

Structural and Biological Assessment of Mg Alloy Surface after Plasma Electrolytic Oxidation in Different Solutions

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Abstract—Plasma electrolytic oxidation (PEO) is a promising method for the development of a new generation of oxide coatings for degradable medical devices made from Mg and its alloys. PEO coatings could protect Mg-based products from fast uncontrolled corrosion and improve biocompatibility. It is still under development the ideal solution that provides a uniform biocompatible and corrosion-resistant layer. Current research investigates the morphology characteristics of silicate and phosphate-based ceramic coatings made by PEO in different modes. PEO in a silicate-based solution leads to the formation of a ceramic layer with a crater-like morphology with uniformly distributed pores and demonstrates high wettability. At the same time, the PEO coating is formed in a phosphate-based solution that characterizes bigger pore size and lower pore distribution. Oxide layer films prevent initial *S. aureus* adhesion on the surfaces of the specimens. This makes it possible to consider the formed PEO coatings for degradable medical implants.

Keywords—Mg, PEO, corrosion resistance, bacterial adhesion.

I. INTRODUCTION

Mg and its alloys have advantages of the physical and mechanical properties over the commercially available stainless steel and titanium implants for surgery [1]. But the corrosion rate for degradable materials has particular interest nowadays. The low corrosion resistance of magnesium is a major drawback in implantable medical devices. The alkalization and evolution of gas during the corrosion process can cause inflammation and delay bone formation. The additional surface layer can control the initial degradation rate and maintain sufficient mechanical strength after implantation. The aim of the modification surface is to maintain the mechanical properties of magnesium during the first time after implantation [2]. There are a large number of surface treatment methods for Mg alloys described in the literature: hydrothermal treatment, plasma electrolytic oxidation (PEO), rare earth laser surface treatment, electrochemical deposition (ED) [3, 4]. ED of HA and PEO are the most widespread techniques. ED forms needle-like blades HA crystal coating on a different kind substrate. While PEO can form porous oxide layer films with different thicknesses, hardness is more attractive for cell adhesion [5].

Therefore pores surface structure more applicable for medical application. Oxide layer films with the addition of the different elements can increase the biocompatibility of implants and control the corrosion process in the physiological environment. Alkaline solutions containing silicate or phosphate are often used as electrolytes in PEO for magnesium and its alloys. However, some research works report a controversial date to the corrosion resistance of PEO coating obtained in silicate-based and phosphate-based electrolytes [6]. Studies have also described no toxic effect this chemical compounds on cells [7]. Both electrolytes reach attention due to the promising results for improving the bioactivity of the oxide coating [8].

The aim of this study was to compare the effect of the silicate and phosphate-based electrolytes on the morphology structure and bacterial adhesional properties of coatings obtained by PEO on a pure Mg surface.

II. MATERIALS AND METHODS

A. Materials and Plasma Electrolytic Oxidation

Na_2SiO_3 , NH_4F , NaOH , Na_2HPO_4 were bought from Sigma–Aldrich (St. Louis, MO, USA).

Magnesium samples with a dimension of $1\text{sm} \times 1\text{sm} \times 1\text{sm}$ were polished and then ultrasonically cleaned in acetone. The first electrolyte was prepared by mixing 10 g/L Na_2SiO_3 , 10g/L NH_4F , and 10 g/L NaOH (silicate-based solution) in distilled water; the second electrolyte contained 5 NaOH 5 g/L, Na_2HPO_4 10 g/L (phosphate-based solution) dissolved in distilled water.

The anodic oxidation was performed under an impulse current of 0.1 A cm^{-2} and up to the final voltage of either 250 V for 10 min. The surface area of the face that was modified was equal to 1 cm^{-2} . The anodized specimens were rinsed with distilled water and in deionized water for 5 min.

B. Scanning Electron Microscopy

The morphology of PEO coatings was investigated with a scanning electron microscope (SEO-SEM Inspect S50-B (FEI, Brno, Czech Republic)). The pores size distribution was analyzed by ImageJ 1.50c. software

(<https://imagej.nih.gov/ij/>). To assess the chemical composition of the surface of the samples, an energy-dispersive X-ray spectrometer (AZtecOne with X-MaxN20 detector (Oxford Instruments plc) accompanied to the SEM was used.

C. SBF Immersion Test

In vitro degradation tests were carried out in stimulating body fluid (SBF). The composition of the SBF is presented elsewhere. Samples with both type coating were immersed in 50 mL of solution (pH 7.4) for 3, 14, and 21 days at 37°C. The liquid was refreshing every two days. After each period, the samples washed in distillate water and then dried over the air to constant weight. The corrosion rate was evaluated by weight loss measurement. The samples were weighed before and after immersion. The weight loss value was calculated as follows:

$$(m_0 - m_1/m_0) \times 100 = \omega(\%) \quad (1)$$

m_0 – weight before treatment, m_1 – weight after immersion test.

D. Contact Angle Measurement

Water contact angle (CA) measurements experiments were made using a video-based optical contact angle measuring instrument (OCA 15 EC, Series GM-10-473 V-5.0, Data Physics, Filderstadt, Germany). The characterization of the wettability of coatings relied on the data static CA measurements. Digital image processing of a sessile drop deionized water was used on the investigated surface. The optical images of the sessile drops were obtained. An average contact angle value was determined by measuring five different spots for each sample.

E. Roughness Measurement

Surface roughness and wear profile characteristics were obtained over a length of 10 mm, using a surface roughness tester (Surftest SJ-301, Mitutoyo, Kawasaki, Kanagawa, Japan). The following roughness parameters were measured: arithmetic mean of the sum of roughness profile values (R_a) and ten-point height (R_z).

F. Bacterial adhesion assay

The bacterial strain of *Staphylococcus aureus* (S. aureus, strain B 918) was cultured overnight in the broth at 37 °C. The inocula were resuspended to a final density of 1x10⁶ colony forming units (CFUs)/mL in TSB using McFarland standards. The specimens (PEO treated and non-coated control) were immersed horizontally in the bacterial suspension in static conditions at 37 °C for 2, 4 6, and 24 h. After co-cultivation, the discs were rinsed three times with 2.0 mL of sterile phosphate buffer saline (PBS; pH=7.4) to remove the loosely-adherent bacteria. After that, the specimens were placed in 1.0 mL of sterile PBS and treated by ultrasonication for 1 min to dislodge the adherent bacteria in an ultrasonic bath (B3500S-MT, Branson Ultrasonics Co., Shanghai, China) followed by vortex mixing (Mini Rocker-Shaker, BioSan MR-1, Riga, Latvia). Bacteria removed from the disk were cultivated on the solid medium for 24 h. CFUs determination was conducted by counting the visible colonies.

III. RESULTS AND DISCUSSION

The PEO modification resulted in a porous coating on the substrate in both variants of solution. Fig. 1 presents SEM images of surface samples anodized at voltages of up to 250 V. The surface of the coatings presents a crater-like morphology. The pores are possibly the result of escaping gases during PEO process. The craters are the result of the cooling and deposition of molten material on the surface [9]. The phosphate coating disclosed bigger pores size and lower pore distribution than the silicate coating. The surface was cover with micro-cracks. For the samples anodized in the silicate solution, the surface is characterized by much more amount of pores and their uniform distribution.

The elements distribution of the coatings was obtained using EDX mapping (Fig. 1). These coatings are primarily composed of Mg and O. This results in oxide coating formation by PEO. Data analysis of the EDX spectra reveals Si and F incorporated from the silicate electrolyte in the coating and presence P atoms in the phosphate coatings.

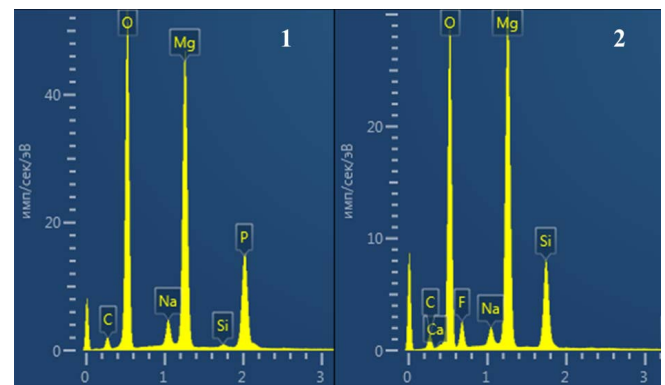


Fig. 1. EDX spectra mapping analyses of 1 silicate and 2 phosphate coatings.

The sample with silicate coating losses 1.1% of weight after 21 days of immersion in SBF, while the sample in which phosphate coating has decrease weight to 0.68%. The dynamic of mass change per day is presented in Table 1.

TABLE I. THE WEIGHT LOSS VALUE SAMPLES WITH SILICATE AND PHOSPHATE COATINGS DURING IMMERSION TEST IN SBF

Name	3d, %	7d, %	21d, %
Silicate solution	-0.32	-0.24	-1.15
Phosphate solution	-0.39	-0.12	-0.68

The roughness value of the PEO coatings is presented in Table 2. R_a and R_z rates for the phosphate coatings increase compare with silicate. This high rate of roughness value is due to the sparking on the surface that leads to appear discharge channels and craters with the bigger size.

TABLE II. AVERAGE ROUGHNESS AND CA OF THE PEO COATINGS FORMED ON Mg IN SILICATE AND PHOSPHATE BASED SOLUTIONS

Name	R_a , μm	R_z , μm	CA
Silicate solution	0.39±0.01	2.83±0.13	22.7 °
Phosphate solution	1.09±0.63	7.79±3.8	70.73 °

Modification of magnesium samples in silicate electrolytes leads to an improvement in the wettability of the

surfaces, which allows the water droplet to be easily wet and to penetrate into the coating quickly. The static contact angle (70.73°) indicates that the surfaces of the phosphate coatings exhibit hydrophobic properties.

In this study, we revealed the time-depending effect of specimens surfaces on bacterial adhesion and viability. There was no significant disparity in the initial adherence after 2-hour incubation between the samples anodized at the same voltage, but in different solutions (Fig. 2).

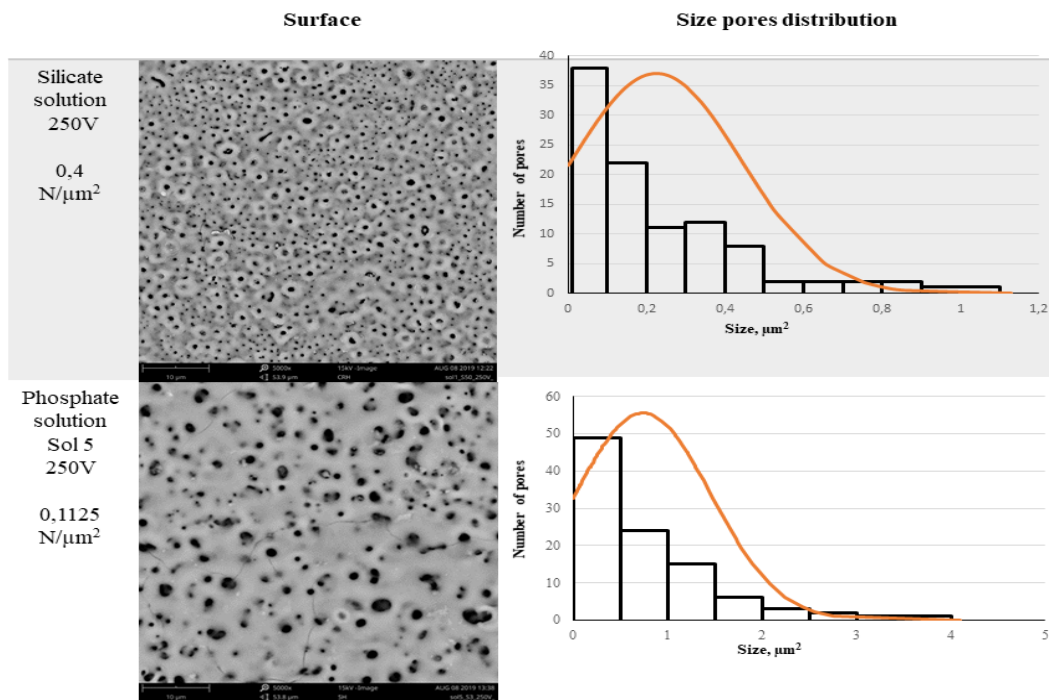
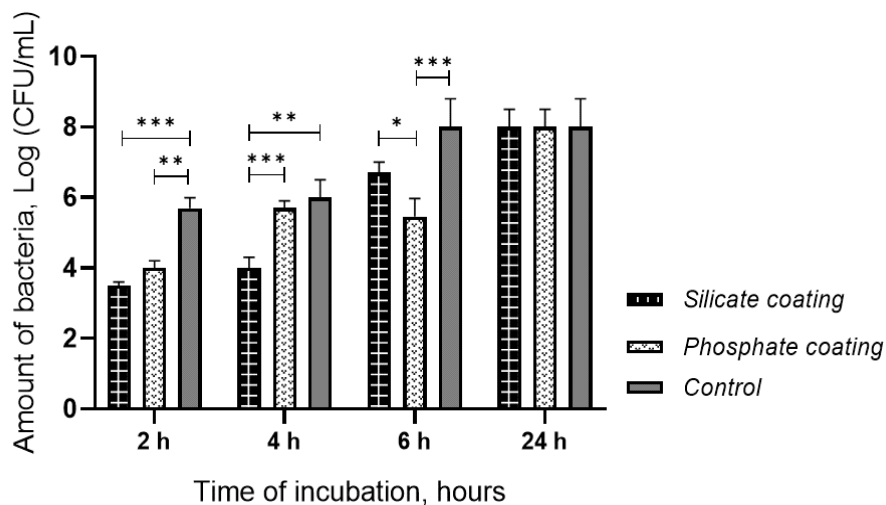


Fig. 2. SEM images of samples anodized at 250v in silicate and phosphate solutions, pore number, and size distribution.

However, there was a significant difference between PEO treated discs and non-coated samples at 2 h time point ($p > 0.0005$ for silicate coating and phosphate coating $p > 0.005$), 4 h time point ($p > 0.005$ for silicate), and 4 h time point ($p > 0.0005$ for phosphate) (Fig. 3). The adherent bacteria amount on the surfaces of specimens treated with silicate during PEO process was significantly decreased compared with that on the samples treated with phosphate after 4 h incubation, respectively. In contrast, the living

bacteria amount was increased considerably on the silicate anodized discs compared with the phosphate at 6 h of the experiment point, respectively. Otherwise, bacteria number increased to 8 Log CFUs after 24 h co-cultivation for both types of samples. Therefore, the results submit that bacterial adhesion a remarkably decreased on the specimens despite the electrolyte solution content at the early stages of the assay.



The amount of viable bacteria adhered on the surfaces of the specimens samples at various time intervals of experiment; asterisks denotes a significant difference (* - $p < 0.05$; ** - $p < 0.005$; *** - $p < 0.0005$)

IV. CONCLUSION

PEO coatings are provided as a uniform porous oxide layer in both variants. The surface chemical composition samples differ additional elements due to the use of disparate compounds electrolyte. The surface structure silicate coating is characterized by uniform pore distribution with a size to 1 μm and high wettability properties. Phosphate coatings have pores to 4 μm with low distribution, hydrophobic properties, and a high rate of roughness value compared to silicate coating. The weight loss results revealed good corrosion resistance in both type electrolytes. Bacterial adhesion test showed that oxide films created with suggested electrolyte compositions by PEO could delay initial *S. aureus* adhesion on the surface of the specimens that can use in dealing with biofilm formation.

According to the results, silicate coating is more appropriate to surgery applications owing to morphology properties and low degradation rate. Moreover, it is necessary to conduct additional studies aimed at determining the cytotoxicity and cytocompatibility of experimental surfaces with different electrolytes using human cells as an example.

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