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Electrodeposited Hydroxyapatite/Graphene Oxide/Zirconia Oxide Composite Coatings: Characterization and Antibacterial Activity

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ABSTRACT

The current study aimed to characterize the hydroxyapatite, zirconia, and graphene oxide nanocomposite coatings on titanium substrate by the use of electrophoretic deposition. In the first stage, besides the characterization of the created composite coating, the thickness and uniformity of the created coating were evaluated by the use of Scanning Electron Microscope (SEM). Also, the distribution of the Nano-powder particles was investigated by the elemental analysis. In the second stage, by the use of X-ray diffraction analysis, the position of the materials used in the coating was drawn and investigated. In the third stage, in order to evaluate the coating's corrosion behavior due to the addition of nanoparticles to the hydroxyapatite and compare it with the non-coated sample, the electrochemical analyses in the form of chemical polarization were investigated and analyzed with drawing the related charts. Finally, in the fourth stage, the antibacterial tests on the Escherichia coli and Staphylococcus bacteria on the coating were conducted and compared to the uncoated alloy samples. The corrosion test results indicated that the use of nano-composite coating leads to the increase in corrosion resistance of the surface. The antibacterial tests results demonstareted that the use of nano-composite coating effectively decreases the bacteria growth on the surface.

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1. INTRODUCTION

Today, pure and alloy titanium are widely used for orthopedic materials and dental applications. The high corrosion resistance (due to formation of titanium oxide layer on the surface), proper mechanical properties (elastic modulus similar to the bone, the high strength to weight ratio), suitable biocompatibility with the tissue, great bone conduction, and the low toxicity are among the reasons behind the extensive use of this type of alloy. The hydroxyapatite coating is used to increase the titanium bonding with the tissue. Chemically and biologically, this material is highly similar to the bone and hard tissues of human. In spite of the proper performance of the hydroxyapatite coatings on the alloy surface from the biological point of view, another challenging subject is the structural fragility of this coating and investigation of its behavior in presence of infectious bacteria. The research show that the use of secondary materials such as titanium oxide, gelatin, chitosan, carbon nanotubes and graphene oxide can properly promote the mechanical behavior of this coating.

According to the research, the use of graphene oxide leads to the creation of a denser coating and sites for the germination of the hydroxyapatite crystals. On the other hand, the increase in the hardness and the elastic modulus are among the advantages of the production of this composite. The use of graphene oxide leads to the increase in coating's corrosion resistance. In the current study, the graphene oxide, zirconium oxide, and hydroxyapatite were combined by Electrophoretic method. Although the basic phenomenon which occurs in the Electrophoretic is well known, and it has been the main subject of many practical and theoretical studies, the ceramics electrophoretic was first studied by Hamaker [1]. The electrophoretic deposition is one of the colloid processes in production of the ceramics and has advantages such as the short formation time, simple facilities, low constraints compared to the substrate

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shape, and no need for binder (which makes the amount of organic matter in the raw material very low or even zero) [2, 3].

The electric field effective on the electrophoretic is reduced by the deposition time, since an insulating layer is made of ceramic particles on the electrodes. However, during the first electrophoretic stage, a linear relationship between the deposition mass and the time is established [4]. Negishsi et al. observed that N-propanol's solvent flow density is comparable with the applied voltage in presence of any powders, and it becomes unstable with the increase in applied voltages. Such stable data are considered as a good guideline for the decision-making on the deposition parameters and the resultant quality of the sediment created by electrophoretic method [5].

The results obtained from different research show that this type of reinforcements causes problems and disorders. For example, addition of the carbon nanotubes causes biological disorders and adverse effects. After the conducted investigations, an absorbent material named graphene was noted. Graphene oxide has unique properties such as proper mechanical properties, great biocompatibility, good antimicrobial property, etc. Addition of graphene oxide to hydroxyapatite leads to the strengthening of the mechanical properties as well as the reduction in the surface cracks and the increase in adhesive strength of the coating. Ming et al. [6] deposed the graphene as the reinforcement to the hydroxyapatite on the titanium substrate by the electrophoretic deposition method. Li and Shei [7] in two articles, studied the antibacterial behavior of four types of graphene (graphite/graphite oxide/ graphene/ graphene derivative) to resist E.coli. It was revealed in this study that graphene oxide solvent has the highest antibacterial activity. Saberi et al. [8] prepared alcoholic suspensions of the zirconium nanoparticles and added Triethanolamine as a stabilizer to it. It was concluded that Triethanolamine is an effective stabilizer for ethanol suspensions with 0.45 density, Isopropanol suspension with 0.3 density, and Butanol suspension with 0.15 density. The current study aimed to characterize the hydroxyapatite, zirconia, and graphene oxide nanocomposite coatings on titanium substrate by the use of the impedance method.

2. MATERIALS AND METHODS/EXPERIMENTAL PROCEDURE

The chemical composition of the alloy used in the current study is provided in Table 1. The samples are abraded by the Sic-contained abrasive papers Nos. 400, 600, 800, and 1200, respectively. The samples were immersed in distilled water for 30 minutes in an ultrasonic apparatus to be degreased, and finally, they were rinsed by acetone.

TABLE 1. The Ti-6AL-4V alloy properties used in the current study

Element	Percentage
Vanadium	4.5
Aluminum	6.75
Iron	3
Oxygen	2
Carbon	0.8
Nitrogen	0.5
Hydrogen	0.15

The properties of the hydroxyapatite powder, zirconium oxide powder, and graphene oxide powder are presented in Table 2.

TABLE 2. The properties of the primary materials' powders used in the current study

Material name	Company	Molecular weight (g/mol)	Chemical formula
Hydroxyapatite	Sigma-Aldrich (US)	502.31	$Ca_{10}(po_4)_6OH_2$
Zirconia oxide	Merck (Germany)	123.21	Zro_2
Graphene oxide	Sigma-Aldrich (US)	120.52	GO

For the preparation of the suspension, the chosen composition powder including the hydroxyapatite is mixed with graphene oxide and zirconium oxide and 50ml of isopropanol obtained from Merck of Germany, and it was put on the magnetic stirrer. In order to better dissolve and create a positive charge on the surface of the hydroxyapatite particles, in addition to increasing the suspension stability, moving the particle toward the cathode and precipitating on its surface in the electrophoretic process of the specified amount, Merck's triethanolamine was added. Then it was put on the stirrer for 48 hours, and by the use of nitric acid, which was again obtained from Merck, the suspension's PH was set as around 3-4.

TABLE 3. The materials used in the suspension for electrophoretic process

Row	Composition name	Amounts
1	Isopropanol	50ml
2	Hydroxyapatite	0.3g
3	Graphene oxide	0.01g
4	Zirconium oxide	0.02g
5	TEA	2ml

In order to create the composite coating, firstly, the intended electrodes were prepared and then, by connecting to the power supply and placing it in a suspension solution with a specified voltage of 60 V and a predetermined time of 180 seconds, the coating was applied to the surface. The voltage selection and coating time were determined based on the optimal conditions. Then, in order to create stability and increase the resistance and adhesion of the applied composite coating, the sintering operation was used. To do so, the coated sample was put in the vacuum oven with a temperature of 950 centigrade for 2 hours, and then it was cooled in the oven.

Elemental analysis was performed by the EDAX EDS Silicon Drift 2017 detector. In order to investigate the crystal structure of the synthesized powders, the X-ray diffraction machine Philips, model Xpert with 31mA current and 41kW voltage was used. The cathode lamp used was copper with a wavelength of 1,4114 angstroms. Scan rate of 1 and step size of 1.11 / s were selected. The phase detection was done by the X-pert software.

For performing the electrochemical tests, first, the metal samples were degreased by the alcohol and then they were rinsed by distilled water. Each of the samples was used as the working electrode after preparation on the cell, and were added to the counter for completion of the circuit and conducting the test. Then, the intended solution was injected into the cell and for setting the temperature, a heating system which included a thermal element and thermometer was used. Potentiostat-galvanostat IVIUMSTAT with IVIUMSOFT software was used for electrochemical measurements by the use of electrochemical polarization spectroscopy method. For performing the antibacterial test, it is required to do the preparations 24 hours prior to the test. To do so, the needed cultivation environments are prepared and for sterilizing these environments, they were put into the autoclave for 15 minutes at a temperature of 121 centigrade. Also, the case and control samples are both sterilized. To prepare the bacteria needed, they are passaged 24 hours before the test.

In order to perform the test by the passaged colonies, we prepare the intended suspension. For suspension preparation with the 10^8 cfu/ml concentration, it is required to be compared to the McFarland standards (to prepare this solution, we add 0.05ml of the $BaCl_2$ 1% solution to 0.95ml of H_2so_4 1% solution and stir well). In order to determine the precise number of the bacteria by the spectrophotometry machine, we measure its absorption rate. The absorption rate required for 10^8 cfu/ml concentration is 625 nanometers. We add 1 cc of the suspension with the intended dilution to the alloy's surface (the pure titanium alloy has been considered as the control sample). Then, it is pit inside an incubator with a temperature of 37 centigrade for 24 hours. In the following, we collect the bacteria from the alloy's surface by a sampler, and add them to a tube which contains 100 cc of ringer cultivation environment, and the vortex it. Then, we prepare the 10^5, 10^6, and 10^7 concentrations through dilution and culture them by the use of spread-plate method. After closing the plates, we put them inside an incubator with a temperature of 37 centigrade, and after 24 hours, we count the colonies by the colony counter and report the number. The bacteria used in this test were gram-positive (E-coli) with ATTC29213 and gram-negative (S-Areus) with ATTC25922.

3. RESULTS AND DISCUSSION

The current study aimed to characterize the hydroxyapatite, zirconia and graphene oxide nanocomposite coatings on titanium substrate by the use of the impedance method. Firstly, besides the characterization of the created composite coating, the thickness and uniformity of this coating were measured by the use of SEM. Then, by the use of X-ray diffraction test, the position of the used materials in the coating was investigated and in the next stage, the electrochemical tests in the form of chemical impedance were performed to evaluate the corrosion behavior of the coating and compare it with the uncoated sample. In the final phase, by performing the antibacterial tests on the E.Coli and S.Areus on the created coating, it was evaluated and compared to the uncoated alloy sample.

3.1. Evaluation of the Coating Structure and Morphology

The images taken from the coating by the SEM are provided in Figures of 1 and 2.

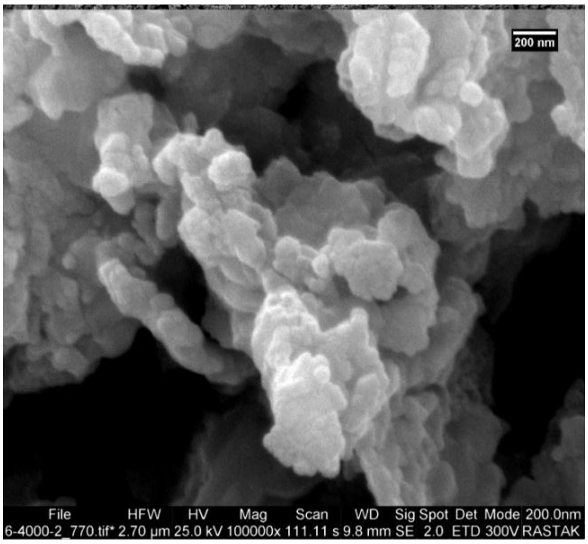

Figure 1. SEM micrograph of the coated sample

The studies show that the hydroxyapatite coating shows weak mechanical and antibacterial properties, and one of the proper ways to promote this costing's properties is the production of the coating with a secondary phase, which shows better properties compared to the pure hydroxyapatite coating. The uniform morphology and the agglomeration of the used powder can be seen in the image. The results indicate that the use of a secondary phase creates the adhesion to the substrate and higher uniformity as well as a surface with no cracks. The investigations show that the factors effective on the thickness of the created composite coatings by the electrophoretic method, depend on the parameters such as the time, voltage, and type of the composition. In Poorraeisi et al study titled "characterization and coating of the hydroxyapatite-zirconium oxide-titanium oxide nanocomposite" observed that by addition of zirconium to pure hydroxyapatite and increasing the voltage, the coating's thickness is increased [9].

As was observed, time and voltage are two effective factors in the electrophoretic method. With the passage of time and increase in the voltage, an optimal coating would be obtained and the more these two parameters are increased, the thicker the sample would be [10].

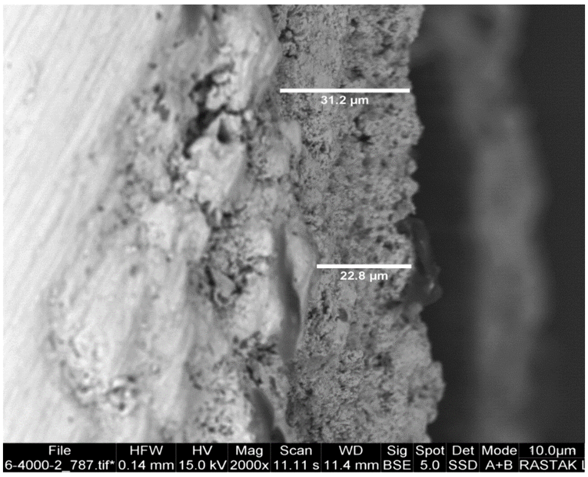

Figure 2. SEM micrograph of the cross-section of composite coating

According to the results, the coating has a thickness of 22.8 to 31.2 micrometers. The results also showed that coating distribution on different sections of the surface has a specific range.

Distribution of the elements in coating was analyzed by the EDAX analyzer for the hydroxyapatite/zirconium oxide composite coating and the graphene oxide on the titanium substrate (Figure 3). Based on the obtained results, we can observe that all elements of the composite have been successfully distributed on the titanium substrate. The peaks intensity was proportionate to the weight percentage of each element in the composite formation. In the composite compound, the amount of the nanohydroxyapatite powder which includes calcium, phosphorus, hydrogen, and oxygen, is higher. The next point is that in these samples, due to having coatings in common with gold peaks, the palladium peak was used to avoid the homogeneity of the peaks.

According to Figure 4, the elemental analysis along the coating has been presented based on different elements. The presence of coating's constituent elements confirms the type of coating's composition.

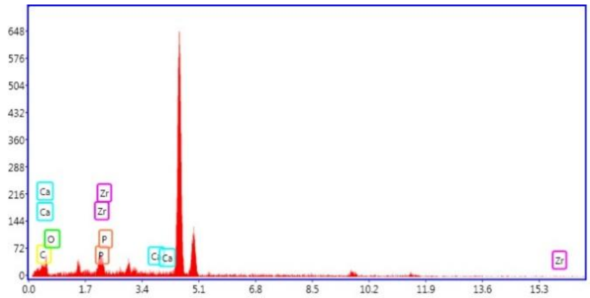

Figure 3. EDAX analysis of the composite coating

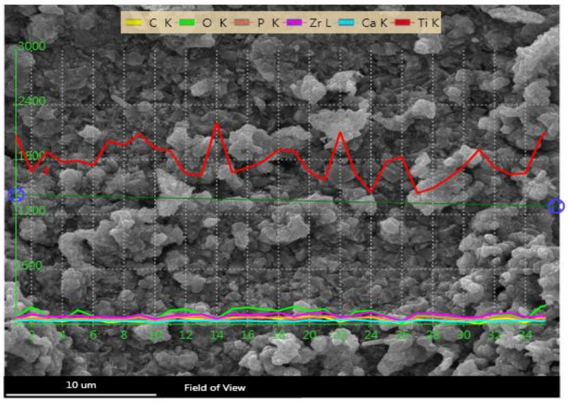

Figure 4. The distribution of the hydroxyapatite, zirconium, and graphene oxide composite coating's elements

According to Figure 5, the distribution of the various elements in the MAP test on the coating's surface has been presented. Also, as observed, the distribution of the secondary phases elements on the surface has been suitable. In the current study, the graphene oxide has been added to the composite as a reinforcement, which can be seen in black.

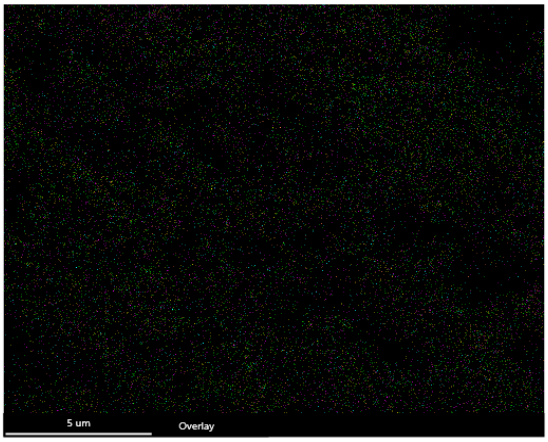

Figure 5. MAP image of various elements of the composite surface

3.2. Phase Evaluation

The X-ray diffraction machine has been used for phase detection of the coatings. Ming et al. in an article titled "characterization of the hydroxyapatite-graphene oxide composite coating by the electrophoretic method" found out that by addition of the graphene oxide to the hydroxyapatite powder, the peaks intensity is increased and the more the graphene oxide is added, the higher the intensity will be.

As observed in Figure 6, all the peaks of the composite coating have been precisely drawn and each of the compositions has been drawn based on the peak's intensity and hardness. It should be noted that regarding the fact that the hydroxyapatite powder in this composite coating has been used more than the other materials, the coating is significant on all the points.

The comparison of the obtained peaks with the JCPDS standard cards shows that all the available phases on the coating are in the XRD spectrum. The existing elements are calcium, phosphorus, oxygen, carbon, zirconium oxide, and titanium.

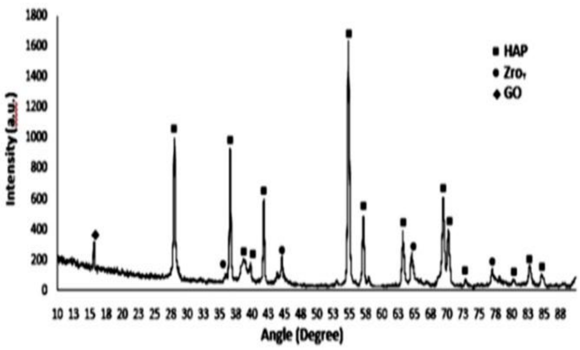

Figure 6. The X-ray diffraction pattern for the hydroxyapatite/graphene oxide/zirconium oxide composite

3.3. Evaluation of Coating Corrosion Resistance

Hadidi et al. in an article titled "synthesis of the hydroxyapatite/copper and hydroxyapatite/copper oxide nanocomposites by the electrophoretic method" concluded that hydroxyapatite -5% copper has the lower corrosion current density and as a result, the highest resistance to the corrosion in the hydroxyapatite/copper oxide coatings [11]. In addition, by increasing the copper oxide percentage in the coating, due to more nobility of the copper oxide, the coatings corrosion potential is more positive.

Evaluation of the two samples showed that in the Figure 7, by the impedance electrochemical tests, it can be concluded that corrosion resistance in the samples with the composite coating is significantly increased compared to the uncoated samples, and it can lead to the increase in the properties and higher efficiency of this type of coating.

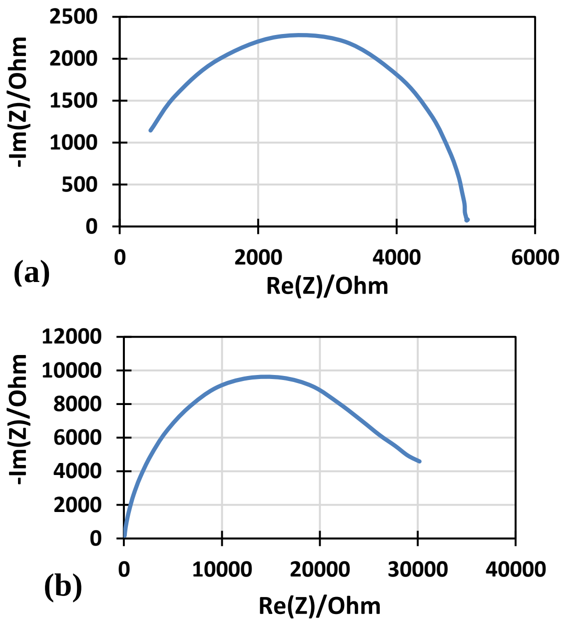

Figure 7. The impedance electrochemical test performed in the study (a) Ti-6Al-4V substrate without coating and (b) the coated sample

3.4. Antibacterial Behavior Evaluation

In the microbial discussion, the bacteria are generally formed on the composite coatings in the form of a biofilm. The formation of the bacteria can damage the coated surface and lead to penetration of the bacteria inside it, which would consequently lead to defections on the surgery point or breakage of the implant. Therefore, it is needed to combine new composite materials which have both bioactive and antibacterial properties. Numerous methods and research have been conducted by the scientists in the microbial fields and the ways to confront them (antibacterial). During the conducted studies, numerous composites such as the hydroxyapatite/graphene, hydroxyapatite/graphene/chitosan, etc. could not increase the antimicrobial property. With the research done by the researcher, a ceramic material named zirconium was added to the hydroxyapatite/graphene oxide composite, and then the results and images of the study were recorded.

Jancovich et al. in a study titled “characterization of the hydroxyapatite/chitosan nanocomposite and graphene oxide coatings by the electrophoretic method” concluded about the antibacterial property that by addition of the graphene oxide powder to the hydroxyapatite/chitosan compound, the number of the bacteria has been significantly reduced compared pure hydroxyapatite [12]. Based on this research, the results show that by addition of the zirconium oxide to the coating’s composite, the antibacterial properties can be improved.

Based on the results obtained, it was observed that the S.Areus bacteria count reached 2.45×10^{6} cfy/ml which means that it has been significantly decreased.

As observed in Figures of 8 and 9, the hydroxyapatite coating is susceptible to microbial contamination. Also, in terms of mechanical properties, it is too weak. The graphene oxide is added to the hydroxyapatite as a reinforcement. Both mechanically and biologically, it has been increased. By addition of the zirconium oxide, the antibacterial properties of the nanocomposite coating have been significantly increased and improved.

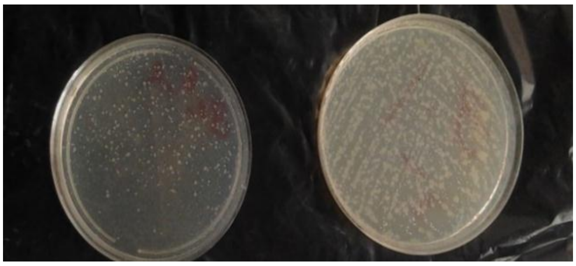

Figure 8. Results of the antibacterial activity tests of the composite coated sample (E. coli bacterial cells)

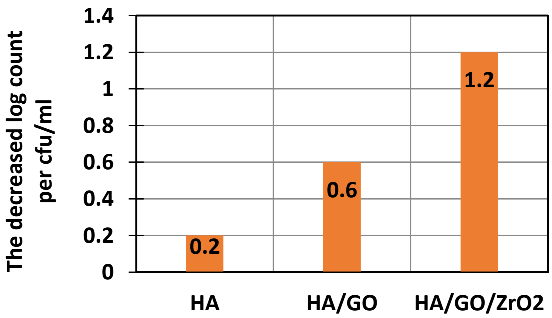

Figure 9. The effects of the use of zirconium oxide on the antimicrobial properties

4. CONCLUSION

The current study aimed to characterize the hydroxyapatite, zirconium oxide, and graphene oxide nanocomposite coating on the titanium substrate by the use of the electrophoretic deposition. The results indicated that this coating has a thickness of around 22.8-31.2 micrometers. Also, the substructure of the created coatings was uniform and free of microscopic cracks. All the elements of the composite coating were also visible. The impedance electrochemical test results also showed that the corrosion resistance of the samples with the mentioned composite coating has been significantly increased compared to the uncoated samples, and it can lead to the increase in the properties of this type of coating and higher efficiency of it. Also, all the peaks of the hydroxyapatite/zirconium oxide/graphene oxide composite elements are visible (carbon, calcium, zirconium, oxygen, phosphorus, hydrogen, and titanium). Finally, the results indicated that the effects of the creation of a hydroxyapatite/zirconium oxide/graphene oxide composite coating increase the antibacterial properties.

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