Functionally Graded Antibacterial Biomaterial Coatings for Titanium Base Alloy

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Abstract. Metal implants made of titanium and its alloys need surface modification operations because they are weak in terms of osseointegration and to protect them from corrosive body fluids and the growth of harmful bacteria that prevent them from performing their function and cause their failure. Hydroxyapatite and titanium dioxide have proven high efficiency by using them as biological and anti-bacterial coating. The basis of the current study is to deposit a functionally graded ceramic coating of titanium dioxide and hydroxyapatite on a Ti6Al4V substrate by electrophoretic deposition (EPD) method at room temperature with optimal conditions of 20 volts and a deposition time of 1 minute, and then sintering in an atmosphere of argon at a temperature of 950ºC for one hour. In addition, polarisation, open circuit, antibacterial activity, XRD, SEM, EDS was conducted. The laboratory results showed that the corrosion rate decreases for the coated samples compared to the uncoated. As for the SEM examination, the cross-section images showed that the gradient coating is dense, cohesive and free from microscopic cracks. Also, the surface images showed that the surface was uniform. In terms of XRD, the results showed the presence of rutile, anatase, and hydroxyapatite phases in addition to α_CaPO and β_TCP as a result of HAp decomposition. For the anti-bacterial examination, the findings revealed that the coating is anti-bacterial.

1. Introduction

Titanium alloys are broadly used nowadays as metallic biomaterials. They are characterised by good biocompatibility, high corrosion resistance, favourable fatigue strength and high strength to weight ratio. In particular, they are applied in orthopaedic and dental implants because their Young’s modules are relatively low among metallic materials [1,2]. Nevertheless, the development of passive oxides layers on titanium’s surface makes it inert in the human body but has weak osteoinductive properties [3,4]. In addition, biofilm formation on titanium implants, which has a decisive influence on the persistence of bacterial infections, is a significant problem [5]. Modifying the surface of titanium biomaterials by coatings allows their anti-bacterial properties, biocompatibility and electrochemical corrosion resistance to be improved [6,7].

Electrophoretic Deposition (EPD) could be defined as the deposition of charged colloidal particles, in stable solutions, on the substrates having opposite charges using DC electric fields [8]. EPD is deemed a simple, quick, and multi-purpose coating method that allows changing molecules or charged particles in solution and sequent combination on the electrode to create different bulk materials and coatings with highly homogenous microstructure and suitable thickness [9].
Nevertheless, high temperatures for the sintering process are required for this type of coating (EPD) to attend mechanical integrity and binding to the substrate. These temperatures on a metallic substrate, which are usually above >950°C, cause the undesired breakdown of HA into the Ca₃(PO₄)₂ phases [10]. The deposits of HAp nano-particles could be sintered at noticeably low temperatures in comparison with the counterparts of micro-particles. The layers of HAp microparticles could be placed from the stable (non-agglomerated) solution, which is synthesised utilising the micro-size starting powders. Owing to the difference between the thermal expansion coefficient of the sintering process and the substrate, the cracks of HAp coating could happen. The considerable structural and chemical properties variance leads to the difficulty of forming the chemical binding between the HAp coating and Ti-alloy substrate at the interface. Titania (TiO₂) is considered a bio-compatible and bio-active material as it is very efficient against bacterial infections, can resist corrosion and has good mechanical properties. Using the titania into the HAp coating could result in strong binding and high mechanical strength of the composite coating and improve the growth and efficiency of the cells against bacterial infections [10,11].

Functionally graded materials could be defined as a particular class of composite materials, as their changing composition and/or microstructure are controllable in the space [12]. In traditional (not graded) composites, the constituent phases are distributed in average uniform in space, compromising their special properties. Instead, the graded structure combines various materials and does not cause serious interior stress in addition to combining diverse properties in one material system. In other words, the desirable properties of the materials could be totally utilised without forming a sudden interface between macro-domains of heterogeneous composition [12,13], particularly the feasibility of a TiO₂-HAp graded coating (as shown in the literature, [14]) by utilising the plasma spraying technology.

In this study, HAp-titania functionally graded coating was synthesised by EPD in solutions with various contents of HAp and titania microparticles. The optimal amounts of powders were calculated by trial and error.

2. Materials and method

2.1. Coating process

In this investigation, Ti-6Al-4V substrate was used as a cathode and commercially available 316L stainless steel as an anode were soaked in the solution with a constant distance of 15 mm. Before the deposition, the grinding process of Ti-6Al-4V substrates was done by 180 to 3000 grit SiC papers; after that, the polishing process for the specimens was achieved using polish cloth and diamond paste 0.5μm. Then the specimens washing was done using distilled water and ultrasonic with acetone to remove the oxide layer; finally, the specimens were dried and kept in a dissection over a pad of silica gel and utilised for microstructure evolution and electrochemical examination.

The EPD process was performed in a glass beaker; magnetic stirring was applied to keep the dispersion of the homogeneous particles during the whole EPD process, as described in Figure 1. The system is equipped with a DC power supply that delivers potential ranging from zero to 60 volts. The voltage could be adjusted as needed to attend the optimal deposition. In order to obtain the best electrophoretic parameters, the deposition was carried out at various voltages (10, 20, 30, 40, 60 V) and several deposition periods (1, 2, 3, 4, 5, 6, 30 and 60 min) from a suspension containing (50 g/L) % TiO2 and 6 g/L PEI.

Gradient coating was done in five consecutive steps. Each step lasted 12 seconds for each layer. The total time was 1 min. The graduated coating was carried out from solution No. 1, 2, 3, 4 and 5 consecutively, as shown in Table 1. Firstly, the deposition of the internal TiO₂ layer was carried out on the Ti-6Al-4V substrate from solution No.1, then three intermediate layers, TiO₂/HAp composite layers, were utilised from their relatively stable solutions (No. 2 and No. 3 and No. 4) respectively. Finally, the upper coating of HAp was placed on the four prior layers from solution No.5; the amounts of HAp vary progressively from 0 % (weight percentage) near the substrates to 100 % (weight percentage) on the upper coating layer.
Careful removal for the coated samples from the EPD cell was done, followed by a drying process in the air at room temperature for 24 hours, and finally sintering process using argon furnace at 950°C for 1 hour. The adopted method in this research is presented in Figure 2, based on previous researches and trail mixes to produce a satisfactory coating.

![Garmenting processes of FG coating samples.](image)

**Table 1**: Chemical compositions of synthesised specimens.

| Solution No. | Layers | TiO₂ (g) | HAp (g) | Absolute Ethanol (ml) | PEI (g/L) |
|--------------|--------|----------|---------|-----------------------|-----------|
| No.1         | 1st    | 10       | 0       | 200                   | 6         |
| No.2         | 2nd    | 7        | 3       |                       |           |
| No.3         | 3rd    | 5        | 5       |                       |           |
| No.4         | 4th    | 3        | 7       |                       |           |
| No.5         | 5th    | 0        | 10      |                       |           |
2.2. Characterisation of the coatings

A scanning electron microscope (VEGA\ TESCAN) equipped with an energy dispersive spectrometer (EDS) was employed to analyse the coating’s microstructural characterisation and elemental composition. In addition, X-ray diffraction (XRD, Philips PW 1480) was also used to analyse Phase compositions of the coated surface.

A potentiodynamic polarisation test was performed in a potentiostat (EG&G 263 A) according to the ASTM G44–99 standard [15] to assess the corrosions resistance of a control (uncoated) and coated specimens, and the samples were immersed into the simulated body fluid Ringer solution electrolyte. A saturated silver/silver chloride electrode was employed as a reference, with a platinum counter electrode; specimens were inserted as a working electrode. The chemical compositions of the ringer solution are illustrated in Table 2. The pH Ringer solution at 37±2°C was 5. The electrochemical test was conducted at a temperature of 37±2°C for all specimens.

**Table 2:** Chemical compositions of ringer’s solution.

| Components                              | Concentrations (g.L⁻¹) | PH |
|-----------------------------------------|------------------------|----|
| Sodium chloride (NaCl)                  | 8.6                    | 5  |
| Potassium chloride (KCl)                | 0.3                    |    |
| Calcium chloride dehydrate (CaCl₂.2H₂O)| 0.33                   |    |
In this study, Gram-positive Escherichia coli (E.coli, ATCC8739) was considered as model cariogenic bacteria to evaluate the anti-bacterial properties of the developed surfaces. The anti-bacterial activity was studied by using the inhibition zone method. This method involves spreading about 1 million cells of a single strain over an agar plate by a sterile swab after that, incubating them in the presence of the suspension. If the strain of bacteria or fungi shows susceptibility to the suspension, then the inhibition zone will appear in the agar plate.

3. Test Results and Discussion

3.1. X-Ray Diffraction Test Result
The XRD pattern of sintered HAp/TiO$_2$ functionally graded coating in argon conditions at 950 °C for 1 hr is given in Figure 3. The pattern proves the presence of HAp and rutile/anatase phases of TiO$_2$ (JCPDS No. 4-0551 and JCPDS No. 21-1272) in the coating. As the phase conversion of anatase to rutile happened at the temperatures range between 400-1000°C (which relays on the microstructural properties, impurity and particles size of anatase particle), the anatase TiO$_2$ in the deposited functionally graded coating begins to transform to rutile during the sintering process and the gained XRD patterns after sintering show the identical reflections of rutile phase.

![Figure 3: XRD pattern for the functionally graded HAp/TiO$_2$ coating specimen.](image)

3.2. Scanning Electron Microscopy Analysis
The cross-sectional image of the HAp/TiO$_2$ functionally graded coating, Figure 4, shows its five layers as TiO$_2$ buried layer is close to the substrate, HAp upper layer and three HAp/TiO$_2$ graded layers in the middle. As could be noticed, the introduced intermediate layer of TiO$_2$ and the graded compositions contributes to improving the adherence and connection of the coatings. The total thickness of the FGC HAp/TiO$_2$ was calculated at about 173.0 μm. The TiO$_2$ layer is a portion of the total coating thickness. As the major purpose of the TiO$_2$ layer is to enhance HAp adherence. Figure 5 shows that the morphology of functionally graded coating, including different layers of HAp layer, TiO$_2$ layer, HAp/TiO$_2$ intermediate graded layers that were deposited at the deposition 20V for 1 minute.
Figure 4: Schematic of SEM cross-section image for functionally graded sample.

A smooth, dense (microstructures with low void ratio), good homogenous surface with fewer pores in structure, no cracks and high uniformity in the coating were attended. The roughness and high porosity of the coating surface make nucleations and osseointegration of bone accumulated easily in forming the bone-producing cells.

Figure 5: SEM micrograph of the surface morphology of functionally graded coating on Ti6Al4V alloy.

3.3. Electrochemical Corrosion
The potentiodynamic polarisation curves of base Ti6Al4V, HAp, TiO₂ and FGC samples in the SBF (Ringer’s suspension) are described in Figure 6. The metal’s corrosion properties are mainly reported in the polarisation curve; this curve is usually utilised in studies of electrochemical corrosion. Higher corrosion resistance can be attended as a higher possibility of free corrosion, and a smaller current passivation yield was achieved.
The corrosion parameters gained from the polarisation curve applying the Tafel least square fitting method are in Table 3.

![Graph showing potentiodynamic polarisation curves of coated and uncoated samples in the ringer’s solution.](image)

**Figure 6:** Potentiodynamic polarisation curves of coated and uncoated samples in the ringer’s solution.

**Table 3:** Electrochemical parameters of Ti6Al4V substrate and coated samples in different media.

| Media          | Sample | \(E_{\text{corr}}\) (V) | \(I_{\text{corr}}\) (\(\mu\text{A/cm}^2\)) | CR (mpy) | \(R_p\times(10^{-3})\) |
|----------------|--------|--------------------------|--------------------------------|----------|--------------------------|
| Ringer's Solution | Base   | -0.84                    | 20.09                          | 0.176    | 1.29                     |
|                | HA     | -0.29                    | 4.00                           | 0.035    | 6.50                     |
|                | TiO\(_2\) | -0.26                    | 3.18                           | 0.027    | 8.17                     |
|                | FGC    | -0.23                    | 2.52                           | 0.022    | 9.90                     |

3.4. *Anti-bacterial Study*

The anti-bacterial effect of FGC HAp/TiO\(_2\) and uncoated specimens were investigated against E. coli culture, as shown in Figure 7. The formation of a clear region around the disc refers to the bacterial inhibition zone. It can be observed that each case exhibited a good anti-bacterial effect towards three types of bacteria after 24 h of the incubation period, but the uncoated specimen has a small inhibition zone compared with the HAp/TiO\(_2\) one. This is attributed to the anti-bacterial activity of the anatase TiO\(_2\) itself [16], and therefore a strong anti-bacterial effect of the composite particles is obtained. Therefore, the presence of TiO\(_2\) particles in the HAp coating increased its effectiveness in preventing the adhesion of bacteria on the surface, which prevented bacterial growth and would improve anti-bacterial activity.

![Image of anti-bacterial test results](image)

**Figure 7:** Images of the anti-bacterial tests of HAp/TiO\(_2\) against E. coli.
4. Conclusions
The following points were obtained from this study:

1. The XRD analysis revealed the desired phases (hydroxyapatite and TiO2 (rutile and anatase)) needed in the coating applying synthesised suspension and heating remediation at 950°C. In addition to some bio-phases resulting from the decomposition of hydroxyapatite at sintering temperature.
2. The added amount of PEI to the HAp/TiO2 EPD bath led to more stability in suspension and deposition of uniform cracks free coating.
3. SEM images approved good adherence and cohesion of gradient coatings.
4. HAp/TiO2 functionally graded coating offers protective effects and could inhibit releasing the ions of toxic metals from Ti6Al4V alloy in physiological environments. The levels of released ions were increased by decreasing the pH value of the solution.
5. The obtained coating could be effectively utilised to alter the surface of Ti6Al4V implants to enhance the biological properties and anti-bacterial activity.

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