The effects of multiple surface treatments with functional surfaces on titanium for enhancing osseointegration and antibacterial

Yun-Jun Lin [1] , Shu-Chuan Liao [2*]

[1] *Department of Materials Science and Engineering, Dayeh University, Changhua, Taiwan*
[2] *Department of Biomedical Engineering, Dayeh University, Changhua, Taiwan*

Abstract: In this study, a zinc ion-containing ceramic coating was first prepared on the surface of pure titanium by plasma electrolytic oxidation and then treated with oxygen plasma activation., Finally, type II collagen was used as a carrier and contained growth factorsso that it endows pure titanium with good biological properties and antibacterial effects. The experimental results discuss the material's physical and chemical properties and the biocompatibility and antibacterial effect.

Keywords: plasma electrolytic oxidation (PEO) , Oxygen Plasma, Growth factor, biocompatibility, antibacterial test

1. Introduction

In biomedical materials, titanium metal and its alloys are the most widely used because of their excellent chemical stability, good mechanical properties and biocompatibility .They are often used in dental or orthopedic implant material[1]. However, titanium for implants is a bioinert material with low bioactivity and lack of osteoinductive properties, resulting in a substantial reduction in titanium implants' stability and long-term success rate. It does not have antibacterial properties and is resistant to physiological and loading conditions.The wear resistance of lower titanium is poor, indirectly affecting its clinical success rate. How to present two functions on its surface at the same time has become the focus of a new generation of research.

Plasma electrolytic oxidation (PEO) is known as micro-arc oxidation (MAO).PEO is to place the metal to be treated, such as Ti, Mg, Zr, Al and other metal substrates, in an aqueous solution environmentand apply a high-frequency DC voltage to the metal surface to generate a slight plasma reaction at the interface between the metal and the solution, under the interaction of the high-temperature plasma and the rapidly cooling solution around it, a porous, wear-resistant, corrosion-resistant ceramic layer will be formed on the metal surface[2,3]. The advantages of PEO are its simple operation, less water consumption, less waste liquid, and no heavy metal ions in the electrolyte.Itwill not cause harm to the environment, so this technology has developed rapidly in recent decades. In addition, adding trace amounts of metals such as calcium and phosphorus in the electrolyte can also help osseointegration, or adding antibacterial elements to the electrolyte can also improve the antibacterial properties of the metal surface[4,5].

In this study, pure titanium was used as the substrate, and a zinc ion-containing ceramics coating was produced on the surface of substrate by PEO technology first. Its formed titanium dioxide (titania, TiO_2) layer can be closely combined with the titanium substrate and used to make strong- metalorganic interfacial layers. Then use oxygen plasma treatment to activate the surface, and then connected growth factor (BMP-2) combined type II collagen slow-release carrier by cross-linking agents. as a by compounding various surface treatment methods to enhance the fuction of titanium metal further,it is expected that through surface modification, two functions (osseointegration and antibacterial) can be given to the surface of titanium metalat the same time. The experimental flow chart for this study is shown in Fig. 1.

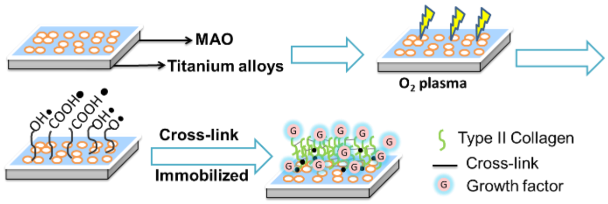

Fig. 1. The schematic illustration of the preparation of the functionalization of titanium surface modification.

2. Materials and methods

2.1 Pretreatment of materials

Chun Kwang Chemical offered pure titanium (99.6%)Industry Co., Ltd. Taiwan, and cut into a suitable size of about 10 × 10 × 2 mm. The substrate surface was sequentially ground from #600 to #2000 with SiC sandpaper and then successively washed with alcohol and deionized water ultrasonically for 15 min to remove dirt and residual fine sand.

2.2 PEO coating for pure titanium

Coatings were fabricated by use of a PEO technique. A DC power supply was used in PEO. The substrates were soaked in an electrolytic solution and kept below 25°C in the water-cooled bath. PEO processes were carried out under the conditions selected in the 400 V. The electrolyte was prepared from a solution of β-sodium glycerophosphate and zinc acetate in distilled water.PEO processes were performed under a treatment time of 5 to 15 min. All treated specimens were removed from the electrolyte, cleaned thoroughly with distilled water, and dried at room temperature for 24 h.

2.3 O_2 plasma activation post-treatment

The O_2 plasma equipment had a chamber and 13.56 MHz radiofrequency generators (UDS, Junsun Tech Co., Ltd) with parallel electrodes. A vacuum pump was employed to provide a low-pressure environment; the after-PEO treatment PEO-Zn substrates specimens were placed on the reaction chamber's lower electrode before being evacuated. The reaction chamber evacuated to less than 0.009 sccm. After the pressure was stabilized, the specimen surface was at an input power of 100 W, using oxygen gas of 20sccm for 1 min. The substrate was subjected to oxygen plasma treatment for active groups (OH−, COO−) on the substrate surface. The material's surface was activated to make it have free radicals and peroxide radicals as the interface layer.

2.4 Growth factor immobilization

After O_2 plasma treatment, use EDC/NHS to crosslink type II collagen as a growth factor (BMP-2) carrier and perform chemical crosslinking treatment; the crosslinking time is 24 hours at 4 °C after the reaction is completed; take it out and dry it in the shade at room temperature for later use. After immobilization, the substrates were mildly rinsed with distilled water for several cycles to remove the loosely attached polymer and dry for 24 h at room temperature.

2.5 Characterization analysis

Analysis of results, the water contact angle was used to measure the surface wettability test. Each sample data were measured three times to take the average. Surface morphologies of substrates were observed by scanning electron microscope. Electron spectroscopy (ESCA, ESCA PHI 1600) was used to characterize the chemical composition of the surface. The X-ray diffraction measurement was taken to analyze the crystal phase of the ceramic oxide layer after PEO treatment, and an electrochemical analyzer tested corrosion resistance. The biocompatibility part is used to cultivate MG63 cells, the influence of growth on the substrate surface and the attachment status, and evaluation of the influence of materials on cell mineralization and antibacterial effect will select Escherichia coli (E. coli) for antibacterial experiments.

3. Results and discussion

3.1 XRD analysis

They are using Regaku XRD to detect the diffraction angle of the prepared titanium substrate, using CuK (λ=1.5432) X light source, Ni filter, voltage 30 KV, current 20 mA, scanning speed 40/min, scanning angle 2θ=10~60° operating conditions for diffraction analysis. Analyze the crystalline phase, crystallinity and product purity of the prepared biological coating to determine the stability of the process product. According to Fig. 2, after PEO treatment, anatase and rutile phases were observed on PEO coatings under different treatment times. It is detected that the content of the anatase phase decreases with the increase in processing time, and the peak intensity change at 25.3° can be seen. The content of the rutile phase increases with the processing time, and the peak intensity change at 27.4° can be seen.

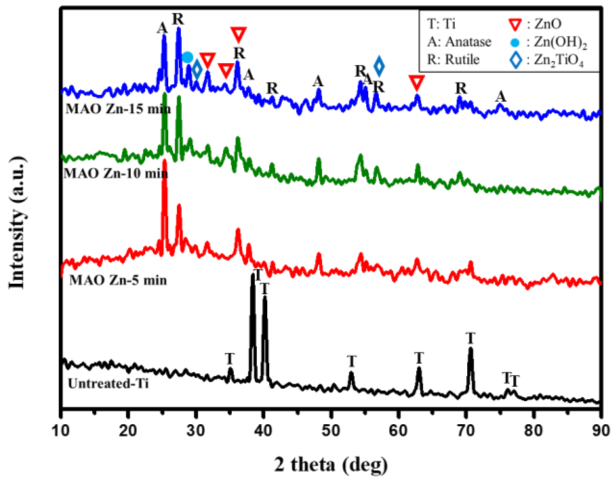

Fig. 2. XRD diffraction pattern of PEO coating.

3.2 Corrosion resistance from potential dynamic polarization test

After the PEO treatment, the substrate was encapsulated with epoxy resin andsoaked in Hank's SBF solution to adjust the pH value to 7.4, The saturated calomel electrode (SCE) was used as the reference electrode, and the platinum electrode as the auxiliary electrode, with the substrate, the working electrode is placed in a constant temperature water tank.The temperature of the water area is set at 37 °C. Use a potentiostat and an AC impedance meter to measure its open circuit potential (corrosion area: 0.07 cm^2, the scan rate is 0.1mV/s, and the potential corrosion ranges from -0.8V~ 0.8 V) analysis. The polarization scan continues toward the anode Fig. 3 is the anti-corrosion test of pure titanium specimens treated with PEO containing zinc ions (PEO-Zn) at different times. The figure shows that after the specimens are treated with PEO, extreme corrosion resistance is obtained by PEO-Zn-15min. The chemical resistance value is the highest, the corrosion current is reduced, and the corrosion current is reduced from 7.46017×10^{-10}A to 5.50492×10^{-10}A. From the results of electrochemical corrosion, it is speculated that the corrosion resistance effect is affected by the number, depth, and density of pores on the surface. Due to many pores on the surface, the corrosive liquid flows into the pores and is blocked or passivated by corrosion products, resulting in current density oscillations. Table 1 shows the polarization curve's corrosion potential and corrosion current measured for different PEO times of pure titanium specimens containing zinc ions (PEO-Zn). When the corrosion current density is low, and the polarization resistance value is high, it means a low corrosion rate, and the formula of the polarization resistance value Rp is shown in (1):

$$Rp = \frac{\beta a \times \beta c}{2.303 icorr(\beta a+\beta c)} \quad (1)$$

Table. 1. Corrosion potential (Vssc) and current density (A/cm2) data of PEO-Zn with different treatment times.

	Ecorr [V]	Log(A)	βa [V/div]	βc [V/div]	Rp [ohm]
Untreatment Ti	-0.255174	2.66803×10^{-10}	0.137	0.086	85.711MΩ
MAO Zn-5min	-0.207978	7.46017×10^{-10}	0.091	0.06	23.688MΩ
MAO Zn-10min	-0.216560	6.12461×10^{-10}	0.083	0.08	15.787MΩ
MAO Zn-15min	-0.232162	5.50492×10^{-10}	0.141	0.061	33.678MΩ

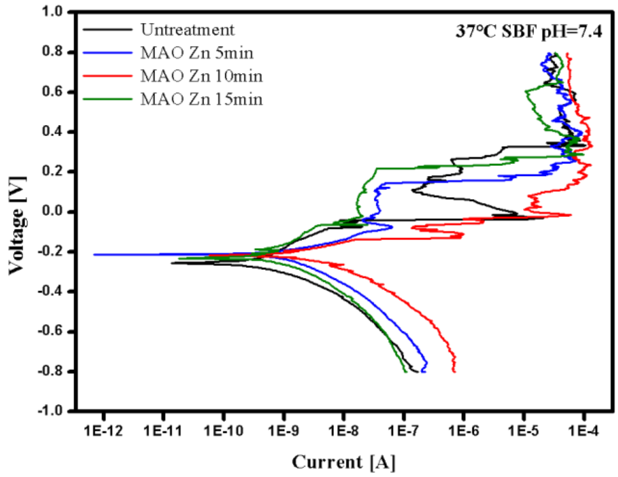

Fig. 3. Polarization curves of PEO coating Ti with various surface modifications in Hank's SBF solution (Temp. 37 °C, pH 7.4)..

3.3 Scanning electron microscope (SEM) morphology

Observed with a scanning electron microscope (SEM), SEM is suitable for high-magnification magnification to observe the surface morphology and porosity distribution of PEO coatings Fig.4 shows the surface morphology and EDS elemental analysis of the pure titanium specimen treated with PEO containing zinc ions (PEO-Zn) for 15 minutes and treated with O_2 plasma.From the experimental results, it can be found that the surface morphology has hardly changed. This phenomenon is because the O_2 plasma only activates the surface and does not damage the material's surface, so it will not affect the original surface structure.

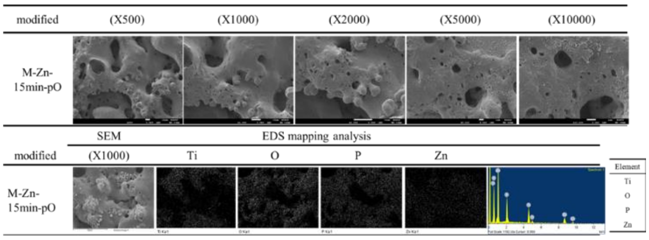

Fig.4 SEM surface morphology and EDS elemental analysis of PEO-Zn-15min after oxygen plasma treatment

3.4 Wettability of the Modified Surface

Fig.5 is the surface hydrophilic and hydrophobic analysis of PEO containing zinc ions(PEO-Zn) and post-treatment. From the analysis of the surface wettability test results, it can be known that the water contact angle of the untreated pure titanium surface is 61.7°±0.4◦ . After being treated with PEO-Zn at different times, its water contact angle is super-hydrophilic. The droplet diffuses on the surface of the substrate in PEO-Zn-5min for 60 seconds. The time for PEO-Zn-10min water droplets to spread entirely on the surface of the substrate was 25 seconds, and PEO-Zn-15min water droplets completely diffused on the surface of the substrate for 15 seconds. From this, it can be known that the roughness after PEO-Zn treatment is significantly improved. As time passes, the surface area increases so water molecules can diffuse quickly. After O2 plasma treatment, the water contact angle is also highly hydrophilic, and it only takes 3 seconds for the droplet to fully diffuse on the surface of the substrate because, after O2 plasma treatment, many oxygen-containing active radical radicals will be generated on the surface to make the surface hydrophilic. The growth factor on the surface of the test piece is also in a hydrophilic state, and the hydrophilic surface is conducive to the differentiation of bone cells in the future.

Modified	Un-modified	M-Zn-5min	M-Zn-10min	M-Zn-15min	M-Zn-15min-pO	M-Zn-15min-O_2-BMP2
WCA(°)	61.7°±0.4	<0	<0	<0	<0	22.1°±0.4
image						

Fig.5 Wettability of various substrates after different treatments

3.5 In Vitro Cytocompatibility Assay of surface-modified Ti sample

In order to understand whether the micro-arc oxidation test piece and the post-treatment test piece are conducive to the growth of cells, a biocompatibility test was carried out. Cell culture testing will be performed using MG-63 osteoblasts. Fig.6 shows the effect of immobilized growth factors on cell growth after PEO treatment (PEO-Zn-15min) and O_2 plasma. It can be observed from the figure that the pure titanium metal substrate has good biocompatibility. It was observed that the biocompatibility did not decrease after modification, and the O.D value was significantly improved after PEO treatment. According to the literature, adding the trace element zinc significantly affects the proliferation and differentiation of bone cells. However, the trace elements calcium and phosphorus are more conducive to the attachment, differentiation, proliferation, and calcium deposition of bone cells, so adding these trace elements will help to improve the biological activity of the pure titanium metal surface. After O_2 plasma treatment to immobilize the growth factor (BMP-2) to test the cell survival rate, it was observed that after the modification, the O.D. value gradually increased with the number of days of culture. However, it needs to be evident that only trace elements are added. A long culture time should be required to see a better cell differentiation effect.

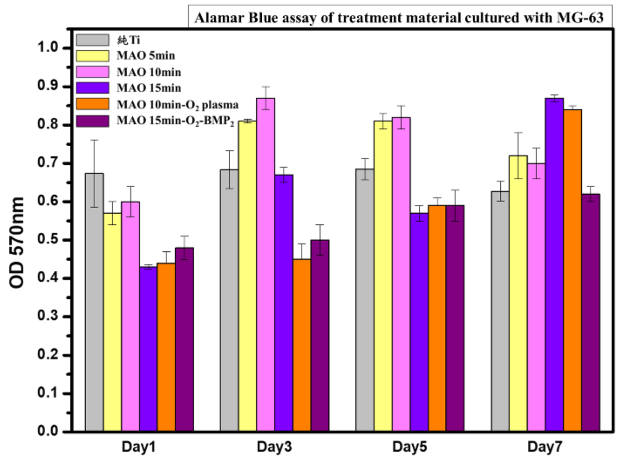

Fig.6 In vitro cytocompatibility of various substrates after different treatments over a period of 1 to 7 days.

4. Conclusions

In this study, PEO technology was used to add different trace elements to enhance the surface bioactivity and antibacterial ability, and then use O_2 plasma to activate the PEO treatment to test the surface to form oxygen-containing polar groups to improve surface hydrophilicity properties, and then use chemical cross-linking to immobilize type II collagen containing growth factors on the surface of the substrates. View of the ideal bone regeneration material, in addition to having good biocompatibility and antibacterial ability, it must be able to promote. The regenerative ability of bone cells, as well as the anti-inflammatory effect, further explore its biological effects: such as the attachment, expansion, growth, and differentiation of osteoblasts. With this composite surface modification method, it is expected that the pure titanium metal substrates can promote osseointegration and antibacterial effect. The following inferences can be drawn from the research results:

(1) Through different surface analysis (XRD/XPS/surface morphology), it can be confirmed that after PEO treatment of zinc (PEO-Zn) titanium specimens, there are indeed TiO_2 rutile phases, anatase, Zn_2TiO_4, HAp crystal phase exists. Using the PEO method to treat the prepared substrates, it can be seen that there are many holes through SEM observation.

(2) According to the electrochemical corrosion results of different treatment times, the polarization impedance value of PEO-Zn-15min is higher, the polarization impedance value of PEO-Zn-10min is the lowest, and the corrosion resistance is the worst. According to the observation of SEM surface morphology, the corrosion resistance may be the worst due to the porosity of the substrates and the number of holes.

(3) According to the results of wettability, the specimens after PEO treatment are all hydrophilic, which is conducive to the adhesion of cells.

(4) The results of the biocompatibility test show that the cell survival rate of the pure titanium substrates is significantly improved after PEO and post-treatment. Observation of the effect of surface modification on pure titanium substrates on bacterial growth. From the observation of the inhibition zone, it can be found that adding a small amount of zinc to the electrolyte is helpful for the antibacterial effect.

5. References

[1] Cheraghali, B., Ghasemi, H., Abedini, M. and Yazdi, R.,2020. A functionalized duplex coating on CP-titanium forbiomedical applications. Surface and Coatings Technology,399, p.126117.

[2] Liao, S., Chang, C., Chen, C., Lee, C. and Lin, W., 2020.Functionalization of pure titanium MAO coatings by surfacemodifications for biomedical applications. Surface and Coatings Technology, 394, p.125812.

[3] Pietro Mandracci.," Surface Treatments and Functional Coatings for Biocompatibility Improvement and Bacterial Adhesion Reduction in Dental Implantology." Coatings., 6, (7):1-22(2016)

[4] Lin Zhu., "Biomimetic coating of compound titania and hydroxyapatite on titanium "Journal of Biomedical Materials Research, 83 (4): 1165–1175 (2007)

[5] GAO A., "The effects of titania nanotubes with embedded silver oxide nanoparticles on bacteria and osteoblasts." Biomaterials, 35: 4223-4235(2014)