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Fabrication and evaluation of porous coatings doped with bioactive elements on titanium surfaces

H.-M. ZHENG[1], S.-J.-J. ZHANG[2], Y. JIAO[3], Y. XIA[1], K.-H. LU[4], Q.-M. ZHAO[4], P.-P. ZHANG[4], X.-L. WU[4], J.-S. WU[2], R.-S. XU[2], L. ZHANG[5], X.-H. NI[5]

[1]Department of Orthopedics Surgery, the People's Hospital of Danyang, Danyang, China
[2]Department of Orthopedics, Affiliated Hospital of Jiangnan University, Wuxi, Jiangsu, China
[3]Department of Stomatology, The Seventh Medical Center of PLA General Hospital, Beijing, China
[4]Department of Orthopedics, Guizhou Provincial People's Hospital, Guiyang, Guizhou, China
[5]Department of Orthopedics, Dafeng People's Hospital, Yancheng, Jiangsu, China

Hongming Zheng, Sujiajun Zhang, and Yang Jiao contributed equally to this work and should be considered co-first authors

Xiaohui Ni and Lu Zhang contributed equally to this work and should be considered co-corresponding authors

Abstract. – OBJECTIVE: Although pure titanium (PT) and its alloys exhibit excellent mechanical properties, they lack biological activity as implants. The purpose of this study was to improve the biological activity of titanium implants through surface modification.

MATERIALS AND METHODS: Titanium was processed into titanium discs, where the titanium discs served as anodes and stainless steel served as cathodes, and a copper- and cobalt-doped porous coating [pure titanium model (PTM)] was prepared on the surface of titanium *via* plasma electrolytic oxidation. The surface characteristics of the coating were evaluated using field emission scanning electron microscopy (FE-SEM), energy dispersive X-ray spectroscopy (EDS), X-ray photoelectron spectroscopy (XPS), atomic force microscopy (AFM), and profilometry. The corrosion resistance of PTM was evaluated with an electrochemical workstation. The biocompatibility and bioactivity of coated bone marrow mesenchymal stem cells (BMSCs) were evaluated through *in vitro* cell experiments.

RESULTS: A copper- and cobalt-doped porous coating was successfully prepared on the surface of titanium, and the doping of copper and cobalt did not change the surface topography of the coating. The porous coating increased the surface roughness of titanium and improved its resistance to corrosion. In addition, the porous coating doped with copper and cobalt promoted the adhesion and spreading of BMSCs.

CONCLUSIONS: A porous coating doped with copper and cobalt was prepared on the surface of titanium through plasma electrolytic oxidation. The coating not only improved the roughness and corrosion resistance of titanium but also exhibited good biological activity.

Key Words:
Titanium, Plasma electrolytic oxidation, Biocompatibility, Surface characteristics, Bioactivity.

Introduction

Titanium has been defined as a suitable biomaterial for orthopedic implants due to its high mechanical strength, corrosion resistance, and biocompatibility. However, the surface of titanium tends to form an inert biological layer, which can cause problems such as poor biological activity, a lack of bone-inducing effects, poor antibacterial activity, and a lack of biological activity after implantation; thus, achieving implant-bone integration in a stable state is difficult[1]. Titanium implants are only mechanically occluded and incarcerated with the surrounding bone tissue in the early stage of implantation, failing to fully mobilize and stimulate the biological activity of the surrounding bone cells; as a result, the implant becomes loose, and repairs fail after long-term application[2]. By optimizing the surface properties of the implant, bone-forming cells can adhere better, promoting early bone integration.

The main research approaches used to improve the bioactivity of titanium and titanium

Corresponding Authors: Lu Zhang, MD; e-mail: 2272283994@qq.com;
Xiaohui Ni, MD; e-mail: nixiaohui@jsmc.edu.cn

alloy materials include the following: first, when designing composite materials, bioactive materials can be alloyed with a titanium matrix; second, surface modification can be performed with titanium alloys to induce biological activity. The latter method is simple and widely used in clinical practice[3]. A variety of surface modification methods can be used to optimize the surface properties and improve the biological activity of titanium. By surface modification, bioactive coatings are prepared on the surface of titanium, which not only retain the original mechanical properties of titanium but also endow titanium with specific biological activities. At present, titanium implants are a hot topic in several fields, including plasma spraying, magnetron sputtering, sol-gel methods, laser cladding and electrochemical technology[4-6]. At present, the most widely used clinical coating technique is plasma spraying with hydroxyapatite (HA) on the surface of titanium alloy implants, which has been demonstrated to achieve direct physicochemical bone bonding and provide reliable interfacial strength with surrounding bone tissue[7]. However, these coatings also have several drawbacks, as the adhesion between the coating and the metal substrate is poor, the coating can be stripped after implantation, active ingredients (such as HA in body fluids) can degrade quickly, and the treatment of complex surface geometry is unfavourable[8].

After biomaterials are implanted in the body, biological cascade reactions, such as cell adhesion, proliferation, differentiation, and tissue formation, occur at the material surface/tissue interface, and these processes are regulated by multiple factors, including the physical and chemical properties of the titanium surface[9]. Therefore, surface modification or modification of titanium and its alloys is essential for regulating the behavior of cells and the surrounding tissues and is an important way to improve bone induction in titanium-based implants. Plasma electrolytic oxidation is a novel, simple and environmentally friendly surface modification method that can produce rough oxide ceramic films on the surface of matrix metals. The inner layer of the ceramic film grows in an interlocking manner on the metal matrix *in situ* with high bonding strength, a dense middle layer, high hardness, and wear resistance, which prevents contact between body fluids and the metal matrix. The outer layer is loose and has a crater-like pipe structure. By optimizing the surface properties of the implant, bone-forming cells can adhere better, promoting early bone integration, which increases the surface roughness[10]. A porous surface structure can improve biocompatibility, regulate cell biological behavior, and facilitate cell adhesion and proliferation.

Copper is an essential trace element distributed in various organs and tissues of the human body. An abnormal copper concentration in the body leads to biochemical and physiological disorders. Copper ions are lipid peroxidation inducible factors, and an appropriate amount of copper can enhance lipid peroxidation and promote cell growth. In addition, copper can inhibit the synthesis of bacterial active DNA and related enzymes, interfere with bacterial energy metabolism, and exhibit good antibacterial effects. Previous studies[11] have utilized copper as an antibacterial coating. Cobalt is also an indispensable trace element in the human body that plays an important role in maintaining human life activities and is a cofactor of many metal proteins in the body; it can upregulate the expression of hypoxia-inducing factors and promote the formation of blood vessels[12]. In addition, cobalt is a component of vitamin B12 and participates in the metabolism of ribonucleic acid and hematopoietic-related substances.

In recent years, a new concept for designing implant surfaces has been proposed, which involves the construction of a multifunctional bioactive surface, and after the surface topography of the material is fully considered, bioactive ions are doped on the surface of the material to play a bioactive role through slow release. Therefore, in the present work, copper- and cobalt-doped porous coatings were prepared on the surface of titanium *via* plasma electrolytic oxidation, and the porous coatings were characterized in detail. Moreover, the biological activity of the coating was evaluated through cell experiments *in vitro*.

The integration of structural/biomedical functions on the surface of titanium implants is an innovative idea, and achieving biological functionalization of titanium implants through the sustained release of beneficial metal elements in the human body is an innovative, clinically valuable, and challenging research direction. The goal of this study was to provide a new method for improving the biological activity of titanium implants and preventing postoperative complications such as loosening of titanium implants by studying the preparation and biological activity of the pure titanium model (PTM) coating, which has important theoretical significance and clinical application prospects.

Materials and Methods

Preparation of the Coating

Pure titanium (PT) rods were cut into discs 13 mm in diameter and 1.5 mm thick. Next, #150, #300, #500, #800, and #1500 silicon carbide sandpapers were used sequentially until the sample was smooth and no visible scratches were observed. After the samples were cleaned with acetone, anhydrous ethanol and deionized water and dried, plasmwa electrolytic oxidation was carried out.

The study adopted a direct-current pulse plasma electrolytic oxidation system. The power supply of this experiment adopts a constant current mode. The voltage was set to 420 V, the time to 5 min, the frequency to 500 Hz, and the current density to 16.5 A/dm^2. After plasma electrolytic oxidation, the samples were washed with deionized water, dried, and reserved.

To prepare the PTM coating, 0.2 mol/L calcium acetate, 0.1 mol/L sodium dihydrogen phosphate, copper gluconate (0.02 mol/L) and cobalt gluconate (0.02 mol/L) were dissolved in 1 L of deionized water, stirred to dissolve the reagent, and left for 1 hour at room temperature. Plasma electrolytic oxidation was carried out with a stainless-steel plate as the cathode and a titanium alloy plate as the anode.

In this study, the PTM coating was used as the experimental group (60 samples), while PT was used as the control group (60 samples). All the samples were cleaned and dried for later use.

Evaluation of Surface Characteristics

Field emission scanning electron microscopy (FE-SEM) was used to observe the surface morphology of the sample, energy dispersive X-ray spectroscopy (EDS) and X-ray photoelectron spectroscopy (XPS) were used to observe the elemental composition and chemical state of the coating, the hydrophilicity was measured by a surface contact angle measurement machine, the roughness of the sample was observed by atomic force microscopy (AFM) and profilometry, and the corrosion resistance of the sample was evaluated by means of an electrochemical workstation.

Corrosion Resistance Test

In this study, the corrosion resistance of the samples was tested by an electrochemical workstation. Nyquist curves were obtained. A saturated calomel electrode (SCE) was used as the reference electrode (RE), a platinum electrode was used as the auxiliary electrode (CE), the sample was used as the working electrode, and simulated body fluid (SBF) was used as the corrosive solution. An impedance test was carried out after the sample was placed in 100 mL of simulated body fluid for 30 minutes. The frequency test range was 90 kHz to 10 MHz, and the scanning speed was 1 mV/s.

Cell Experiments

Cell culture

In this study, bone marrow mesenchymal stem cells (BMSCs) were subjected to *in vitro* cell tests, and α-Minimum Essential Medium (α-MEM) with 10% Fetal Bovine Serum (FBS) and 1% antibiotics was added and incubated at 37°C in a 5% CO_2 humidified atmosphere. The culture medium was changed every other day. The 2nd to 3rd generations of the cells were used for follow-up experiments.

Cell adhesion

The cell inoculation density and culture method used were the same as those described above. The culture was terminated after 2 hours, and the cells were fixed with paraformaldehyde. The surface of the sample was stained with 50 μl of DAPI for 10 min, observed and counted by fluorescence microscopy.

Observation of cell morphology

The cell inoculation density and culture method were the same as those described above. The culture procedure was terminated after 72 h, after which the cells were washed with PBS, fixed with paraformaldehyde, permeated with 0.5% Triton X-100, incubated with rhodamine-labeled minocycline for 30 min, stained with DAPI, and observed *via* fluorescence microscopy.

Statistical Analysis

The obtained data are presented as the means ± standard deviations. One-way ANOVA and the SNK test were used to compare the differences between groups. $p < 0.05$ was considered to indicate statistical significance, and $p < 0.01$ was considered to indicate high statistical significance. The statistical analysis was performed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA).

Results

Figure 1 shows the overall surface morphologies of the PT and the PTM coatings. It is obvious that PT had a silver-white metallic luster, and

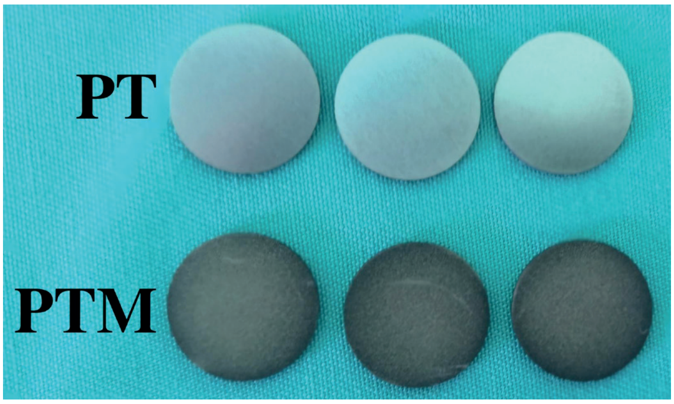

Figure 1. Overall surface morphologies of PT and the PTM.

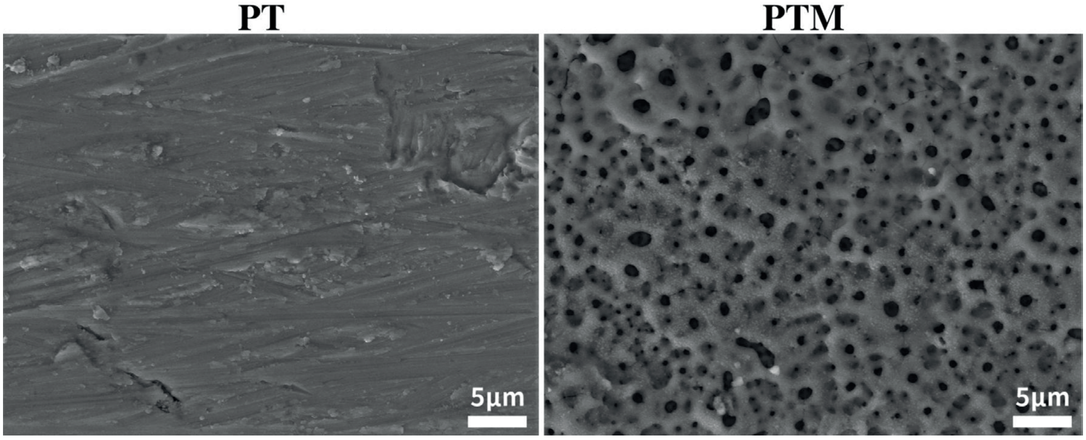

Figure 2. SEM images of PT and PTM.

no obvious polishing marks were observed. The PTM coatings were brown with darker edges and a flat surface that lost its metallic luster.

Figure 2 shows the surface morphology of the PT and the PTM coatings. The surface of the Ti group samples was smooth, but there were sandpaper polishing marks, and the surface of PT did not exhibit a microporous structure.

The surface of the PTM coating was covered with holes of different sizes; the large holes were

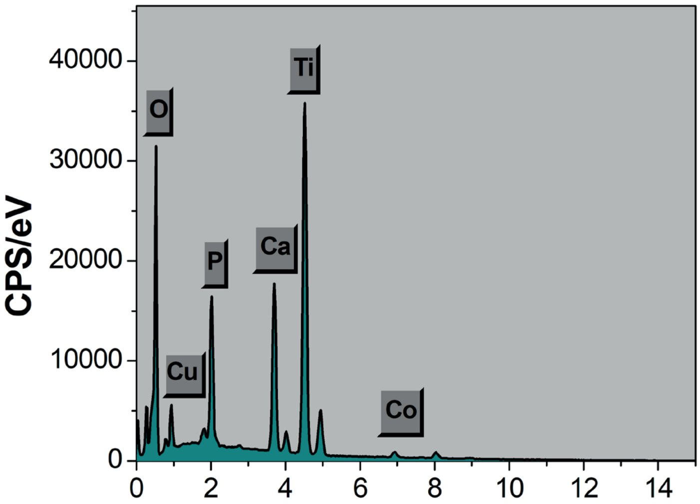

Figure 3. EDS results of the PTM coating.

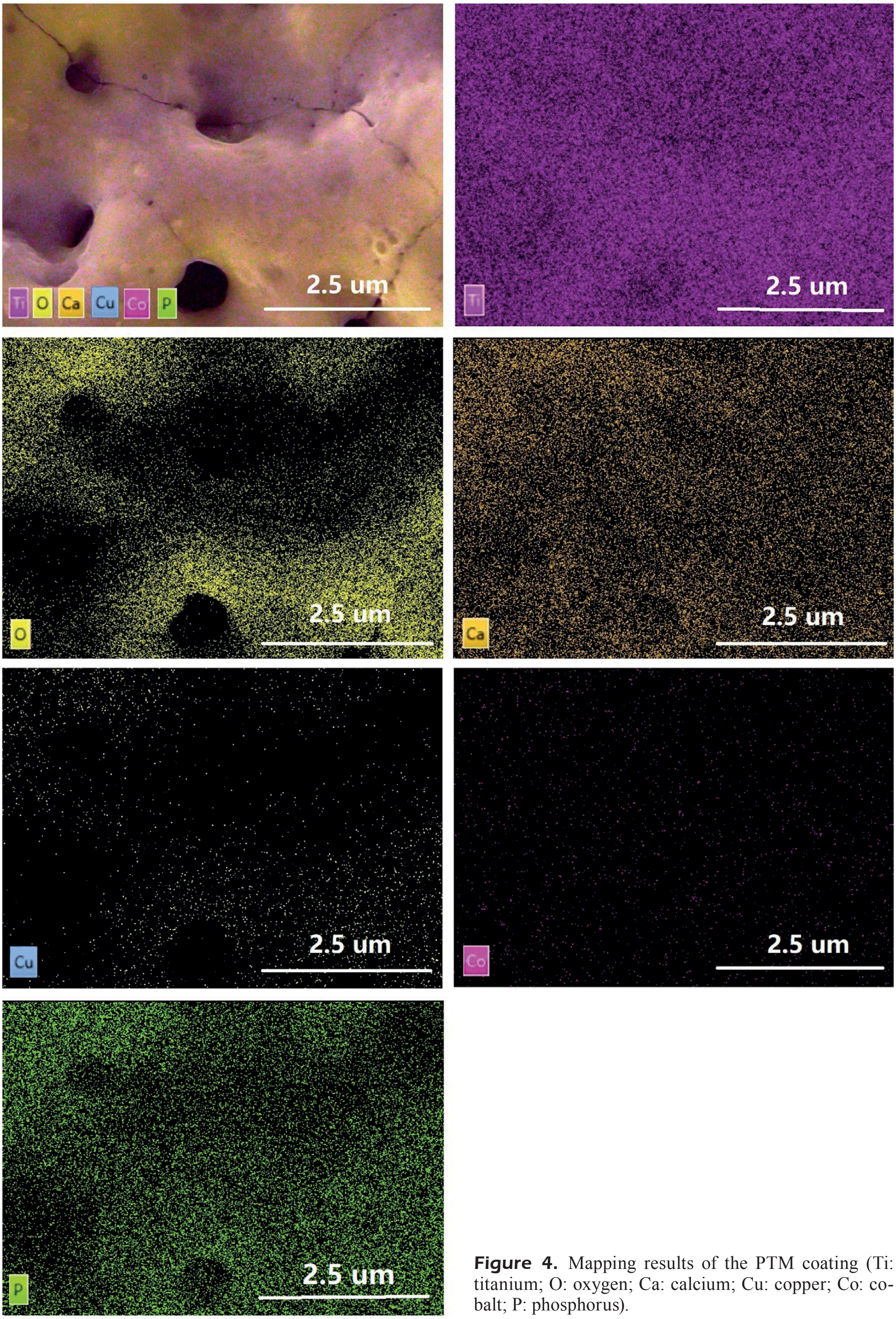

Figure 4. Mapping results of the PTM coating (Ti: titanium; O: oxygen; Ca: calcium; Cu: copper; Co: cobalt; P: phosphorus).

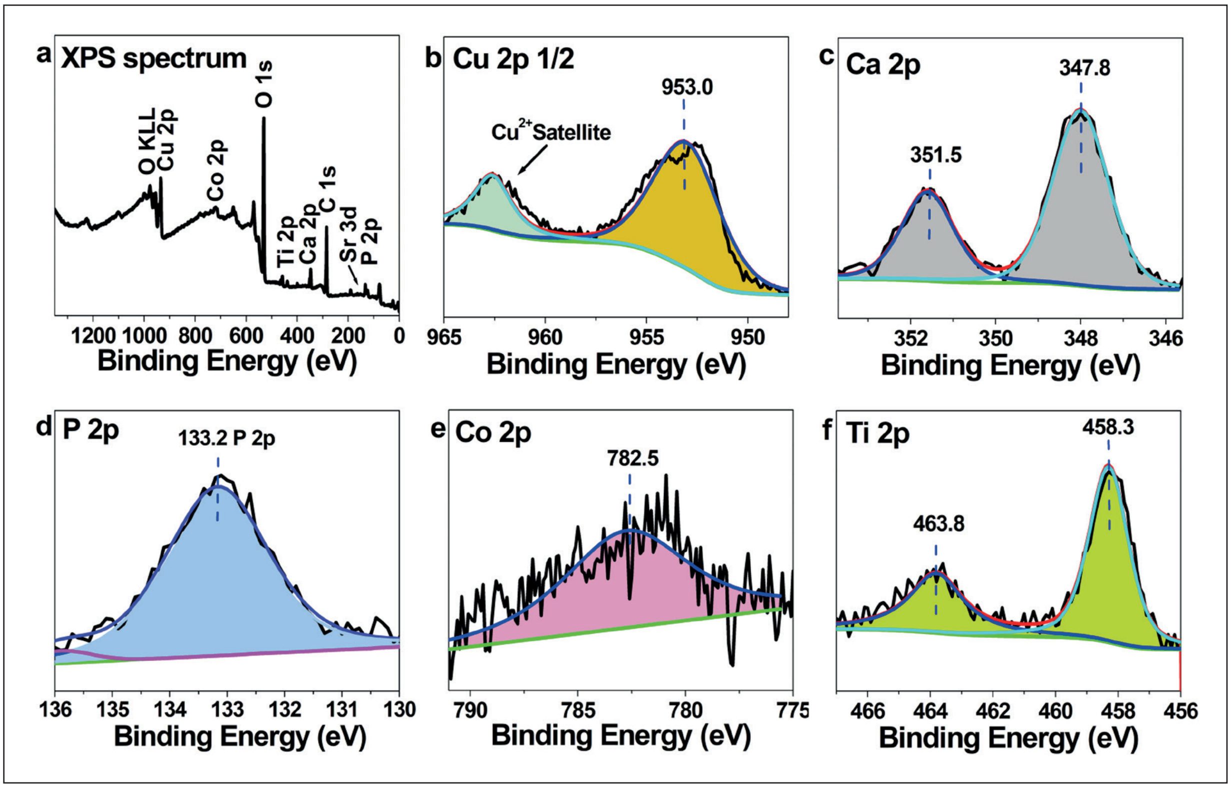

Figure 5. **a**, XPS spectra, (**b**) Cu2p, (**c**) Ca2p , (**d**) P2p, (**e**) Co2p, and (**f**) Ti2p high-resolution spectra of the PTM coating.

covered with small holes, and each hole was connected.

Figure 3 shows the EDS results for the PTM coating. The PTM coating contained elements such as Ti, calcium, phosphorus, copper, cobalt, and oxygen, and the copper and cobalt in the electrolyte solution were introduced into the coating.

Figure 4 shows the elemental mapping results for the PTM coating. As determined by EDS, the coating contained Ti, calcium, phosphorus, copper, cobalt and oxygen, which were evenly distributed on the surface and inside the holes in the coating.

Figure 5 shows an XPS image of the PTM coating. The PTM coating was mainly composed of Ti, calcium, phosphorus, copper, cobalt and oxygen. The peak in the Ti 2p spectrum corresponds to titanium dioxide, and a single peak in the P 2p spectrum was located at 133.2 eV, which is consistent with the P-O bond of PO_4^{3-}. In the Ca 2p spectrum, peaks were ob-

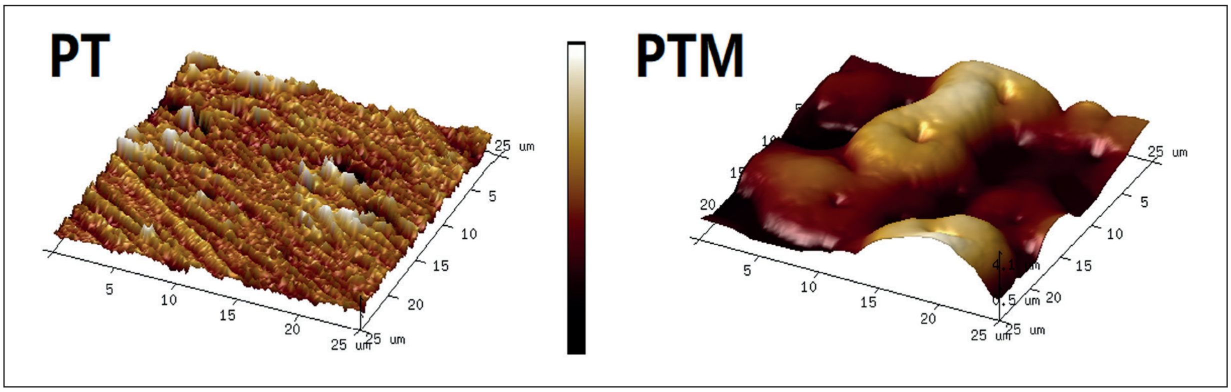

Figure 6. AFM morphology of the PT and PTM coatings.

Table I. Comparison of roughness and contact angles between PT and PTM.

Groups	Roughness (nm)	Contact angles (deg.)
PT	102 ± 18	67.5 ± 3.8
PTM	453 ± 46*	52. 6 ± 3.1*

*$p < 0.05$ (compared with PT, $p < 0.05$).

served at 347.8 eV and 351.5 eV, corresponding to Ca 2p in $Ca_3(PO_4)_2$.

Figure 6 shows the AFM morphology of the PT and the PTM coating. The surface fluctuation of Ti was not obvious, and the surface of the PTM coating was convex, showing a typical "crater" morphology. This roughness increased the surface roughness of titanium.

The roughness values of the PT and PTM measured by AFM are listed in Table I. There were significant differences in the microscale roughness between PT and PTM, as characterized by the average roughness (Ra). It is clear that the porous coating doped with copper and cobalt significantly improved the roughness of the titanium. In addition, the hydrophilicity of PT and PTM was measured by a surface contact angle measurement machine. The water contact angles on PT and PTM differed (Table I), with the PTM group having a smaller contact angle than the PT group. Overall, the porous coating doped with copper and cobalt changed the surface roughness and wettability of titanium.

Figure 7 shows the profiles of the PT and the PTM coating. As observed by AFM, the surface of Ti was flat, and the surface of the PTM coating was rough. Quantitative analysis revealed that the roughness of the PTM coating group significantly increased compared with that of the Ti group; this result was closely related to the formation of a porous coating structure due to the formation of discharge channels during the plasma electrolytic oxidation process.

Figure 8 shows the corrosion resistance of the PT and the PTM coating. As seen from the Nyquist curves of the PT and PTM coatings, the impedance shapes of the PT and the PTM coating were the same, and the arc resistance of the PTM coating was greater than that of PT, indicating that the PTM coating exhibited better corrosion resistance. Therefore, the corrosion rate of the PTM coating was slower than that of PT, and the surface PTM coating exhibited better corrosion resistance.

Figure 9 shows the BMSC adhesion results on the surface of the PT and PTM coatings. There was a significant difference in the number of cells adhered on the surface between the PT group and the PTM coating group. Compared with that of the PT group, the number of cells adhered to the surface of the PTM coating was significantly greater, indicating that the PTM coating promoted the adhesion of BMSCs.

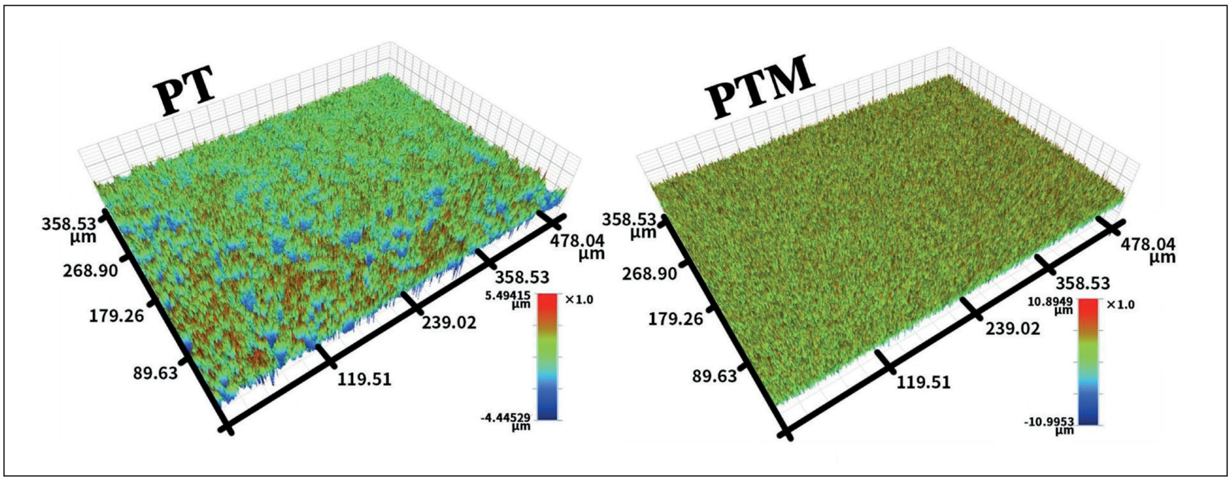

Figure 7. Profiles of the PT and PTM coating. The data are expressed as the mean ± SD (n = 3). **$p < 0.05$.

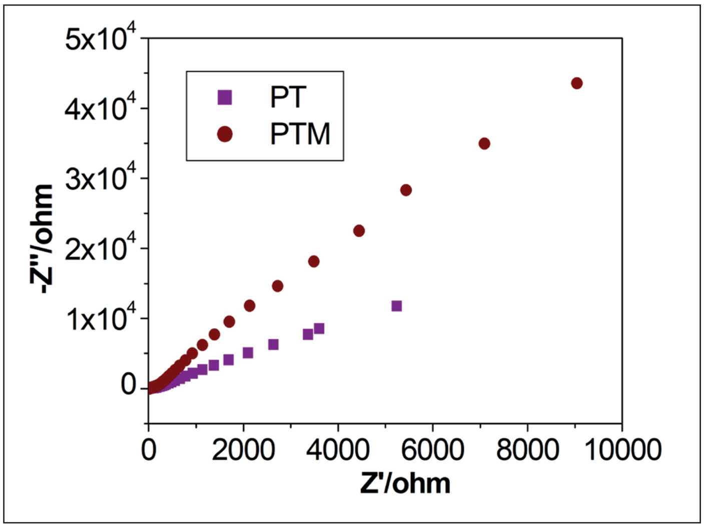

Figure 8. Nyquist curves of the PT and PTM coatings.

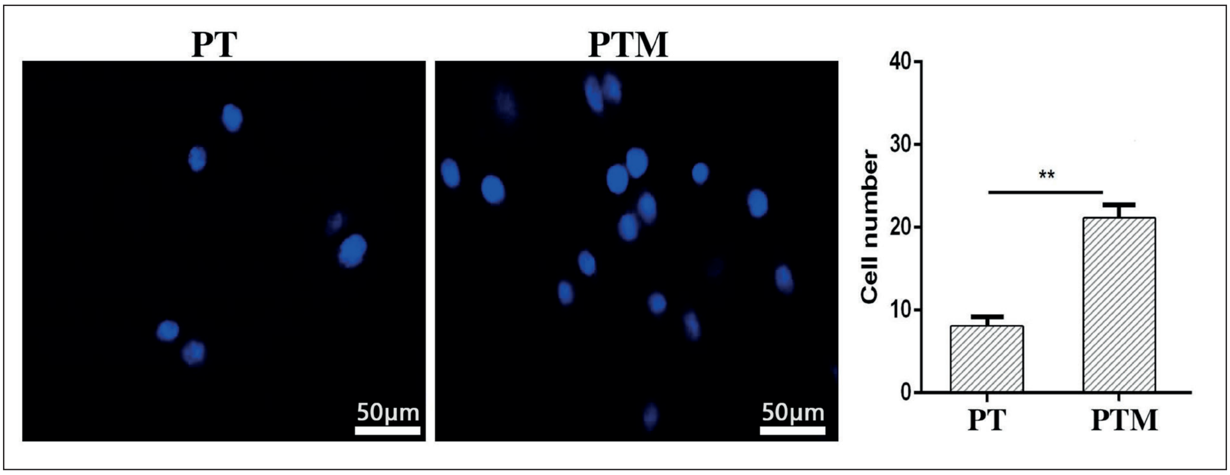

Figure 9. BMSC adhesion on the surface of the PT and PTM coatings. The data are expressed as the mean ± SD (n = 3). **$p < 0.01$.

Figure 10 shows cytoskeleton staining on the surface of the PT and PTM coatings. The spread area of the PT cytoskeleton was small, the spread area of cells on the surface of the PTM coating was greater, and the PTM coating promoted the spread of BMSCs.

Discussion

The fabrication of coatings with excellent morphological characteristics and good biological activity on the surface of titanium by surface modification technology to achieve the integration of surface structure and biomedical function of titanium implants is currently an important research topic in the field of titanium implants. The key to achieving integration of the surface structure and biomedical function of titanium implants lies in the preparation of target materials for clinical applications. To maintain the performance levels of titanium and achieve both mechanical load-bearing capacity and biomedical function, bioactive elements were introduced into specific structures, and bioactive elements were continuously released. Given the numerous biological activities of copper and cobalt, a porous coating structure was prepared using PEO technology. In this study, we successfully prepared copper- and cobalt-doped porous coatings on a titanium surface using PEO, achieving the integration of the surface structure and biomedical function of titanium implants.

PEO, also known as micro-arc oxidation (MAO) and anodic spark deposition (ASD), was developed from anodic oxidation and uses a micro-arc oxidation power supply to apply voltage (DC, AC, or pulse) based on an ordinary anodic oxidation process. As a result, the surface of valve metals (titanium, magnesium, aluminum, etc.) interacts with electrolyte solutions (generally weak alkaline solutions), forming micro-arc discharge. At high temperatures under the influence of electric fields and other factors, a ceramic film on the surface of titanium and other metals can be formed by using arc discharge to enhance and activate the reaction at the anode; this film can improve the wear resistance, corrosion resistance, and high-temperature resistance of a material[13]. In this study, a microporous structure was formed on the surface after plasma electrolytic oxidation. The formation of a microporous structure results from the melting of the oxide film and the formation of gas when the film cools during the plasma electrolytic oxidation process. The surface first solidified, causing internal gas to overflow and forming loose micropores similar to those formed after volcanic eruptions. These micropores were discharge channels for plasma electrolytic oxidation, and due to the extremely high temperature at the moment of discharge, the metal around the discharge channel melted.

In addition, plasma electrolytic oxidation can introduce bioactive elements (such as osteogenic, vasogenic and antibacterial elements) into the surface of titanium[14,15] and exert bioactive effects by slowly releasing these bioactive elements *in vivo*. In this study, copper acetate and cobalt acetate were added to the electrolyte, and copper and

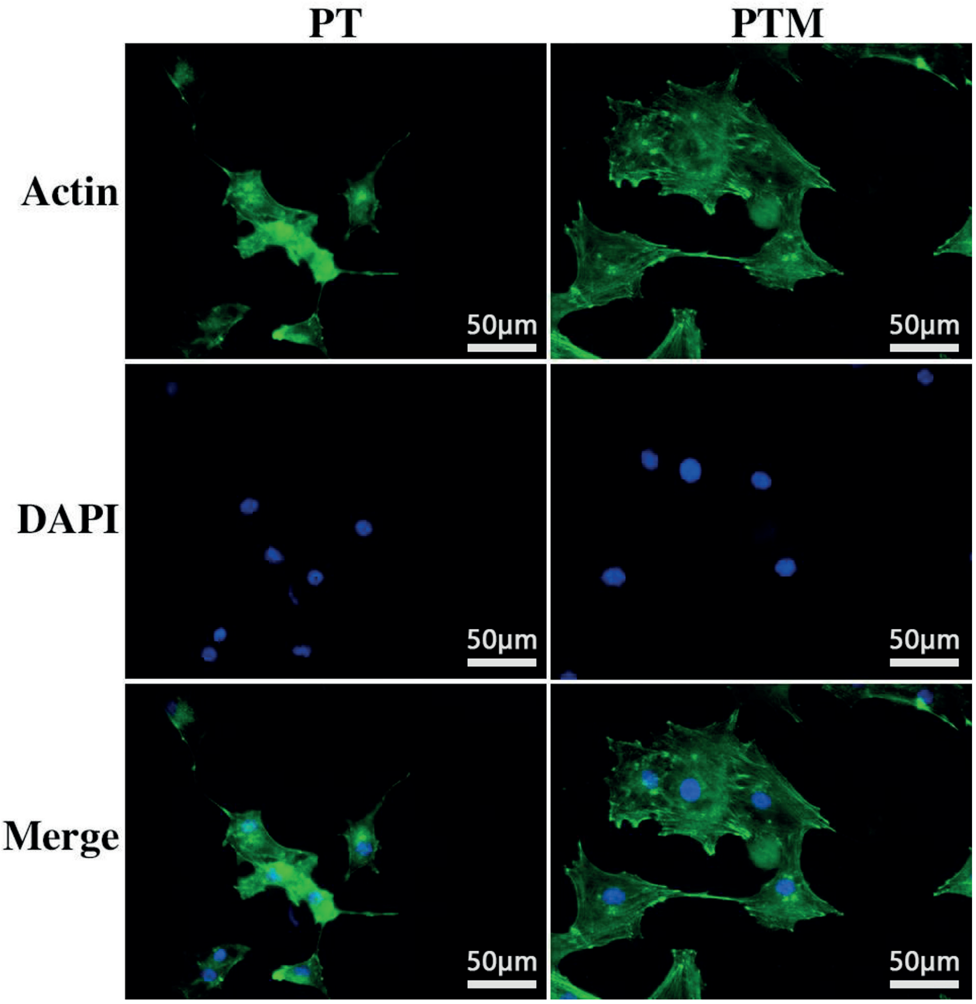

Figure 10. Cytoskeleton staining on the surface of PT and PTM coatings.

cobalt were successfully introduced into the microporous coating, which played a biological role through sustained release.

Studies[16] have shown that the rougher the surface of titanium implants is, the larger the specific surface area and the better the hydrophilicity, which is conducive to cell adhesion on the surface of titanium implants. Compared to the Ti group, the PTM coating group was significantly rougher in this study, which was related to the porous structure of the PTM coating surface. The literature shows that smooth surfaces are not conducive to cell adhesion, while rough surfaces are more conducive to cell adhesion and proliferation in the later stage of osteogenic differentiation, which is highly important for increasing bone integration in titanium implants.

Corrosion resistance is an important index used to evaluate biological materials, and good corrosion resistance is the basis for the long-term stability of materials after implantation[17], which is highly important for the repair of hard tissues (especially bone tissues). In this study, the corrosion resistance of the PTM coating was better than that of PT, indicating that the PTM coating on the titanium surface improved the corrosion resistance of titanium. On the one hand, the main component of the PTM coating was titanium dioxide, which is a ceramic and exhibits good corrosion resistance. On the other hand, although the PTM coating has a porous structure, these holes are mainly located in the loose layer, the dense layers of the coating do not communicate with each other, and the integrity of the ceramic coating is not damaged. The above reasons explain the improvement in the corrosion resistance of the PTM coating.

The material interface results from the interaction between materials and cells, and titanium surface characteristics, including surface morphology, chemical composition, roughness, chemical composition, and hydrophilic/hydrophobic properties, play an important role in cell adhesion and extension[18]. In the present study, the PTM coating exhibited a porous structure, which was conducive to the adhesion and extension of BMSCs. Moreover, the PTM coating increased the roughness of the titanium coating and promoted the adhesion and extension of BMSCs. However, copper and cobalt ions were slowly released and have been demonstrated to exhibit good biological activity and promote cell adhesion.

Like cell adhesion, cell spreading on the surface of a material reflects the biological activity of the material and is used for evaluation. Many factors, including surface morphology, roughness, chemical composition, and surface energy, affect cell spreading on the surface of materials[18]. Among these factors, surface morphology and chemical composition are the main factors that affect cell adhesion and spreading on the surface of materials. In the present study, BMSCs exhibited greater extension in the PTM group than in the PT group, which was related to the porous structure of the PTM group and the sustained release of copper and cobalt ions. Our research and published reports are consistent. Huang et al[19] fabricated Cu-containing ceramic coatings on titanium substrates by PEO and found that the integration of Cu in titanium implants could lead to enhanced macrophage-mediated osteogenesis and bactericidal capacity.

Likewise, Zhou et al[20] generated coatings on a titanium surface *via* micro-arc oxidation, incorporating Co, F, and Sr doping, and *in vitro* experiments validated the coatings' antibacterial, angiogenic, and osteogenic properties.

Limitations

This study has several limitations that should be considered. The molecular mechanism by which the PTM coating promotes BMSC adhesion and extension still needs to be further explored. In addition, the current study lacked *in vivo* animal experiments, which are also crucial for titanium implants. In addition, the long-term biological activity of PTM coatings also needs further exploration.

Conclusions

We prepared a PTM coating on the surface of a titanium implant by plasma electrolytic oxidation, and copper and cobalt were successfully doped into the coating surface. The copper- and cobalt-doped porous coating not only exhibited good surface topography but also improved the corrosion resistance of titanium. More importantly, the PTM coating promoted BMSC adhesion and spreading and showed good biological activity. This study may provide a new method for improving the biological activity of titanium.

Conflict of Interest

The authors declare that they have no conflicts of interest to disclose.

Ethics Approval

The study was carried out after approval was obtained from the Medical Ethics Committee of Dafeng People's Hospital (DFRMYY2023006).

Informed Consent

Not applicable due to the design of the study.

Funding

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Authors' Contributions

Xiaohui Ni and Lu Zhang designed the study; Hongming Zheng, Yang Jiao and Yan Xia evaluated the surface coating properties; and Quanming Zhao, Pengpeng Zhang and Ruisheng Xu performed the cell culture and cell experiments. Sujiajun Zhang and Jieshi Wu carried out the data analyses, Hongming Zheng, Yang Jiao and Sujiajun Zhang wrote the draft of the manuscript, and Xinglin Wu and Kaihang Lu prepared the figures. Xiaohui Ni and Lu Zhang provided guidance and supervision for the execution of the experiments. All the authors read and approved the final manuscript.

ORCID ID

Xiaohui Ni: 0000-0001-8642-2770

Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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