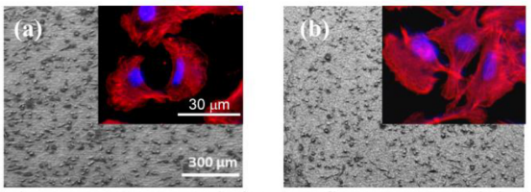


Figure 2 SEM and immunofluorescent staining micrographs of test materials: (a) Ti; (b) TiGC.

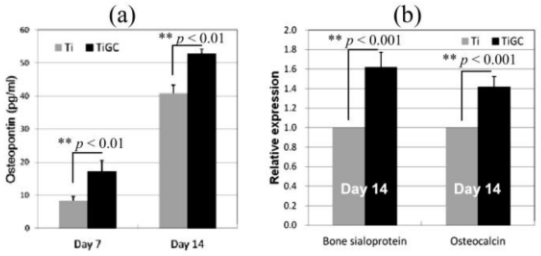


Figure 3 Expression levels of osteogenic markers, in terms of osteopontin (a), bone sialoprotein, and osteocalcin (b), of test materials Ti and TiGC.

四、 建議

為了鼓勵國內生醫材料相關領域的研究及提升研發技術的水準，舉辦國際性的會議與積極參與國際性相關領域的重要會議，是達到上述目標有效的方法之一。此次第27屆歐洲生醫材料會議是全世界醫材領域學者都相當關注的學術會議，不論是活動內容或是學術交流，皆是值得參加此會議的特色。此外，科技部長期在學術研究上所付出的心力，筆者個人持非常高度的肯定。同時也感謝科技部在經費上的補助 (MOST 104-2221-E-040 -003)，讓筆者有幸能參與此重要的學術交流活動，激發出更多的研究構想。

五、 攜回資料名稱及內容

筆者於會後攜回資料豐富，包括所參加的會議論文集（包含口頭及海報論文之摘要），以及會議材料展示會的寶貴資料（包含最新的各項生醫材料研究產品及技術資訊），上述資料將可做為國內相關學者及業界研發新生醫材料及技術重要參考。

附件

科技部補助專題研究計畫出席國際學術會議心得報告

日期：2016 年 10 月 31 日

計畫編號	MOST 104-2221-E-040 -003		
計畫名稱	應用電化學陰極沉積鈣磷處理提升具超低彈性係數新型Ti-Nb-Zr-Sn鈦合金表面之骨細胞相容性質		
出國人員姓名	黃何雄	服務機構及職稱	國立陽明大學牙醫系/教授
會議時間	2015年 8月 30日 至 2015年 9月 03日	會議地點	波蘭、克拉科夫
會議名稱	(中文) 2015年第27屆歐洲生醫材料會議 (英文) 27th European Conference on Biomaterials (ESB2015)		
發表題目	(中文) 探討天然交聯劑京尼平接枝第一型膠原蛋白於鈦表面之人類骨髓間葉幹細胞反應 (英文) Human Bone Marrow Mesenchymal Stem Cells Responses to Titanium Surface Coated with Type I Collagen Using Natural Cross-linker Genipin		

一、 參加會議經過

筆者於2015年09月至波蘭克拉科夫參加27th European Conference on Biomaterials (ESB 2015)，發表科技部研究成果，會議期間為2015年08月30~09月03日。

二、 與會心得

本次「2015年第27屆歐洲生醫材料會議」會場位於波蘭克拉科夫中心 (圖一)，附近交通相當便捷，非常適合舉行國際性會議。該會議會場的設施完善，令筆者印象非常深刻。大會於08月31日舉行開幕式(圖二)。並邀請許多世界上知名的學者進行大會特邀報告，其中加拿大學者Michael V. SEFTON教授演講的"Vascularization in Tissue Engineering: Alternative Foreign Body Responses"、波蘭學者Małgorzata LEWANDOWSKA-SZUMIEŁ教授演講的"Cell-made or Man-made Materials for Bone Reconstruction"及四川大學顧忠偉教授演講的" Bioinspired Design of Dynamic Macromolecular for Gene Delivery"(圖三)皆讓筆者大有所獲。

會議進行的同時，會場外面亦同時舉行了廠商產品展示會及貼示報告交流，此為一般國際會議中常見到的安排，其目的是增加與會者更多的交流管道。會議中也舉辦許多比賽，促進大家在研究領域中的認真與積極，讓年輕學者有許多實質的交流機會。大體而言，會場的布置及展示會的內容而言，主辦單位非常用心的投入。筆者於09月01日參加poster session (圖四)，會場交流相當熱絡，筆者也與相關領域學者們進行學術交流(圖五)。筆者所發表的oral報告題目為"Human Bone Marrow Mesenchymal Stem Cells Responses to Titanium Surface Coated with Type I Collagen

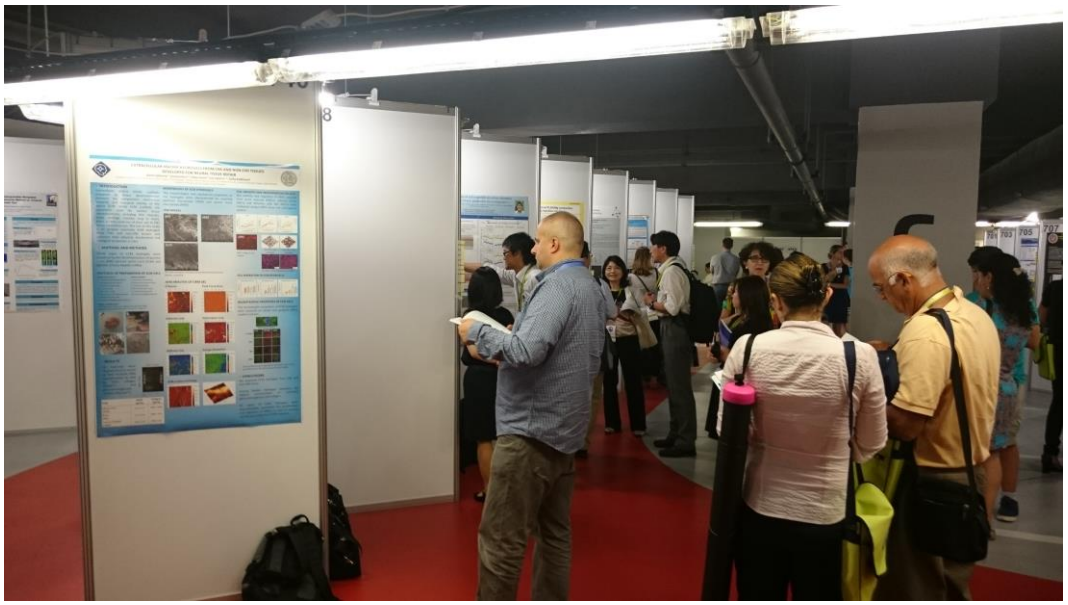
Using Natural Cross-linker Genipin”(圖六)，為科技部計畫之研究成果。

本次會議的主題眾多，包括Bone Tissue Engineering、Cartilage Tissue Engineering、Drug Delivery、Surface Modification、Cell Instructive Materials、Advanced Manufacturing、Osteointegration、Neural Regeneration等數專業領域。筆者主要參加Synthesis and Fabrication of Biomaterials and Devices及Interactions of Biomaterials and cells等主題的論文發表。

圖一、ESB 2015 會場外觀。

圖二、ESB 2015 大會主席於 08 月 31 日會議開幕式中致詞。

圖三、筆者聆聽四川大學顧忠偉教授演講。



圖四、會議poster session會場一角。

(a)

(b)

圖五、筆者在會場與中國科學院金屬研究所徐堅教授 ((a)圖中) 及 (b) 四川大學顧忠偉教授((b)圖中)、浙江大學高長有教授((b)圖右)進行學術交流與合影。

圖六、筆者於 08 月 31 日進行的口頭報告。

三、 發表論文摘要

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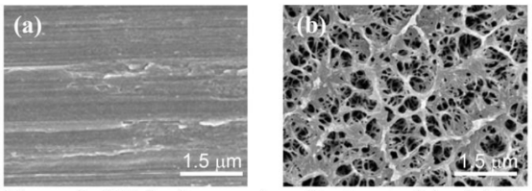

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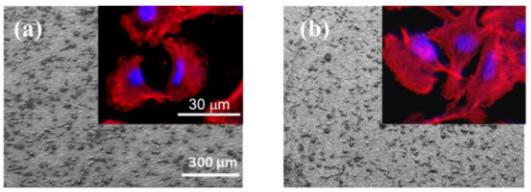

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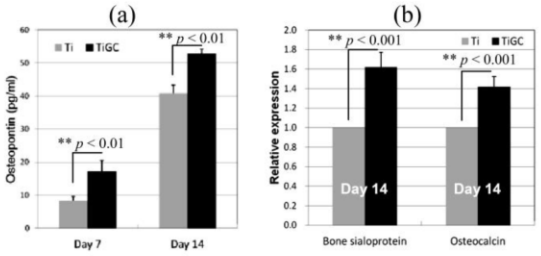

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科技部補助計畫衍生研發成果推廣資料表

日期:2016/11/14

科技部補助計畫	計畫名稱: 應用電化學陰極沉積鈣磷處理提升具超低彈性係數新型Ti-Nb-Zr-Sn鈦合金表面之骨細胞相容性質
	計畫主持人: 李慈心
	計畫編號: 104-2221-E-040-003- 學門領域: 生醫材料

無研發成果推廣資料

104年度專題研究計畫成果彙整表

計畫主持人：李慈心	**計畫編號：**104-2221-E-040-003-
計畫名稱：應用電化學陰極沉積鈣磷處理提升具超低彈性係數新型Ti-Nb-Zr-Sn鈦合金表面之骨細胞相容性質	

成果項目					量化	單位	質化 （說明：各成果項目請附佐證資料或細項說明，如期刊名稱、年份、卷期、起訖頁數、證號...等）
國內	學術性論文	期刊論文			0	篇	
		研討會論文			0		
		專書			0	本	
		專書論文			0	章	
		技術報告			0	篇	
		其他			0	篇	
	智慧財產權及成果	專利權	發明專利	申請中	0	件	
				已獲得	0		
			新型/設計專利		0		
		商標權			0		
		營業秘密			0		
		積體電路電路布局權			0		
		著作權			0		
		品種權			0		
		其他			0		
	技術移轉	件數			0	件	
		收入			0	千元	
國外	學術性論文	期刊論文			0	篇	
		研討會論文			1		參加"""2015年第27屆歐洲生醫材料會議"""，口頭發表文章一篇。
		專書			0	本	
		專書論文			0	章	
		技術報告			0	篇	
		其他			0	篇	
	智慧財產權及成果	專利權	發明專利	申請中	0	件	
				已獲得	0		
			新型/設計專利		0		
		商標權			0		
		營業秘密			0		
		積體電路電路布局權			0		
		著作權			0		

		品種權	0		
		其他	0		
	技術移轉	件數	0	件	
		收入	0	千元	
參與計畫人力	本國籍	大專生	0	人次	
		碩士生	0		
		博士生	1		不支薪兼任研究助理
		博士後研究員	0		
		專任助理	0		
	非本國籍	大專生	0		
		碩士生	0		
		博士生	0		
		博士後研究員	0		
		專任助理	0		
其他成果 （無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。）					

科技部補助專題研究計畫成果自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現（簡要敘述成果是否具有政策應用參考價值及具影響公共利益之重大發現）或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估
   ■達成目標
   □未達成目標（請說明，以100字為限）
   　□實驗失敗
   　□因故實驗中斷
   　□其他原因
   說明：

2. 研究成果在學術期刊發表或申請專利等情形（請於其他欄註明專利及技轉之證號、合約、申請及洽談等詳細資訊）
   論文：□已發表　□未發表之文稿　■撰寫中　□無
   專利：□已獲得　□申請中　■無
   技轉：□已技轉　□洽談中　■無
   其他：（以200字為限）

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性，以500字為限）
   A simple and fast electrochemical anodization treatment, a nanoporous oxide multilayer could be successfully produced and thickened on the surface of the Ti-24Nb-4Zr-8Sn alloy with a low elastic modulus of 45 GPa.
   We suggest that the surface-modified Ti2448 alloys with low elastic modulus have a great potential for biomedical implant applications. Further in vivo study on the osseointegration of the developed materials is suggested.

4. 主要發現
   本研究具有政策應用參考價值：■否　□是，建議提供機關
   （勾選「是」者，請列舉建議可提供施政參考之業務主管機關）
   本研究具影響公共利益之重大發現：■否　□是
   說明：（以150字為限）