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Fabrication and *in vitro* properties of zinc-based superhydrophilic bioceramic coatings on zirconium

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ABSTRACT

In this study, as the first step, the bioceramic coatings were fabricated on Zr (commercial pure zirconium) in calcium acetate and β-calcium glycerophosphate salt-based electrolyte by MAO (micro arc oxidation). And then, as the second step, the Zn (zinc) thin film layer measured an average thickness of 5 nm was coated on the MAO surface by TE (thermal evaporation). The phase composition, surface and cross sectional morphology, elemental composition and wettability of the MAO and Zn-based MAO coatings were analyzed by powder-XRD and TF-XRD, SEM, EDS-mapping and contact angle goniometer, respectively. The phases of cubic-ZrO_2, perovskite-$CaZrO_3$ and HA (hydroxyapatite) were observed on the surfaces by powder and TF-XRD analyses. The morphology of the MAO surface was not changed by TE although the chemistry of it was different from the MAO surface. The surfaces of both coatings were porous and rough. The Zn was uniformly deposited through the whole surface. After TE process, the wettability of the surface decreased and the Zn-based surface exhibited superhydrophilicity compared to MAO surface. *In vitro* bioactivity test of both coatings were investigated by immersion test in SBF (simulated body fluid) up to 10 days. The bone like apatite layer (secondary apatite) on the Zn-based bioceramic surface was compact and uniform compared the one on the MAO surface although it was completely propagated through both surfaces. *In vitro* anti-bacterial test of both coatings were carried out by microbial adhesion for Gram positive and Gram negative bacteria. It was observed that the microbial adhesions of the Zn-based bioceramic surfaces were lower than ones of the MAO bioceramic surfaces for all Gram-positive and Gram-negative bacteria.

1. Introduction

The commercial Zr has high mechanical strength and good biocompatibility is widely preferred for biomedical implant applications such as orthopedic implant, dental implant and building prosthetic devices in last decades [1–4]. The Zr-based implant materials promote excellent ossoeintegration under *in vivo* studies and its cytotoxicity is lower than one of titanium in some cases [5–7]. However, they are bioinert within the highly corrosive and demanding environment of the human body [8]. Therefore, they cannot chemically bond to bone tissue.

The MAO coatings strongly adhere to metallic substrates such as Zr, Ti, Mg, Al *etc*. with complex geometries and they have elemental homogeneity and great mechanical properties compared to other coating systems such as plasma spray, sol-gel, electrophoretic deposition. The porous MAO surface accelerates bone attachment improving the interfacial bonding strength between the implant and bone. Many investigations about bioactive and biocompatible ZrO_2 and ZrO_2/HA-based fabricated on Zr by MAO process was carried out [9–17]. However, the studies on the production of antibacterial bioceramic coatings are still limited. Especially, antibacterial bioceramic coatings are essential to prevent/decrease the bacterial colonization for the implant applications although the bioceramic surfaces do not contain antibacterial compounds have greater bioactivity and biocompatibility than plain Zr surfaces.

After implantation, one of the most important problems observed for the coatings fabricated on dental and orthopedic implants by various surface modification techniques is bacterial colonization under

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body conditions. The bacteria proliferated around dental and orthopedic implants could penetrate into peri-implantitis or peri-implantoclasia. The bacterial infections led to devastating complications could be resulted in implant failure [18]. Many chemical forms of Ag such as metallic, silver salt and silver nanoparticles are widely preferred in biomedical field due to their anti-bacterial properties. However, some studies verified that it indicated potentially cytotoxicity [19–22]. The diffused Ag into the cells may act as Trojan horses under body conditions. And then, the ionized Ag cause damage intracellular functions [23].

It is well known that the trace elements such as Zn in human bone had an important role in the biological research fields such as bone formation and organism essential elements. *In vitro* studies proved that Zn is an essential trace element for promoting osteoblast cell adhesion, proliferation and differentiation [24–26]. The Zn element which inhibited bone resorbing activity play diverse roles in biological functions such as DNA synthesis, enzyme activity, nucleic acid metabolism, biomineralization and hormonal activity [27,28]. Moreover, the existence of it on the implant exhibited excellent antibacterial properties [29–31].

Especially, the biological properties such as bioactivity, biocompatibility and bacterial adhesion of the Ag-based MAO coatings produced on Zr were studied in last decades [32,33] Fidan et al. fabricated the Ag nanoparticles-based ZrO_2 surface within an Ag-$C_2H_3O_2$–based electrolyte on Zr by a single step MAO process. Afterward, the bioactivity and the bacterial activity in MRSA suspension of the Ag nanoparticles-based MAO surface were investigated [33]. In order to produce homogeneous anti-bacterial layer on the MAO surface and to preserve porous morphology, TE process was preferred by our research group. TE technique works under vacuum conditions does not contain any impurity through process. Moreover, the morphology of surface is not changed and the MAO surface was homogeneously coated at nanometer scaled under vacuum conditions. In our previous study, Ag-based hydrophobic bioceramic coatings on Zr were fabricated by combination of MAO and TE processes. And then, they were systematically characterized and their biological properties such as *in vitro* bioactivity and the bacterial adhesion for 10 different types of bacteria of them were investigated and discussed [32]. These studies indicated that Ag-based surfaces enhanced anti-bacterial and *in vitro* bioactivity properties compared other control samples. However, the Zn structure were not been formed on Zr-based MAO coatings. Moreover, the formation and the effect such as *in vitro* biological properties of Zn on Zr-based ZrO_2/HA oxide coatings layer was not reported so far whereas some literature studies were carried out for the Zn-based TiO_2 coatings on titanium by MAO [34–37]. The effects of Zn incorporation on the phase composition, morphology, surface chemistry, surface energy and biological responses of ZrO_2/HA have not been examined even so far. Thus, the fabrication and investigation of the properties of anti-bacterial Zn-based superhydrophilic bioceramic coatings on Zr were decided by our research group.

In this study, the Zn-incorporated superhydrophilic ZrO_2/HA bioceramic coatings on Zr were produced by MAO and TE techniques. And then, their phase compositions, surface and cross sectional morphologies, elemental amount and distribution and wettability were systematically characterized and investigated by powder and TF-XRD, SEM, EDS-area and EDS-mapping and wetting tests, respectively. Moreover, *in vitro* bioactivity and the bacterial adhesion properties for Gram-negative bacteria as *E. coli*, *P. aureginosa*, *P. vulgaris*, *S. typhimurium*, *P. putida* and Gram-positive bacteria as *B. subtilis*, *S. aureus*, *B. cereus*, *M. luteus*, *S. pyogenes* of both surfaces were investigated and discussed in detail.

2. Experimental details

2.1. Micro arc oxidation (MAO) process

The pure zirconium substrates were the sizes of 45 mm × 25 mm × 5 mm were used for the MAO process. All surfaces of the rectangular substrates were ground by using of the number of 400, 800 and 1200 SiC abrasive papers. After grinding, all samples were cleaned in an ultrasonic bath containing acetone through 15 min and dried under warm air by using heat gun. In order to fabricate the oxide structures on Zr substrate, the MAO system (MDO-100WS) had an AC power supply was used. This system could be run up to maximum 100 kW. The MAO system mainly consisted of a stainless steel container, water cooler and air flow stirrer equipment. The stainless steel container and Zr substrate were used as a cathode and an anode during MAO process, respectively. The alkaline electrolyte was prepared by the dissolution of 0.25 M calcium acetate and 0.06 M β-calcium glycerophosphate salt in distilled water, respectively. The pH value of electrolyte was measured as 8.4. The electrolyte temperature was continuously kept below 40 °C by using a water circulation pump cooler system during MAO process. The MAO process was carried out at 0.260 A/cm^2 for 15 min. Immediately afterwards completing of the MAO process, the coatings' surfaces were cleaned in an ultrasonic bath consisting distilled water for 30 min. And then, they dried in desiccators. In order to provide reproducibility, three MAO coatings were fabricated on three pure zirconium substrates by using same MAO parameters.

2.2. Physical vapor deposition-thermal evaporation (PVD-TE) process

A zinc thin film layer (Alfa Aesar 99.90% purity zinc powder) was coated on bioceramic surfaces at room temperature at a base pressure of 3×10^{-5} mbar by PVD-TE technique (Vaksis PVD/2T) in a vacuum evaporation system. The base pressure of vacuum chamber was kept as about 1×10^{-6} mbar. A tungsten boat which was about 18 cm away from the MAO surfaces was used for the evaporation of Zn. An attached XTM was employed to measure average thickness Zn film with about 5 nm. The deposition rate was kept as 0.5 nm/s during PVD-TE process. In order to prevent the evaporation of zinc, the variable current was gradually increased up to 15 A. Afterwards, the vapor products were deposited onto the bioceramic surfaces. In order to provide reproducibility, three zinc layers were deposited on three MAO surfaces by using same PVD-TE parameters as written above.

2.3. Characterization of the coatings

The phase compositions of the MAO and Zn-based MAO coatings were scanned by powder XRD (X-ray diffractometer, Bruker D8 Advance) and TF-XRD (Thin film-X ray diffractometer, PANalytical X'Pert PRO MPD) with Cu-Kα radiation at a scanning speed of 0.1° min^{-1} from 20° to 90°, respectively. The surface and cross sectional morphologies of the both coatings were observed by using SEM (Scanning electron microscope, Philips XL30S FEG). The elemental amount and distribution analyses of the both coatings were carried out by using EDS-area and EDS-mapping (Energy dispersive spectrometer) attached to the SEM. The average thickness of the MAO coatings was determined by using an ECD (Eddy current device, Fischer Dualscope MP40) at least different 30 points through the surface. The average contact angles and wettability of the both surfaces were examined by using CAG (Contact Angle Goniometer, Dataphysics OCA 15EC) sessile drop technique at room temperature. The average contact measurements were conducted using a light microscope equipped with a video camera and an image analyzer system. The wettability data was measured during each 10 s up to 60 s after the contacting of 1 μL distilled water drop onto the surface.