Electrochemical and biological characterization HA/Al₂O₃-YSZ nano-composite coatings using electrophoretic process

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Abstract: In this research work, hydroxyapatite/alumina/YSZ bio nanocomposite coatings on titanium substrate were created by electrophoretic deposition (EPD) and reaction bonding process. By using the EPD process, uniform green form coatings containing HA, yttria-stabilized zirconia (YSZ), and aluminum particles were produced on titanium. After oxidation of aluminum at 660°C and sintering at 850°C, a dense and adherent HA/Al₂O₃/YSZ coating was produced. Scanning electron microscopy, X-ray diffractometric and mechanical tests were employed to investigate the morphologies, compositions, hardness, toughness and bonding strength of the coatings. The corrosion studies and cell culturing experiment were carried out and the results show that the HA/YSZ/Al₂O₃ coatings are more bioactive and more resistance to corrosion than HA coatings.

Key Words: Electrophoretic deposition, electrochemical, cell culture, YSZ, suspensions, hydroxyapatite

INTRODUCTION
To reconstruct bioactive ceramics are generally utilized as coatings on metals and reconstruct skeletal muscle tissue. Among different Caps, HA [Ca₁₀(PO₄)₆(OH)₂] is widely used due to its osseous nature and suitability to strong bonding with host bone tissues.¹–³ Different coating methods have been utilized such as plasma spraying, dip coating, hot isostatic pressing, ion beam-assisted deposition, pulsed laser deposition, sol-gel technique and electrophoretic deposition (EPD).⁴–¹³ Among of these methods, EPD has several benefits such as better control thickness and morphology of the coating, components with complex shapes are deposited uniform, higher deposition rate than other coating technics and cheap equipment required.¹⁴–¹⁶ However, deposited pure HA by the EPD method has defects that limit the applications of this method. The main limitation is low sintering temperature that applied for this technique while full densification coatings required high sintering temperature.¹⁷ High temperature can cause destruction of the metal substrate and disintegration of HA coating.¹⁸ Disintegration of the HA coating is unfavorable because it produces tricalcium phosphate phase that is biodegradable in vivo. Sintering temperatures ideally should be below 1000°C.⁹,¹⁹ Furthermore, HA coatings are produced by the EPD which has little adhesive strength and due to large differences in physical and thermal properties between HA and Ti.²⁰,²¹ Coefficient of thermal expansion of HA is much more than that of titanium substrate. Thus, when sintered pieces make cold large thermal contraction mismatch would arise and lead to the formation of cracks.²² To overcome this problem, HA-alumina composite coatings were utilized on Ti.²²,²³ Reaction bonding of Al₂O₃ was developed to produce near-net-shape ceramics to overcome problems caused by the shrinkage during sintering.²⁴,²⁵ Zirconia (ZrO₂) is a bio ceramic and is bio inert, which shows superior mechanical behavior and biocompatibility. Therefore, adding ZrO₂ into HAP improves the interfacial bonding strength of the Ti substrate and coating.²⁶–²⁸ Zirconia is superior to alumina in terms of mechanical properties and bio compatibility.²⁹ Conversions of zirconia from tetragonal to monoclinic phase induces the increase in its strength and fracture toughness.³⁰ In this research, hydroxyapatite/alumina/YSZ bio nanocomposite coating to improve the mechanical properties and biocompatibility on titanium substrate was carried out by electrophoretic deposition and reaction bonding process.

EXPERIMENTAL
Materials
For this study, HA nano powder was synthesized by chemical precipitation method.³¹ The synthesized nanoparticles were characterized by X-ray diffractometric (XRD) and scanning electron microscope (SEM) analysis (Fig. 1).³¹ Al nano powder prepared with milling by satellite mill (Tajhiz Ceram

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Com., Iran) for 10 hours of Al powder (purity >99.5%, PMC, Iran, with D <60 μm). YSZ –ZrO₂ nano powder (purity 99%, APS <50 nm, SSA 17m²/g star chemical china) was used as the coating material too.

Suspension preparation
The suspensions containing 20 g/L of HA, Al and YSZ nanoparticles were prepared with different compositions according to Table I. Isopropanol (Merck) and acetone (Merck) were used as the solvent mediums and iodine (Merck) as the dispersant. For suspensions preparation, 0.6 g/L of iodine was added into 60 mL of isopropanol-acetone with ratio 50/50 and dissolved by stirring after 15 min.

EPD process
The titanium sheets were used as cathode electrode and the stainless steel plate was used as anode electrode for EPD process. The separation distance between electrodes was 2 cm. EPD process was carried out in suspensions at a constant voltage of 10 V for 2 min using a laboratory D.C. power supply. After the EPD process, the coated titanium substrates were removed from the suspension and were dried at room temperature. Then specimens were placed in a furnace in air and heated at a rate of 2°C/min up to 660°C and held at this temperature for 2 h in order to oxidize the Al powders. After that specimens were placed in a tube furnace and temperature was increased at the same rate up to 850°C and held for 2 h in an argon atmosphere for sintering. Finally, the furnace was cooled down to room temperature at a rate of 1°C/min.

Characterization
Phase composition of the coatings was tested by the XRD (Bruker model D8Tools) by using a Cu Kα radiation, with scan step size of 0.02 before and after of sintering. Surface morphologies of coatings were observed by the field emission scanning electron microscopy (FE-SEM) (Mira3Tescan, Czech Republic). Topography and roughness of the sintered coatings surfaces were investigated by an atomic force microscope (AFM) (Dualscope/Rasterscope C26, DME, Denmark). The adhesion strength of the coatings to the substrate was measured according to ASTM F1044-87.19 The coatings were stuck to Ti plates with the same dimension as the substrate using epoxy resin (Uhu Plus Endfest 300). The epoxy resin was cured in an oven at 180°C for 5 min. The value of bond strength was measured using a Universal Testing Machine (Zwick/Roell Z100, Germany) at a crosshead speed of 1 mm.min⁻¹. Three specimens were used in the adhesion tests and the average adhesion strength was calculated. To calculate the fracture toughness of the samples, the experimenters used the Vickers hardness tester at

<table>
<thead>
<tr>
<th>Suspension</th>
<th>HA Powder (wt %)</th>
<th>Al Powder (wt %)</th>
<th>YSZ Powder (wt %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S2</td>
<td>50</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>S3</td>
<td>35</td>
<td>35</td>
<td>30</td>
</tr>
</tbody>
</table>

FIGURE 1. The X-ray diffraction pattern and FE-SEM of hydroxyapatite nano powder.

FIGURE 2. XRD patterns of green coatings.
a load of 49 N for specimens over a 10 s loading duration. Vickers hardness was determined by means of an instrumented hardness tester (Walter model 4021). Fracture toughness was determined by the Vickers indentation technique developed by Evans and Charles and modified by Niihara et al. The fracture toughness $K_{IC}$ was estimated using the Eq. (1):

$$K_{IC} = 0.055H_a L^{-0.5}$$

where $H$ is the Vickers hardness, $a$ is the half diagonal length of the indentation and $L$ is radial crack length from the corner of the indentation.

**Cell culture**

MG-63 osteoblast cells (NCBI C555 Pasteur Institute of Iran) were used in cell culture. After defreezing cells, they were transferred to flask containing RPMI medium with 10% FBS (Fetal bovine serum). The flask was placed in the incubator at 37°C, 90% humidity and 5% oxygen concentration. To determine of samples cell proliferation was used of direct contact method. One of the best existing indirect methods to determine the cell proliferation, is Tyazol dimethyl diphenyl tetrazolium bromide (MTT, Sigma) test, which is based on change on the yellow powder insoluble to purple-black formozan crystals. This phenomenon happens only found in the mitochondria of living cells using enzyme called succinate dehydrogenase. Formozan crystals were dissolved using an organic solvent such as isopropanol. A allays reader was used to the optical density (OD). The OD is directly proportional to the concentration of
Formozan which is proportional to the metabolic activity of living cells.

**MTT test.** The first, $2 \times 10^4$ cells were placed in a volume of 100 mL culture medium on each of the sterilized samples in 12-well plates. Then for 4 h they were placed in an incubator at 37°C until the cells attached to the surface of specimens. After confidence of adherence of the cells, 2 mL of culture medium was added to each well again. The culture medium on cells was discharged as much as possible after 2 and 4 days. 400 μL of 5.0 mg/mL MTT were poured in each well then were placed in incubator for 4 h. After 4 h, solution on the cells were removed and isopropanol were added into them to solving created purple crystals. The plate was placed on a shaker device for 15 min to well dissolve precipitated MTT. Then, 100 μL of purple solution of each well were transferred on 96-well plate. Furthermore, the amount of material dissolved in isopropanol was measured using Micro plate reader (STAT FAX 2100) at a wavelength of 570 nm. The wells with most cells show OD higher than the wells with lower cell. Therefore, wells with higher cell amount was determined the following equation Eq. (2) and compared with control sample. It should be noted that each sample was repeated three times.

$$\text{Toxicity\%} = \left(1 - \frac{\text{mean OD of sample}}{\text{mean OD of control}}\right) \times 100$$

$$\text{Viability\%} = 100 - \text{Toxicity\%}$$

**Cell adhesion test.** To study cell adhesion, sterilized samples were placed in six well plates. Then 20,000–30,000 cells in a volume of 100 μL were poured on each sample and were incubated for 5–4 h. After adhesion cells, a certain amount of culture medium containing 10% fetal bovine serum (FBS) was added to each well. After 24 h, the culture medium was out on samples and for 30 s was washed with PBS (phosphate buffered saline). Then, the cells were fixed using Glutaraldehyde 3.5%. After pouring a certain amount of fixator on each sample was placed in the refrigerator for 2 h. Furthermore, the fixator matter was got out and samples were washed with deionized water two times. The cell adhesion was assessed by the SEM.

**Electrochemical measurements**

The electrochemical and corrosion studies on Ti substrate and coated samples(HA, HA-Al2O3, HA-Al2O3-Y SZ bio nano coatings) were performed using a potentiostat (Versastat-3, Prinston Applied Research) conforming to ASTM Standard G61–94. To investigate electrochemical corrosion behavior a conventional three electrode set up was used with Pt as counter electrode and a saturate calomel electrode as the reference electrode.
reference electrode. The Ringer’s stimulated body fluid (SBF), the aqueous solution containing 960 mL deionized water, 6.5456 g NaCl, 0.373 g KCl, 0.1419 g Na2HPO4, 0.3049 g MgCl2·6H2O, 9 mL of 1 M HCl solution, 0.3675 g CaCl2·2H2O, 0.071 g Na2SO4 and 6.057 g Tris [(CH2OH)3CNH2] was used as corrosion medium.35 The corrosion behavior of all the samples was tested at 37°C. The exposed area of working electrode was 100 mm². The open circuit potential (OCP) was recorded for 30 min. Polarization curves were obtained with a scan rate of 2 mV/s from -0.8 V to +0.8 V. The corrosion potential (Ecorr) and corrosion current density (Icorr) were extracted by Tafel extrapolation method from polarization curves. Additionally, the polarization resistance (Rp) is estimated by the use of Stern–Geary equation:36

\[ Rp = \frac{b_a b_c}{(2.303 I_{corr})(b_a + b_c)} \]  

RESULTS AND DISCUSSION

Figure 2 shows XRD pattern of green coatings obtained from suspensions of S1-S3. In the S1 coating only hydroxyapatite peaks were observed. Furthermore, in the S2 coating hydroxyapatite and aluminum (2θ = 25.56°, 37.7°) peaks can be seen as aluminum in green coatings was completely converted to alumina phase after sintering process. Also, in S3 coating hydroxyapatite, alumina and YSZ peaks can be observed. The presence of rutile peaks demonstrates oxidizing of titanium substrate. Figure 4 shows FE-SEM images of green coatings. Figure 4(a,b) shows pure nano hydroxyapatite coating and hydroxyapatite-aluminum nano composite coating prepared by S1 and S2 suspensions, respectively. Also, Figure 4(c) illustrates hydroxyapatite-aluminum-YSZ nano composite coating prepared by S3 suspension. Furthermore, Figure 5 shows FE-SEM images of samples after sintering in 850°C. In Figures 4 and 5, the background is related to hydroxyapatite phase. Dark polygon shape particles in Figures 4 and 5 dispersed on hydroxyapatite background phase are alumina (Fig. 4), and alumina (Fig. 5). Bright and fine spherical particles are yttria stabilized zirconia. It can be seen that the particle size increased after the sintering of coatings.
TABLE III. Electrochemical Corrosion Parameters

<table>
<thead>
<tr>
<th>Specimens</th>
<th>$i_{corr}$ (A)</th>
<th>$E_{corr}$ (V)</th>
<th>$R_{corr}$ (ohm/cm$^2$)</th>
<th>Corrosion Rate (mpy)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ti substrate</td>
<td>$2.783 \times 10^{-6}$</td>
<td>$-0.381$</td>
<td>7801</td>
<td>0.3382</td>
</tr>
<tr>
<td>HA coating</td>
<td>$2.204 \times 10^{-6}$</td>
<td>$-0.3$</td>
<td>9949</td>
<td>0.2836</td>
</tr>
<tr>
<td>HA-Al$_2$O$_3$ coating</td>
<td>$2.098 \times 10^{-6}$</td>
<td>$0.223$</td>
<td>$1.035 \times 10^{-4}$</td>
<td>0.2700</td>
</tr>
<tr>
<td>HA-Al$_2$O$_3$-YSZ coating</td>
<td>$1.403 \times 10^{-7}$</td>
<td>$0.397$</td>
<td>$1.548 \times 10^{-5}$</td>
<td>0.0180</td>
</tr>
</tbody>
</table>

[Fig. 5(a–c)]. By addition of aluminum to coatings the porosity decreased and dense structure was obtained resulting in improved mechanical properties. Bonding strength, hardness and toughness of coatings are shown in Table II. As can be seen with addition of aluminum and YSZ (S2-S3) to HA coatings adhesion strength, hardness and toughness coatings were increased. In S3 coating maximum result of bonding strength, hardness and toughness are observed. As can be observed in FE-SEM images, there are uniform distribution of YSZ particles in this coating. Surface roughness of nano-composite coatings was analyzed by an AFM, which was coupled with the software. Root mean square roughness ($S_q$) and average roughness ($S_a$) were measured for each sample. Figure 6 shows the three-dimensional surface topographies of HA, HA/Al$_2$O$_3$ and HA/Al$_2$O$_3$/YSZ nano-composite coatings, which were sintered at 850°C for 2 h. Figure 7 demonstrate the surface roughness parameters ($S_a$ and $S_q$) of HA, HA/Al2O3 and HA/Al$_2$O$_3$/YSZ nano-composite coatings. It can be seen that $S_a$ and $S_q$ for HA/Al$_2$O$_3$/YSZ surface coatings are 218 and 263 nm, respectively and it has the highest roughness in comparison with other coatings. The surface roughness investigation is of great importance for a cell culture application where an appropriate roughened surface is highly desirable due to its higher bonding strength of cells with the coating.37–41 Cell viability expressed by MTT assay after 2 and 4 days incubation is shown in Figure 8. Titanium sheet was considered as control process (blank). As can be seen the most cell proliferation has occurred on the S3 coating, which was due to the high surface roughness of this coating (HA/Al$_2$O$_3$/YSZ) in comparison to the other coatings. Figure 9 shows FE-SEM adhesion images of MG63 bone cell to HA, HA/Al$_2$O$_3$ and HA/Al$_2$O$_3$/YSZ coatings. As can be seen in the images, coupling and adhesion of cells to HA/Al$_2$O$_3$/YSZ coatings are more than other coatings. The potentiodynamic polarization curves of the samples in a SBF solution are illustrated Figure 10. The corrosion parameters, adapted from polarization curves using the extrapolation Tafel method, which are listed in Table III. The results corresponding to HA-Al$_2$O$_3$/YSZ coating mention a significant decrease in the corrosion current ($i_{corr}$) and an increase in the corrosion potential ($E_{corr}$), increase polarization resistance ($R_p$) and decrease corrosion rate.

CONCLUSION

Fabrication of HA/Al$_2$O$_3$/YSZ bio nano composite coating was carried out with combination of electrophoretic deposition (EPD) and reaction bonding process.

1. XRD results indicate that there is no HA decomposition during the sintering process and aluminum particles in green coating were completely converted to alumina phase after sintering process.
2. HA/Al$_2$O$_3$/YSZ bio nano composite coating show best mechanical properties.
3. Cell culturing results show that the HA/Al$_2$O$_3$/YSZ nano composite coatings are more bioactive in compare with HA and HA/Al$_2$O$_3$ coatings.
4. The electrochemical and corrosion studies show that Corrosion resistance of HA/Al$_2$O$_3$/YSZ nano composite coating is more than of others.
5. In comparison to HA coatings, HA/Al$_2$O$_3$/YSZ bio nano composite coatings have higher bonding strength. Adhesion of cells to HA/Al$_2$O$_3$/YSZ coatings is more than other coatings. The most cell proliferation has happened on the HA/Al$_2$O$_3$/YSZ coating which was due to the high surface roughness of this coating in comparison to the others.

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REFERENCES


