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# Micro-arc oxidation treatment enhanced the biological performance of human osteosarcoma cell line and human skin fibroblasts cultured on titanium–zirconium films

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ABSTRACT

Development of oxide films on metallic implants with a desirable combination of mechanical properties and biocompatibility can greatly improve its functionality and application to the medical implant field. The present work is aimed to fabricate titanium (Ti) or zirconium (Zr) oxide films with porous structures onto Ti substrates by micro-arc oxidation (MAO) procedure. Before the MAO treatment, a series of Ti—Zr alloy films were deposited on polished pure Ti substrates using a cathodic arc deposition system with different deposition cathode current ratios of Zr/Ti. The post-MAO-treated surface layers were characterized for its composition, crystalline structure, bonding states, surface morphology, and wettability. The results showed that porous crystalline titanium and zirconium oxides were formed using the MAO treatment for the Ti—Zr films. To evaluate the bioactivity, cell viability and gene expression of human osteosarcoma cell line (MG-63) and human skin fibroblast cell line (SKF) cultured on different Ti—Zr oxide films were determined. The results of MTT tests showed that the higher cell viability of MG-63 and SKF cells was found in MAO-treated Ti—Zr films with higher Zr content. Gene expression data by RT-PCR and agarose gel electrophoresis showed that MG-63 cells and SKF cells exhibited notable osteogenic gene expression, such as Runx2, ALP, Dlx-5, OCN, BMP-2 and BMPR1A. The cell adhesion-related genes, such as fibronectin, collagen types I and III, and laminin were also remarkably expressed in the gel imaging. It suggested that the MAO-treated Ti—Zr films were suitable for bone tissue- or soft tissue-derived cell growth and differentiation. The design and fabrication of Ti—Zr oxide films with specific composition and porous surface layer with MAO treatment may provide a better material environment for cell bonding, living, and differentiation in dental and orthopedic implants.


## 1. Introduction

An appropriate surface treatment is critically required for the biomedical implants to improve their bioactivity and mechanical properties, such as dental implants, artificial hips and knee components…, etc. Titanium (Ti) and zirconium (Zr) have been widely used as biomedical materials for decades due to their great biocompatibility, high mechanical strength, reliable property of corrosion resistance [1–5]. Although Ti is in widespread clinical use, its poor wear resistance prevents its application in some medical fields. Adding alloying elements to Ti to produce Ti alloys can improve this shortcoming; for example, Ti—Al—V alloy has good corrosion resistance and biocompatibility, but some concerns remain its clinical use because of the possibility of toxic aluminum being released. In addition, the increasing use of computer-aided design (CAD) and computer-aided manufacturing (CAM) techniques in recent years has led to the increased use of Zr-based materials in medical applications, including dental implants. Ti and Zr have similar chemical properties because they are both Group IV elements in the periodic table. Zr has been used as an alloying element to improve the properties of Ti in the development of new TiZr-based alloys. Its excellent biomechanical properties compared to Ti results in acceptable performance in dental implants. A native oxide film ($TiO_2$ or $ZrO_2$) naturally formed onto the metal surface provides bioactivity for cell adhesion, migration, and growth. However, these native oxide films are too thin (2–5 nm) to bear the wear or corrosion. Therefore, $TiO_2$ surface coatings can be deposited to improve the cellular behaviors of the osteoblast, which is the major cell responsible for bone formation and osteoblast proliferation rate [6]. In addition, $ZrO_2$ is also a promising material due to its superior mechanical properties and biocompatibility. Brakel et al. recently [7] showed similar bioactivities of $ZrO_2$ and Ti surfaces on soft tissues.

To improve the chemical features and biocompatible abilities, numerous surface modification techniques have been progressively

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developed and applied to different metals, alloys or ceramics for biomedical purposes. The surface topography and composition play important roles on guiding the bone integration between the implant surface and cells. Moderately rough surface usually provides higher bone-implant integration compared to smooth or minimally rough surfaces [6,7]. Due to the surface modifications are available to alter the original material characteristics, such as surface topography, compositions, chemical bonding, wettability, bioactivity, and the costs, different combinations have been developed to provide a better choice.

Micro-arc oxidation (MAO), also named as plasma electrolytic oxidation or anodic spark oxidation, is a technique using high voltages (several hundreds of voltage) to fabricate porous, crystalline, and thick oxide coatings on metals [8,9]. The synthesized oxide coatings have strong adhesion to the substrates. In addition, the morphology and thickness of the oxide films can be controlled by MAO by changing different parameters, such as voltage, temperature, and treatment time. The manufacturing process can improve the corrosion resistance, wear behavior and various other functional properties [10,11]. The pore size and surface roughness formed by MAO would also play an important role in the adsorption of proteins, adhesion of cells, and the rate of osseointegration [12]. Therefore, MAO has become a promising technique for surface modifications. The mechanical properties of Ti6Al4V alloy foams are significantly improved after MAO treatment [13]. Moreover, a MAO-treated coating deposited onto a pre-sandblasted surface shows a favorable combination of rough and residual stresses as well as better bioactivity [14].

Due to Ti and Zr have been widely used as biomedical materials for decades resulting from their great biocompatibility, high mechanical strength, reliable property of corrosion resistance, a series of Ti—Zr alloy films were deposited on polished pure Ti substrates using a cathodic arc deposition system with different deposition cathode current ratios of Zr/Ti. To further improve the biocompatibility of these Ti—Zr alloy films, a post-MAO treatment was produced. Therefore, the fabrication and biocompatibility tests of a series of TiZr alloy films with post-MAO treatment were the main originalities and objectives performed in this study. The post-MAO surface modification using a pulsed power supply was used to form a porous oxide layer on the Ti. An oxide surface layer including $TiO_2$ and $ZrO_2$ is therefore can be synthesized. To evaluate the effect of the post-MAO- treated Ti—Zr oxide films on the bioactivity of hard tissue- and soft tissue-derived cells, the cell viability of human osteosarcoma cell line (MG-63) and human skin fibroblast cell line (SKF) cultured on each MAO-treated film was determined, individually. The osteogenic and cell adhesion-related gene expression were demonstrated by RT-PCR and agarose gel electrophoresis analyses.

## 2. Experimental details

### 2.1. Sample preparation and characterization

Pure Ti plate samples with square size (15 mm × 15 mm × 1 mm, Surface roughness Ra = 0.8 μm, bio-grade 2, Uniti Titanium, Moon Township, PA, USA) were used as substrates. Table 1 shows the deposition and MAO parameters of the Ti—Zr alloy coatings. At first, Ti—Zr alloy coatings with different alloy contents were deposited on the polished pure Ti plate samples using a cathodic arc deposition system. Cathodes of pure Ti and Zr cathode targets (diameter = 150 mm) were set on the chamber wall to deposit the Ti—Zr alloy coatings. A base pressure prior to deposition was $<1 \times 10^{-3}$ Pa. The samples were mounted on a rotational substrate holder for the deposition. The distance between the target and the Ti substrate was 160 mm. Ar was introduced around the target to enhance the plasma reaction. The total thickness of the coatings was 5 ~ 6 μm, which was controlled by a deposition time of 60 min. A dc cathode current was applied between the anode and the cathode using a welding power supply (Miller XMT 304 CC/CV). The total cathode current of both Ti and Zr cathodes was 160–170 A and the ratios of Zr/Ti cathode current were 0.7 (TiZr-0.7), 1.0 (TiZr-1), and 1.7 (TiZr-1.7). Substrate bias voltage of −80 V and deposition pressure of 1.2 Pa were used. For the MAO treatment, the bath contained an electrolyte consisting of 0.02 M $K_3PO_4$ and 0.11 M KOH. The pH value of both electrolytes was ~14. The MAO treatment has the potential to form an oxide layer on the Ti—Zr coated Ti. Using a pulsed power supply attached to an electrolyte cell consisting of titanium anode and stainless steel cathode, a series of MAO treated Ti—Zr coatings were prepared for 300 s at an average current of 2 A, frequency of 1000 Hz, and duty cycle of 20%. After MAO treatment, the samples were taken out of electrolyte, washed thoroughly in cold running water, ultrasonically cleaned in acetone and air-dried before performing material characterization.

**Table 1**
Deposition and MAO parameters of the Ti—Zr coatings.

| Parameters | Values |
|---|---|
| Deposition using CAE | |
| CAE target | Ti and Zr (150 mm in diameter) |
| Cathode current ratio of Zr/Ti | TiZr-0.7 (Ti = 100 A/Zr = 70 A), TiZr-1 (Ti = 80 A/Zr = 80 A), TiZr-1.67 (Ti = 60 A/Zr = 100 A) |
| Distance between cathode and substrate (mm) | 160 |
| Base pressure (Pa) | $1.0 \times 10^{-3}$ |
| Working pressure (Pa), Ar | 1.2 |
| Bias voltage during deposition (V) | −80 |
| Deposition time (min) | 60 |
| MAO treatment | |
| Electrolyte | 0.02 M of $K_3PO_4$ + 0.11 M of KOH |
| Current (A) | 2 |
| Pulse frequency (Hz) | 1000 |
| Duty cycle (%) | 20 |
| Oxidizing time (min) | 5 |

The surface morphology of MAO-treated Ti—Zr coatings was observed using a JEOL JSM-7000F high resolution field emission scanning electron microscope (FESEM). An X-ray photoelectron spectroscope (PHI1600 XPS) with non-monochromatized Mg Kα radiation was used to identify the composition and chemical binding of the MAO-treated Ti—Zr coatings. It was performed with 3 kV Ar ions to sputter the surface oxide layer for 1 min and reveal the chemical composition of the MAO-treated Ti—Zr coatings. The survey spectra in the range of 0–1000 eV were recorded for each sample, followed by high-resolution spectra over different elemental peaks, from which the composition was calculated. The spectral ranges at 460 ± 12 eV, 533 ± 10 eV, and 182 ± 12 eV, corresponded to the binding energies of Ti2p, O1s, and Zr3d. An X-ray diffractometer (PANalytical X'pert Pro) with a high-resolution ψ goniometer and Cu radiation under a glancing angle (5°) was employed for phase identification. The diffractometer was operated at 40 kV and 30 mA.

The static contact angle was measured using an instrument for measuring contact angles (FTA-125, First Ten Angstroms, Portsmouth, VA, USA). The obtained images were analyzed to calculate the contact angle of the deionized water of each sample at room temperature. Each reported contact angle is the mean of at least three independent measurements.

### 2.2. Contact angle measurement

The static contact angle of the deionized water on each sample at room temperature was measured after all specimens were washed alternately in containers with ethanol and deionized water in an ultrasonic cleaner for 30 min each. After samples were dried in a clean dry oven at 55 °C for 6 h, drops generated using a micrometric syringe were deposited onto the surface. The height-to-width ratios of the samples were measured and they were immediately photographed using an instrument for measuring contact angles (FTA-125, First Ten Angstroms,