Fabrication of oxide layer on zirconium by micro-arc oxidation: Structural and antimicrobial characteristics


A Department of Metallurgical and Materials Engineering, Istanbul Technical University, Sariyer, 34469 Istanbul, Turkey
B Department of Medical Microbiology, Center for Infection and Immunity Amsterdam (CINIMA), Academic Medical Center, University of Amsterdam, Meibergdreef 15, 1105 AZ, Amsterdam, The Netherlands
C International Centre of Electron Microscopy for Materials Science, AGH University of Science and Technology, PL, 30-059 Kraków, Poland

**Abstract**

The aim of this study was to cover the surfaces of zirconium (Zr) with an antimicrobial layer for biomedical applications. For this purpose, the micro-arc oxidation (MAO) process was employed in a sodium silicate and sodium hydroxide containing base electrolyte with and without addition of silver acetate (AgC2H3O2). In general, synthesized MAO layers were composed of zirconium oxide (ZrO2) and zircon (ZrSiO4). Addition of AgC2H3O2 into the base electrolyte caused homogenous precipitation of silver-containing particles in the MAO layer, which exhibited excellent antibacterial efficiency against methicillin-resistant *Staphylococcus aureus* (MRSA) as compared to the untreated and MAO-treated Zr.

**Keywords:** Biomaterials, Micro-arc oxidation, Surfaces, Thick film, Antibacterial

1. **Introduction**

Zirconium (Zr) and its alloys can be alternative metals for dental and orthopedic implants because of their high strength and fracture toughness, low modulus and good corrosion resistance, but they are in need of surface modification for good bone integration owing to their bio-inert nature [1,2]. From this point of view the micro-arc oxidation (MAO) process, which is successfully applied to titanium and its alloys to form titanium oxide (TiO2) based biocompatible surface layers [3–6], can offer attractive technical solutions for Zr-based implants also. Indeed, several studies demonstrate the possibility for fabrication of biocompatible zirconium oxide (ZrO2) layers on Zr via MAO process [3–7,14].

Despite the enhancement in biocompatibility, one of the major concerns of surface modification techniques is bacterial colonization around the implants. There are reports showing antibacterial activity of TiO2 based MAO layers upon incorporation of silver (Ag) as an antibacterial agent [5,15,16]. To the best of our knowledge, studies to fabricate antibacterial layers on Zr and its alloys using the MAO process are scarce.

Earlier study showed good antibacterial activity of TiO2 based MAO layers fabricated in silver acetate (AgC2H3O2) added electrolyte against *Escherichia coli* and *Staphylococcus aureus* (S. aureus) [5]. Therefore, we used AgC2H3O2 as the antibacterial chemical in this study for the MAO of Zr samples. Then we characterized the MAO layer structure, and evaluated the antibacterial activity against methicillin-resistant S. aureus (MRSA), one of the major difficulties to treat pathogens in infections associated with metallic implants [17,18].

2. **Materials and methods**

A pulsed DC power supply was employed for three minutes at positive and negative voltages of 400 and 80 V, respectively, for the MAO of Zr samples (9 × 5 mm). The base electrolyte consisted of 15 g/L sodium silicate (NaSiO3) and 2 g/L sodium hydroxide (NaOH) in 2 L distilled water. Some of the samples were subjected to MAO in base electrolyte containing 0.025 M AgC2H3O2 [19]. It is noticed that, addition of AgC2H3O2 changed the color of the electrolyte to light yellow at the beginning. Later this light yellow color turned to brownish color suggesting formation of silver oxide (in the form of Ag2O) [20]. It is noticed that, addition of AgC2H3O2 changed the color of the electrolyte to light yellow at the beginning. Later this light yellow color turned to brownish color suggesting formation of silver oxide (in the form of Ag2O) [20].

The structural features of the layers were analyzed by X-ray diffraction (XRD) technique using Cu-Kα radiation and energy dispersive X-ray spectrometer (EDX) equipped scanning electron microscopy.
(SEM) surveys. The average surface roughness (Ra) of the layers was measured by using a surface profilometer. In order to evaluate the bioactivity and stability of the fabricated layers, Zr-MAO and Zr-MAO-Ag samples were immersed in 1.5x simulated body fluid (SBF) for one week. 1.5x SBF was prepared by dissolving reagent-grade chemicals of NaCl, NaHCO₃, KCl, Na₃PO₄, MgCl₂·6H₂O, CaCl₂·2H₂O and Na₂SO₄ in deionized water buffered at pH 7.40 with ((CH₂OH)₃CNH₂) and 1.0 mol/L HCl at 36.5 °C [21]. Afterwards, surfaces of the samples were examined by SEM.

The bactericidal activity of the triplicate samples of Zr, Zr-MAO and Zr-MAO-Ag was evaluated essentially according to the Japanese Industrial Standard Test (JIS Z 2801:2000) for surface microbicidal activity using the clinical MRSA isolate AMC201 [22]. Four μL of an MRSA suspension containing 4 × 10³ CFU, was pipetted on the surface of each sample and parafilm (8 × 4 mm) was carefully placed on top. After incubation for 20 h at 37 °C in a humid atmosphere, each sample was placed in 500 μL of phosphate buffered saline and sonicated for 30 s in a sonicator water bath. Ten-fold serial dilutions were made, and 10 μL aliquots of the undiluted suspension and of the dilutions were pipetted onto blood agar plates. The plates were incubated overnight at 37 °C and the numbers of colonies were counted the following day.

3. Results and discussion

Surface appearances of the Zr-MAO and Zr-MAO-Ag samples are presented in Fig. 1. MAO process in the base electrolyte generated a matt grey colored surface layer on the Zr-MAO sample. In accordance with the brownish color of AgC₂H₃O₂ added electrolyte, Zr-MAO-Ag sample also appeared in brownish color, which implied incorporation of Ag₂O into the surface layer. It is suggested that Ag and hydroxide ions formed, upon dissolution of AgC₂H₃O₂ and NaOH, respectively, which in turn favored sequential formation of unstable silver hydroxide and thermodynamically stable brownish Ag₂O in the electrolyte [24]. At prolonged times of the MAO process, these Ag₂O particles migrated towards the sample and generated brownish colored layer on the surface of the Zr-MAO-Ag sample.

Although MAO samples appeared in different colors, XRD analyses (Fig. 2) revealed that their phase structures were identical and composed of monoclinic and tetragonal forms of ZrO₂, and ZrSiO₄. However, Sandhyarani et al. [13] did not detect ZrSiO₄ in the biocompatible MAO layer synthesized in an electrolyte system containing phosphate, silicate, and potassium hydroxide. This implies that the MAO parameters of the present study introduced higher amounts of silicon (Si) into the ZrO₂ based surface layers. Since Si is one of the essential elements for the normal growth and development of bone and connective tissues, Si enriched surface layers are expected to exhibit enhanced bioactivity [11,23].

Low magnification SEM surveys revealed the rough and micro-porous (having 2–3 μm diameter) morphology of the MAO layers. Profilometric measurements revealed that the surface roughness of the Zr-MAO and Zr-MAO-Ag samples were in the same range (Ra ≅ 1.1 μm). EDX analysis confirmed the presence of high amount of Si (17–20 wt.%) in the MAO layers. Addition of AgC₂H₃O₂ into the base electrolyte caused incorporation of Ag (0.45 wt.%) into the MAO layer at a concentration well below the cytotoxic limit [24,25]. High-magnification SEM examinations showed that spherical Ag-containing particles (~50 nm) were homogenously precipitated on the surface of the Zr-MAO-Ag sample, unlike Zr-MAO sample. Although no extra examinations have been made other than high magnification SEM surveys and EDX analyses however, these nano-sized particles were suggested to be Ag₂O according to the explanations given above.

Cross-sectional SEM micrographs revealed good bonding of MAO layers to the Zr substrate (Fig. 4). The Zr-MAO-Ag layer was more porous and slightly thinner than the Zr-MAO layer (12–13 μm and 15–16 μm, respectively). The elemental mapping analysis confirmed
homogeneous distribution of Zr and oxygen (O) in the MAO layers (in the form of ZrO₂), while Si tended to be localized at the outer sections of the MAO layers and around the pores (in the form of ZrSiO₄). In the case of the surface layer formed in AgC₂H₃O₂ added electrolyte (Zr-MAO-Ag sample), Ag mostly accumulated at the outermost sections.

Surface appearances of the Zr-MAO and Zr-MAO-Ag samples immersed in 1.5 × SBF for one week are presented in Fig. 5. Calcium- and phosphorous-rich deposits (e.g. hydroxyapatite) have been detected on the surfaces of the examined samples as the primary indication of good bioactivity of the fabricated oxide layers[26]. Furthermore, any evidence showing deterioration of the MAO layers in SBF has not been detected.

The results of the surface microbicidal activity assay showed that Zr-MAO-Ag samples completely killed the 4 × 10³ CFU MRSA inoculum after 20 h of incubation (Fig. 6), with a significant reduction in numbers of CFU when compared to both the non-treated Zr (p = 0.019) and Zr-MAO samples (p = 0.0017). This superior antibacterial effect detected at low Ag concentration (0.45 wt.%) might be associated with the size of the Ag-containing particles (< 50 nm) precipitated in the MAO layer (Figs. 1 and 2). Nano-sized Ag-containing particles have a high ability to penetrate into the contacting bacteria and accelerate release of Ag⁺ ions owing to the greater surface/volume ratio[27]. It should be noted that the Zr-MAO sample showed a minor, non-significant, reduction in numbers of CFU compared to the non-treated Zr sample.

4. Conclusion

Micro-porous and thick oxide layer (mainly consisting of monoclinic and tetragonal forms of ZrO₂, and ZrSiO₄ with Ag containing nano-particles) has been successfully fabricated on Zr via employing MAO in a 0.025 M AgC₂H₃O₂ containing silicate-based electrolyte. Incorporation of Ag in the form of nano-particles at Ag concentration lower than toxicity limit instigated excellent antibacterial characteristics against MRSA as the predominant cause of implant-associated infections. Therefore, MAO appeared as a promising surface modification process for the Zr-based implants.

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Fig. 4. Cross-sectional SEM micrographs and elemental mapping of O, Zr, Si, and Ag for the Zr-MAO and Zr-MAO-Ag samples.
of CFU retrieved. The horizontal line represents the mean log CFU. *, p ≤ 0.05 and **, p ≤ 0.01.

References


