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Identify in Vitro Behaviour of Composite Coating Hydroxyapatite-Nano Silver on Titanium Substrate by Electrophoretic Technic for Biomedical Applications

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ABSTRACT

To develop Titanium implants that are biocompatible, non-toxic and exhibit antibacterial properties, it is imperative to apply biomaterial coatings that meet the stringent standards required for biomedical applications. Hydroxyapatite (HA) is extensively used as a coating for bone implants due to its exceptional biocompatibility. However, Titanium's vulnerability to corrosion and bacterial colonization within the physiological environment poses significant challenges that can undermine the long-term success of implants. This study explores the in vitro behaviour of a composite coating composed of hydroxyapatite (HA) and varying concentrations of nano-silver (0, 10, 20, 30 and 40 wt.%) on a Titanium substrate, utilizing the electrophoretic deposition (EPD) technique. Given Titanium's prevalent use in orthopaedic and dental implants for its favourable mechanical properties and biocompatibility, enhancing its surface characteristics is essential for implant longevity. The HA-nano silver composite coating is designed to augment Titanium's surface by improving its resistance to corrosion, enhancing biocompatibility and providing antibacterial protection. The coatings were applied at 30V for 30 minutes and their crystallinity, morphology and microstructure were systematically characterized using X-ray diffraction (XRD), Scanning Electron Microscopy (SEM) and energy dispersive spectrometer (EDS). Corrosion resistance was evaluated through potentiodynamic polarization in simulated body fluid (SBF). The findings indicate that the HA-nano silver coating markedly enhances the corrosion resistance of Titanium, with the 10% silver coating showing a significant reduction in corrosion current density. These results underscore the potential of HA-nano silver coatings in advancing the performance of Titanium-based surgical instruments, particularly in improving both biocompatibility and corrosion resistance.

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1. Introduction

The global incidence of implant surgeries, including those involving artificial joints, exceeds hundreds of thousands. Consequently, the prevention of implant rejection assumes significant relevance [1,2]. It is anticipated that a permanent implant inside the human body would establish a physical connection with the bone tissue within a few timeframe [3]. To achieve optimal adhesion between the bone tissue and permanent implant, it is necessary to fill the interfacial area between the implant and bone with endogenously synthesized bone tissue [4]. Due to their limited bioactivity, metallic implants need surface modification via the application of a bioactive coating in order to effectively interact with bone-forming cells [5]. Hydroxyapatite has garnered significant interest within the field of bio-ceramics owing to its notable bioactivity, osteoinductive properties and osteoconducting capabilities, making it a promising candidate for use as a coating for permanent implants [6,7]. Nevertheless, hydroxyapatite coatings are plagued by two issues. Initially, it should be noted that hydroxyapatite does not possess inherent high mechanical toughness or strength [7-10]. One potential approach to enhance the mechanical characteristics of hydroxyapatite coatings is the incorporation of bio-ceramics, such as titanium oxide, zirconium oxide and carbon nanomaterials like carbon nanotubes [11]. The second concern is the many coating processes used, including electrophoretic deposition [12], electrodeposition [13], magnetron sputtering [14], cold spray [15] and sol-gel technologies [16].

Electrophoretic deposition (EPD) is a coating technique widely utilized in many applications, including intricate microstructures and functionally graded nanostructures. The benefits derived from the EPD were significant, leading to widespread adoption of the approach since its simplicity, ease of use and scalability. Significant benefits include cost-effectiveness, adaptable microstructure, precise control over intricate forms and even dispersion of particles [17]. The technique involves the movement of charged particles toward an electrode in an electrical field to accomplish deposition by particle agglomeration. The EPD process often requires adequate heat treatment to improve mechanical characteristics. The factors, including voltage and duration, are crucial in the EPD process and are typically maintained consistently [18].

Many researchers try to improve implements and metallic biomedical applications [19], a composite coating comprising silver, fluoride and hydroxyapatite (Ag-FHA) was developed on a Titanium substrate using the sol-gel method. The precursors used included triethylphosphite, hydrated calcium nitrate, ammonium fluoride and silver nitrate, serving as sources of phosphorus, calcium, fluoride and silver, respectively. The coating was formulated with a Ca:P ratio of 1.67 and a silver concentration of 0.3 wt%. The characterization of the coatings was performed using Fourier transform infrared spectroscopy (FTIR), XRD, field-emission scanning electron microscopy (FE-SEM) and atomic absorption spectrometry (AAS). Potentiodynamic polarization measurements in simulated body fluid demonstrated that the Ag-FHA coatings provided effective corrosion protection. Additionally, the antibacterial activity of the coatings against Escherichia coli was significantly enhanced with increasing fluoride content. The findings suggest that these Ag-FHA coatings hold the potential for preventing bacterial infections in implants.

Olgun *et al.,* [20] used the atmospheric plasma spray technique to deposit nanosilver particles onto hydroxyapatite (HAP) microparticles. Subsequently, the resulting nanoAg-HAP particles were coated onto the surface of Ti metal. Subsequently, the nanoAg-HAP coating developed on the Ti substrate underwent annealing at a temperature of 700°C. The antimicrobial efficacy of the nanoAg-HAP coatings on Titanium (Ti) was also evaluated against various microorganisms. A comprehensive analysis of the nanoAg-HAP particles was conducted utilizing several analytical techniques, including optic microscopy, FE-SEM, HR-TEM, XRD and UV-vis absorption spectroscopy. The nanoAg particles

displayed crystalline spherical structures, characterised by particle sizes less than 30 nm. The experiments conducted using FE-SEM-EDS revealed a uniform distribution of nanoAg particles on the HAP microparticles, with Ag concentrations ranging from 0.153% to 0.313%. The electron transmission (HR-TEM) tests revealed that the nanoAg particles exhibit a hexagonal tight packing (hcp) crystal structure, characterized by d-spacing magnitudes of 0.23 nm (111), 0.21 nm (002) and 0.14 nm (022). Following the application of heat treatment at a temperature of 700°C, the nanoAg-HAP coating that was produced on Ti metal exhibited complete antibacterial efficacy against both E. coli and S. epidermidis bacteria. Consequently, the nanoAg-HAP coated Ti metal may be efficiently used as a self-disinfecting biomedical implant material for orthopaedic bone repair of external anatomical structures.

Göncü *et al.,* [21] used EPD to apply a nano hydroxyapatite-nano hexagonal boron nitride (nano HA-nano hBN) composite onto commercially available pure Titanium specimens. A systematic investigation was conducted to examine the impact of process parameters, namely applied voltage, deposition time and solid amount, on the morphology, thickness and adhesion behaviour of the coating. The experimental results demonstrated the successful production of crack-free nano hBN-nano HA composite coatings. These coatings were utilized in the development of bioactive coatings for orthopaedic applications on Titanium substrates. To investigate the structural and morphological properties of the coated surfaces, a range of complimentary analytical techniques were used. For structural characterization, XRD and Raman spectroscopy had been utilized. Additionally, morphological characterization was conducted using SEM coupled with EDS and Transmission Electron Microscopy (TEM) methods. The experimental findings demonstrated the effective deposition of nano HA-nano hBN on the Ti surface using EPD, resulting in a uniform and crack-free coating. The present study suggests that the concentrations of hBN in suspension do not have any influence on the thickness of the coating. The introduction of hBN into HA did not result in any alterations to the morphology of HA and the presence of hBN did not have a substantial influence on the porous structure. The anticipated characteristics of these nanostructured surfaces include their capacity to facilitate cell proliferation and their significant potential for the development of bioactive materials.

The current study aims to improve the corrosion resistance and biocompatibility of a Titanium substrate by applying a composite coating composed of hydroxyapatite and nano-silver. This coating is deposited onto the Titanium substrate using the EPD technique.

2. Experimental Part

Nano hydroxyapatite (Shanghai Hualan Chemical Technology Co., Ltd; Chain; mean particle size 20 nm, 96% purity) and nano silver particles (Hongwu International Group Ltd; Chain; mean particle size 20 nm, 99.99% purity), nitric acid (69-72%, Central Drag House, Daryaganj, New Delhi-110002, India) and hydrochloric acid (35-38%, Thomas Baker Chemicals Pvt Ltd (Factory), B3 & B4, Midc, Chemical Zone, Ambernath 421501, India) were utilized to produce coatings on Titanium plates as shown in Figure 1.

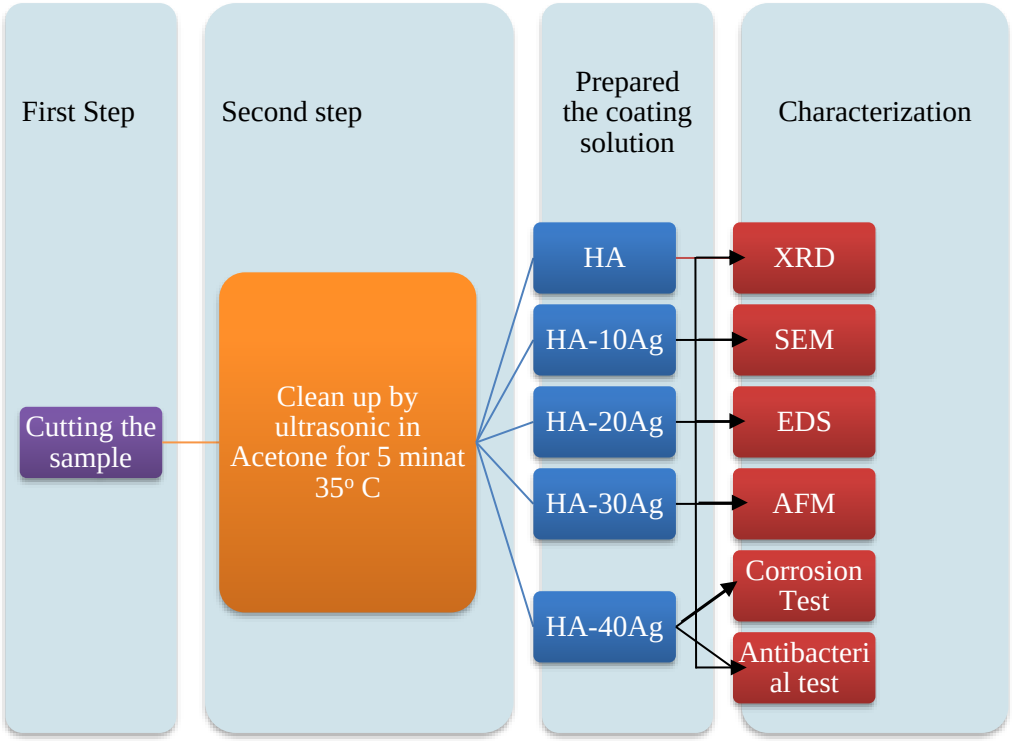


Fig. 1. Working methodology flowchart

The chemical composition of the Titanium substrate is listed in Table 1. Ethanol (Scharlab S.L., Spain) was applied as the suspension medium. Acetic acid (Hopkin and Williams, Britain) has been added to suspensions to enhance particle charging as a dispersion material.

Table 1
The chemical composition of Titanium plate

Elements	V	Cu	Mn	Mg	Ni	Mo	Fe	Zr	Ti
Percent%	0.06	0.01	0.01	0.04	0.01	0.04	0.05	0.05	Rem.

Suspensions for electrophoretic experiments have been produced by ultrasonic agitation of HA powders (synthesized in our laboratory) in ethanol. A minimal amount of 1 M HCl and HNO_3 was added to the ethanol alcohol to conduct stable suspensions and gain the desired electrophoretic mobility level. The suspensions were sealed in glass bottles and aged for various periods ranging from 1 to 30 days.

The TP120-5S power supply was utilized as a DC source in the EPD process. The electrophoretic cell (shown in Figure 2) consisted of a 50 ml cylindrical glass beaker and Titanium plates measuring 2.5×1×0.1 cm were utilized as electrodes. The separation between the two electrodes was 1.5 cm. The solid was added 1wt% percent to the ethanol solution. Five groups of hydroxyapatites coated on Titanium plates were prepared with (0, 10, 20, 30 and 40) wt% silver addition. During the 30-minute procedure, a voltage of 30V is supplied, resulting in the deposition of particles on the cathode electrode. The coated specimens were sintered at 450°C for 1 hour and then heated to 800°C for 3 hours in a vacuum furnace using Argon gas. XRD and potentiodynamic polarization experiments were conducted on both uncoated and hydroxyapatite-nanosilver (HAP-nAg) coated specimens in simulated bodily fluid at a pH of 7.4 and a temp of 37±1°C. The breakdown potential (Eb) and corrosion potential (Ecorr) were determined from the polarization curves.

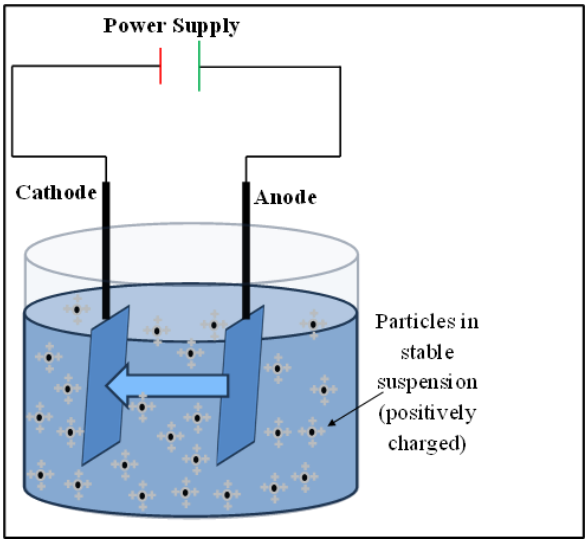


Fig. 2. ElectroPheritic deposition cell

2.1 Tests

2.1.1 X-ray diffraction (XRD)

X-ray diffraction (XRD) techniques define the presence phases in Titanium before coating and after EPD with hydroxyapatite (0, 10, 20, 30 and 40), wt. % silver. The "SHIMADZU Lab X XRD-6000", Japan. X-ray with a nickel filter and generator copper Kα radiation (λ=1.5406). The scanning speed of the diffract meter was adjusted to 60 per minute with a diffraction angle range 2θº was (0-80) º. The resulting peaks are then compared to standard peaks for each phase appearing in the Titanium alloy and phases appearing since surface modifications.

2.1.2 Scan electrons microscopy/energy dispersive spectroscopy (SEM/EDS)

SEM offers topographical and elemental data at magnifications ranging from 10x to 300,000x with an almost infinite depth of focus. Scanning electron microscopy (SEM) provides higher-quality pictures with less distortion and improved spatial resolution of up to 1.5 nanometres, three to six times superior to conventional SEM. This test utilized specimens of Titanium coated with hydroxyapatite with varying percentages of silver (0, 10, 20, 30 and 40) wt.

2.1.3 Atomic force microscopy

The deposition layer's surface image feature and nano roughness are observed using the atomic force microscope (AFM). AFM is a beneficial tool for detecting the high-resolution topography of coated films. This technique is very effective for characterizing the material on the nanoscale. The kind of AFM was (SPM AA3000, Angstrom Advanced Inc., USA). Titanium coating samples with hydroxyapatite (0, 10, 20, 30 and 40) wt. % silver, tested.

2.1.4 Electrochemical test

Studying the electrochemical corrosion tests for the specimens involved conducting polarization tests (potentiodynamic) before and after surface modifications. These tests are conducted in "Ringer's solution" with the chemical composition provided in Table 2. The test uses

potentiostats/Galvanostats from MLab Bank Elektronik in Germany. There are three electrodes in the corrosion cells:

i. The electrode can work with "Titanium the specimen or coated specimens".
ii. The counter electrode (platinum rod).
iii. The saturated calomel electrode (SCE). The surface area subjected was 1.767 cm2 for the electrode utilized for every specimen.

Table 2
Simulated body fluid solution chemical ingredients

Components	Amount (M)
NaCl	0.137
KCl	0.027
$CaCl_2.2H_2O$	0.014
$MgCl_2.6H_2O$	0.003
KH_2PO_4	0.05
$NaHCO_3$	0.05
pH	7.4

The corrosion rate is calculated using the equation below [22]

$$CR\ (mpy) = 0.13 \text{icorr (E.W.) A.}\rho \quad (1)$$

E. W. = equivalent weights (g/ eq) A = areas (cm^2)
ρ = density's (g/ cm^3)
0.13 = metric and duration conversion parameter
icorr. = The current density (µA / cm^2)

Potentiodynamic Polarization, the corrosion behaviour of Titanium, was studied here both before and after the surface changes (samples of Titanium coating with hydroxyapatite of (0, 10, 20, 30 and 40) wt. % silver) were made by using an electrochemical cell (three-electrode Polarization experiments were carried out using a potentiate of the kind "Winking M Lab 200." The potentiodynamic polarization curves were drawn. Tafel plots were utilized to quantify the potential of corrosion (Ecorr) and the current density of corrosion (Icorr) using both anodal and cathodic polarization branches. The experiments were performed at 37 ±1 °C in a "Simulated Body Fluid solution".

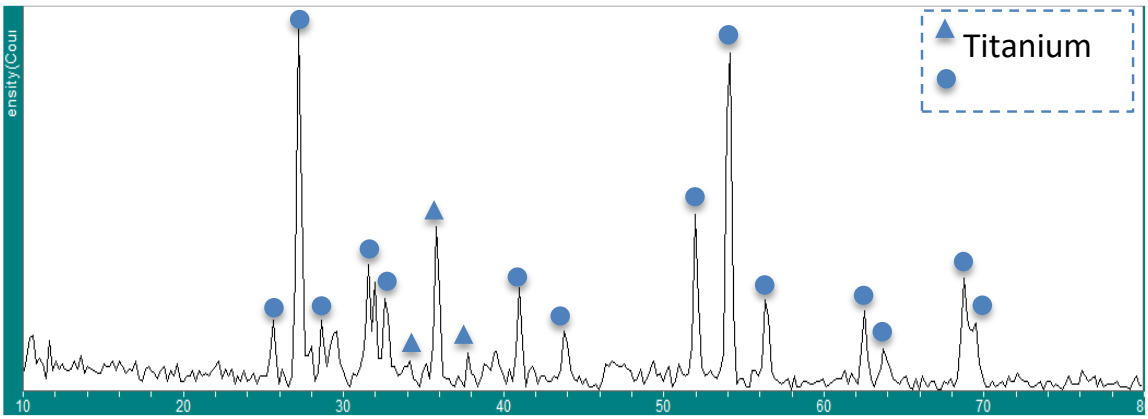
2.1.5 Anti-bacterial test

Microbial colonization and bacterial assault may occur on the surface of biomaterial. An investigation was conducted to assess the anti-bacterial properties of the surface layer of modified Titanium to determine its anti-bacterial efficacy. The inhibition zone approach assesses anti-bacterial activity. An anti-bacterial kinetic test is conducted using the bacterial strain "E. coli" (Escherichia coli, American Kind Culture Collection 5922), a gram-negative bacterium, at the College of Girls Science, University of Babylon. E. coli bacteria were cultured on nutrient agar and incubated for 24 hours. The bacteria were then distributed on a petri dish and kept at 37 degrees Celsius for another 24 hours. Subsequently, specimens were put in three distinct places on the petri dish and the inhibitory zones were detected.

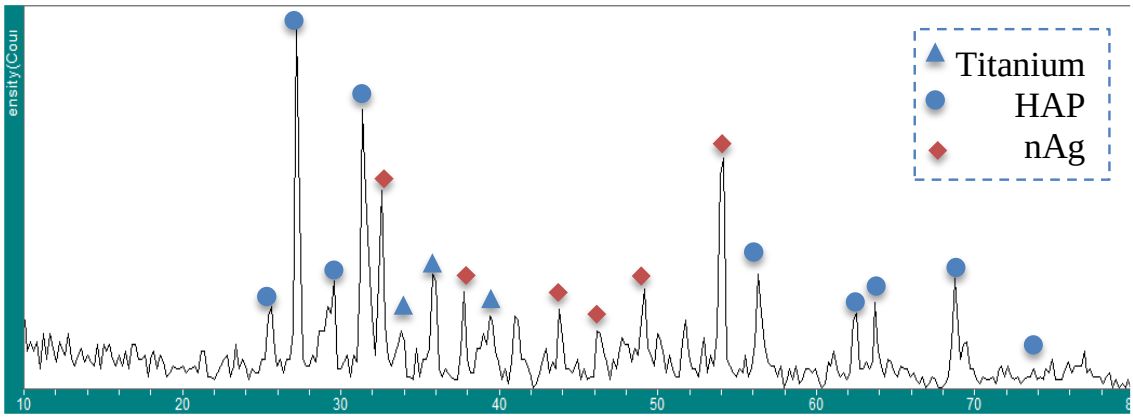
3. Results and Discussion

3.1 XRD Analyses

The level of crystallinity affects the dissolution and biological response of hydroxyapatite (HAP) coatings. Prior research indicates that a covering with a highly crystalline structure results in reduced dissolving [23]. Figure 3 displays the XRD pattern for a Titanium specimen. The displayed patterns are compared to the standard patterns for the specified alloy. The specimens were coated electrophoretically at 30 V for 30 minutes with hydroxyapatite containing varying percentages of silver (0, 10, 20, 30 and 40) g/l. The specimens were then sintered in a vacuum furnace at 450 degrees Celsius for 1 hour, followed by an increase to 800 degrees Celsius for 3 hours in a vacuum furnace with Argon gas, as depicted in Figure 3(b) to 3(f) [24]. These patterns exhibit similarities in terms of the coating crystallographic structure. The XRD patterns include diffraction peaks with minimum line broadening and high intensities, reflecting the highly crystalline and stoichiometric hydroxyapatite (HAP). No further $Ca_3(PO_4)_2$ phases were detected. The prominent peaks in the XRD patterns are attributed to reflections from certain crystallographic planes of hydroxyapatite (HAP), as identified by JCPDS file #09-0432: (002), (211), (112), (300), (202), (222) and (213). A strong peak at around 26° indicates the preferred crystal orientation of hydroxyapatite (HAP) along the [002] direction, as prior studies on HAP coatings described. Other crystalline phases, such as tricalcium phosphate, are not found [25].



(a)



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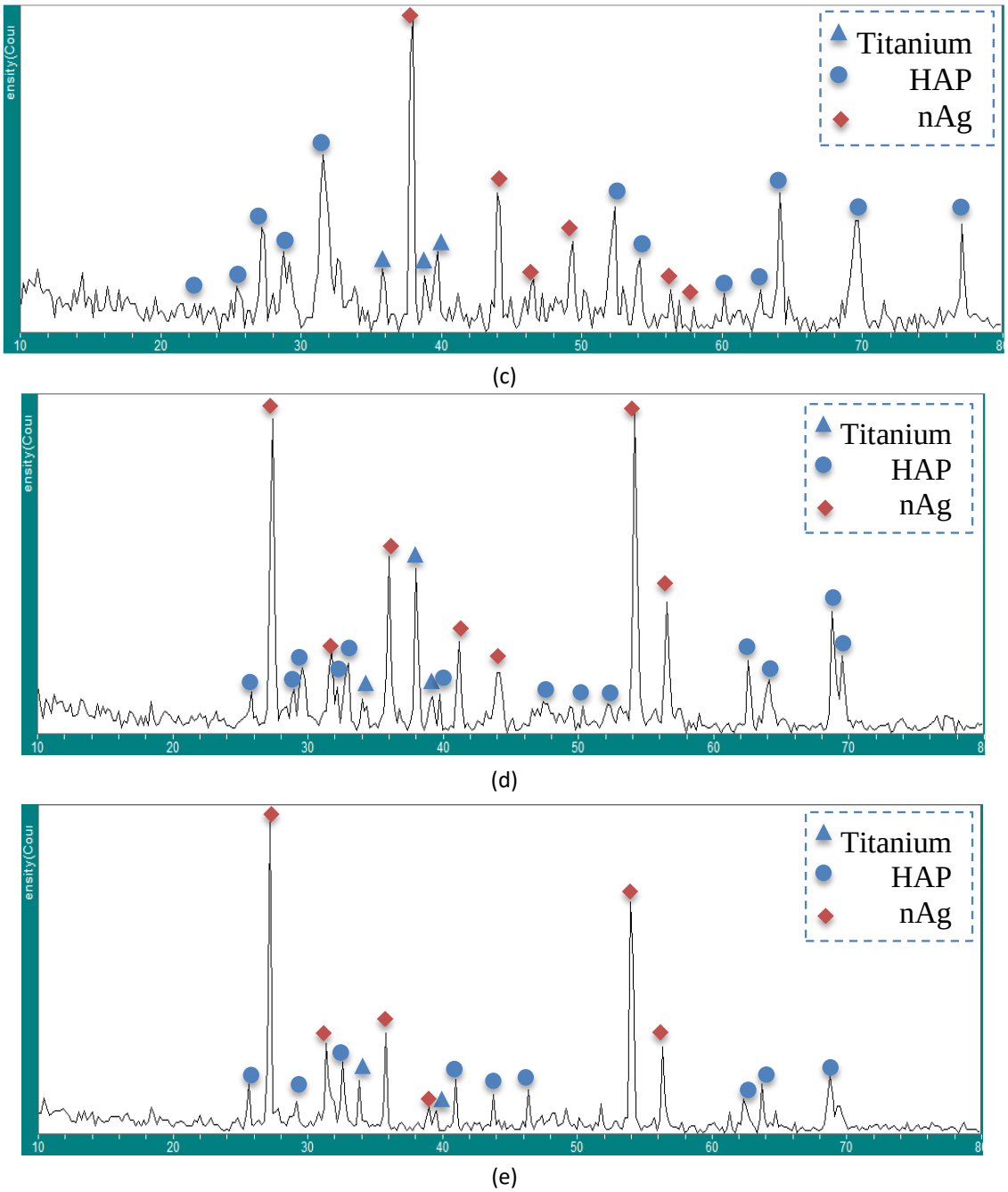


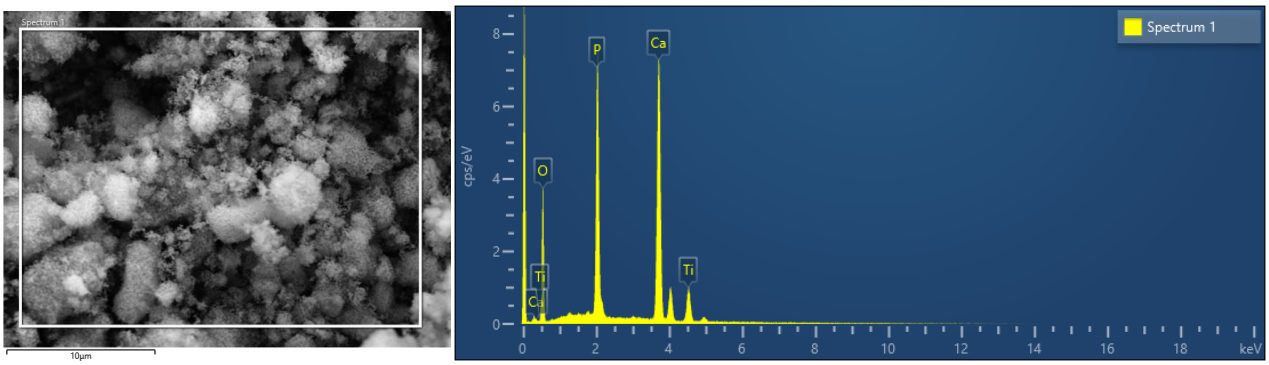
Fig. 3. XRD pattern for utilized samples: (a) Titanium coated by HAP (b) Titanium coated by HAP-10%nAg (c) Titanium coated by HAP-20% nAg (d) Titanium coated by HAP-30%nAg (e) Titanium coated by HAP-40%nAg

3.2 SEM Results

Figure 4 shows the surface morphologies of the coated specimens produced using EPD. Figure 4(a) to 4(d) displays a homogeneous distribution of particles, suggesting that the coatings are compact and microporous. No cracks in the coating indicate that there was little shrinking of the coating. This finding validates the convenience of using the electrophoretic processing method to produce dense specimens post-sintering. The coating comprises a network of evenly distributed big granules, each made of smaller equiaxed nanometric crystals as seen using atomic force microscopy, showing uniformity and absence of fissures. The findings confirm the prior XRD data, showing the

presence of solely the stoichiometric HAP phase and silver. The EDS measurements of the ECD HAP align well with the FDA recommendations for a Ca/P ratio of 1.67 to 1.76 and a max of 50 ppm heavy metals in the coating—measurements of open circuit potential over duration. Figure 4(a) displays the open circuit potential (OCP)–duration plots for both uncoated and hydroxyapatite (HAP) coated Titanium samples, which were prepared using EPD with 30 volts for 30 minutes. The uncoated specimen's open circuit potential (OCP) decreases in the active direction until it almost reaches a stable state potential.

On the other hand, the open circuit potential (OCP) of all hydroxyapatites (HAP) coated specimens gradually shifts towards a more noble potential over duration, reaching a stable condition rather quickly, suggesting that the coatings remained undamaged. The specimen coated for 30 minutes reached a steady state in a much shorter duration [26], which may result from a rise in the weight of the covering and its porous characteristics. The exemplary conduct of the specimen coated at 30 V for 30 minutes might be ascribed to the consistent behaviour of the coated surface.



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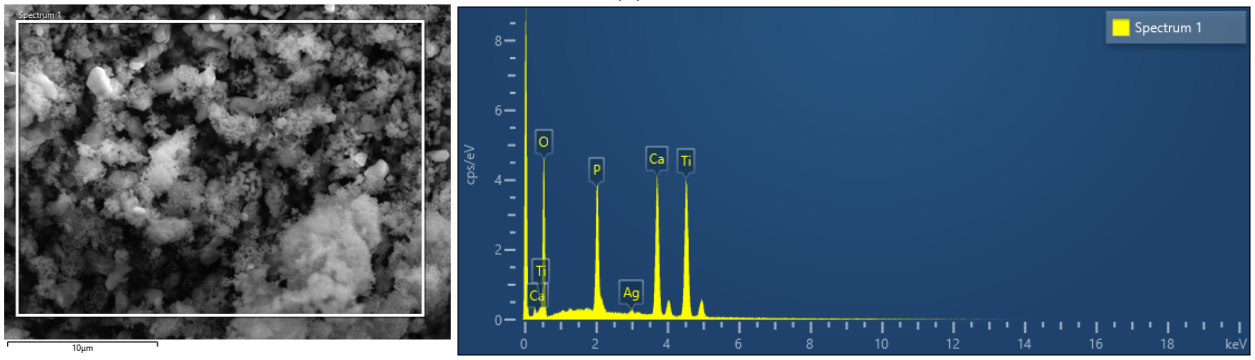

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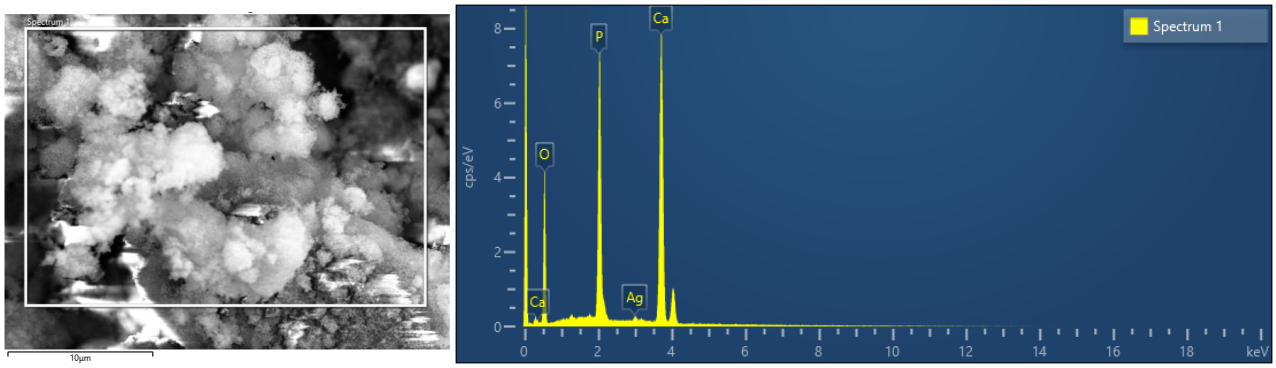

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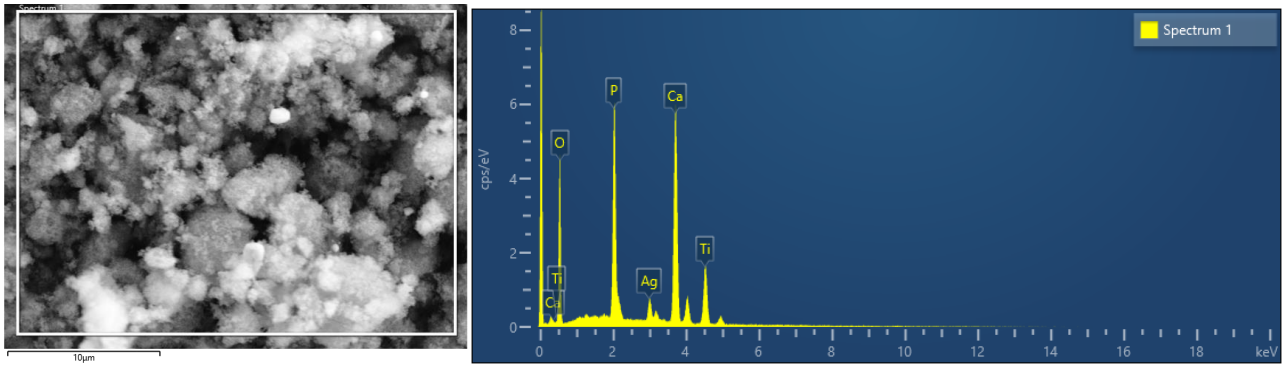
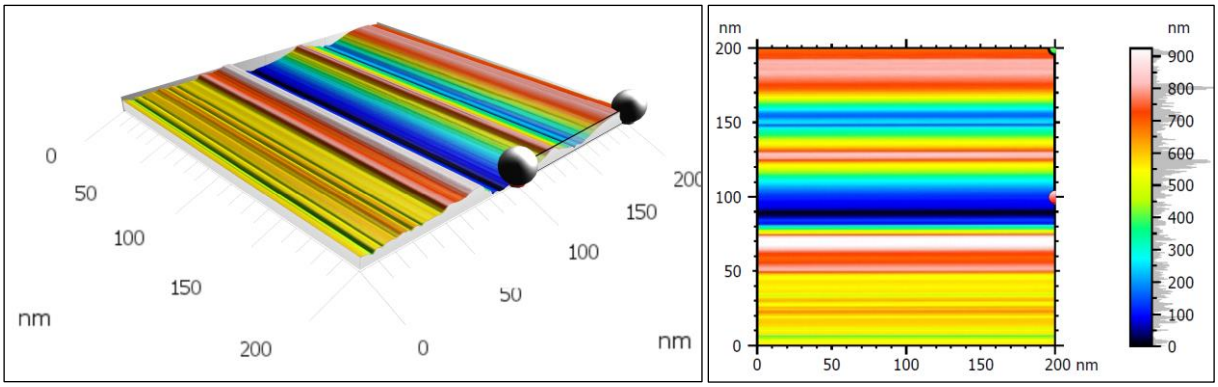


(d)

Fig. 4. SEM and EDS for utilized samples: (a) Titanium coated by HAP (b) Titanium coated by HAP-10%nAg (c) Titanium coated by HAP-20% nAg (d) Titanium coated by HAP-30%nAg

3.3 AFM

Figure 5 illustrates the surface roughness analysis of the Titanium coated by HAP only and HAP with (0, 10, 20, 30 and 40) g/l nano-Ag. The surface morphologies of silver-substituted films indicate high-quality thick films with a relatively large surface area, which is attributed to the more significant amount of silver.



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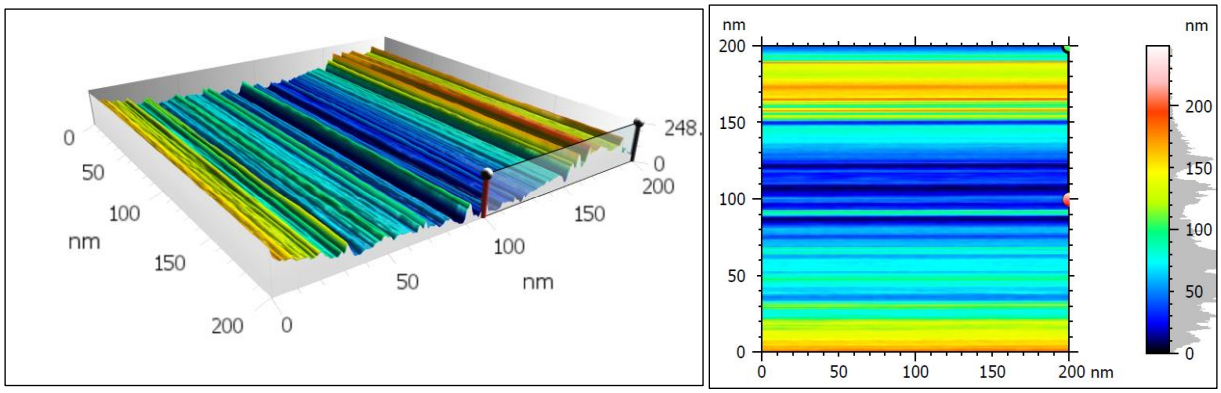

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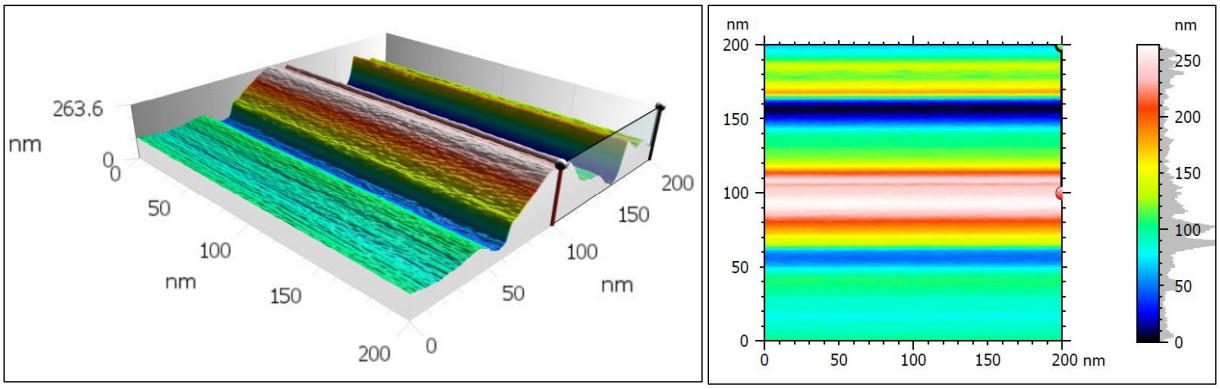

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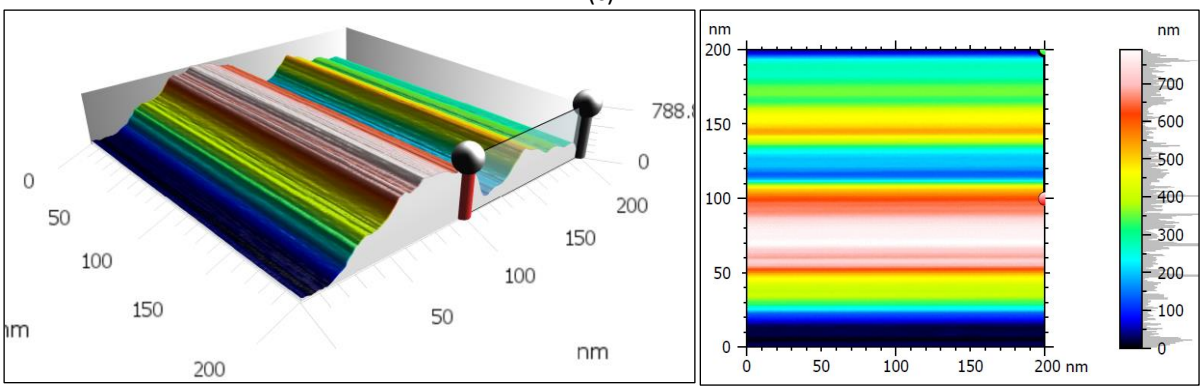

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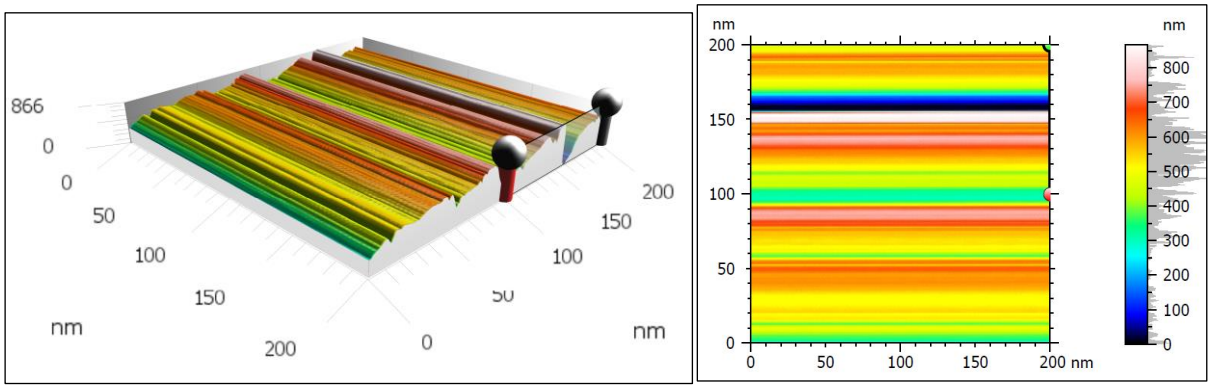

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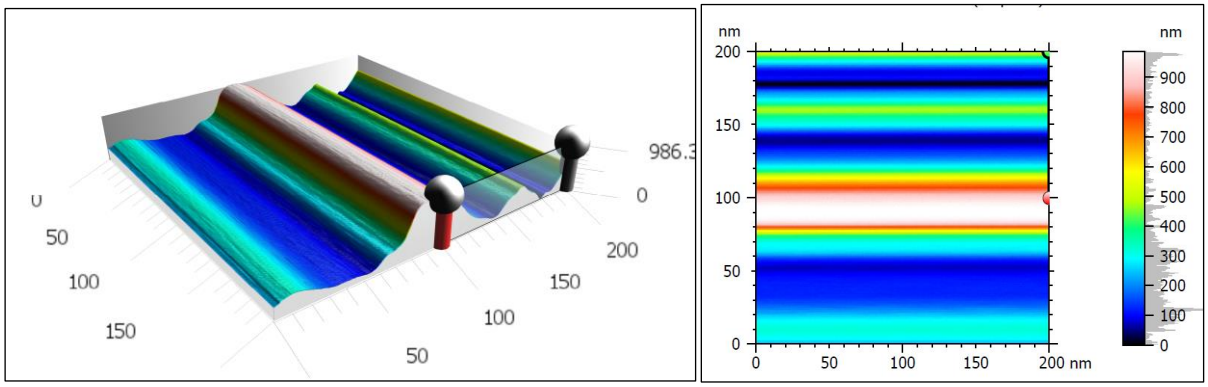


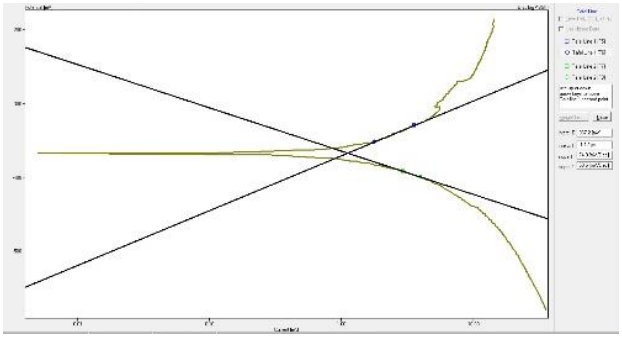
(f)

Fig. 5. AFM for utilized specimens: (a) Titanium (b) Titanium coated by HAP (c) Titanium coated by HAP-10%nAg (d) Titanium coated by HAP-20% nAg (e) Titanium coated by HAP-30%nAg (f) Titanium coated by HAP-40%nAg

3.4 Linear Polarization Results

To sum up, achieving stoichiometric HAP coatings is possible through EPD processes. When electrochemical deposition is utilized to create hydroxyapatite coatings on Titanium, the choice of applied potential and deposition duration has a crucial function in the development of the coatings. The coating weight and thickness rose as the applied potential and deposition period increased [27]. Increasing the weight of the coating on the HAP may not be ideal, as it can lead to more noticeable delamination from the substrate. Lab tests indicate a change in the OCP and pitting potential magnitudes favouring the HAP-coated specimens over the uncoated Titanium. Redepenning *et al.,* [32] have documented a process for electrodepositing HAP that consists of two main steps: the initial $Ca_3(PO_4)_2$ apatite mineral formation on the surface of the metal, followed by the continuous growth of the mineral layer through precipitation with constant composition. Throughout this procedure, water electrolysis causes a localized rise in pH at the cathode surface since the creation of OH^- and H_2. When present in a stable $Ca_3(PO_4)_2$ solution supersaturated with apatite, the pH increases, resulting in higher $(PO_4)^{3-}$ concentration, causing further $Ca_3(PO_4)_2$ salt supersaturation and resulting in uniform precipitation. It is unlikely that the $Ca_3(PO_4)_2$ is nucleated on the metallic surface. On the other hand, precipitation could occur in the solution and the mineral particles move to the metal surface through gravity and electrophoretic attraction. This process will lead to a relatively consistent mean spherules size over duration, as demonstrated in this study.

Nevertheless, as the bulk solution remained clear throughout the deposition, it can be inferred that the $Ca_3(PO_4)_2$ precipitation did not occur far from the metal's surface. To enhance the HAP coatings quality and optimize their formation k, gaining a deeper insight into the reactions of electrochemical occurring at various pH levels and possible magnitude is essential. The findings suggest that coatings obtained by EPD could be a promising option for enhancing implant device corrosion resistance and biocompatibility. The polarization diagrams of all samples and the Ecorr. (mV), I corr. (µA) are shown in Figure 6.



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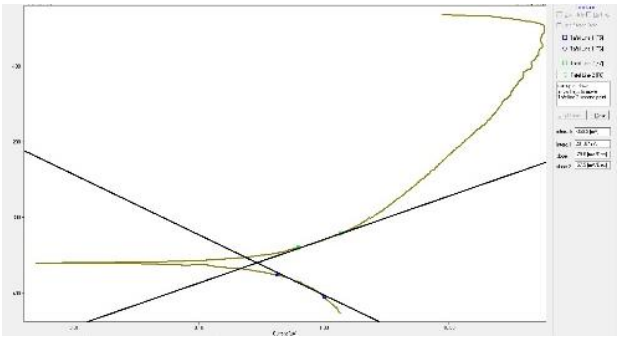

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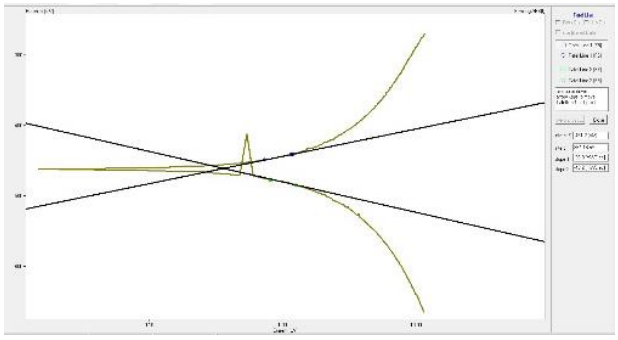
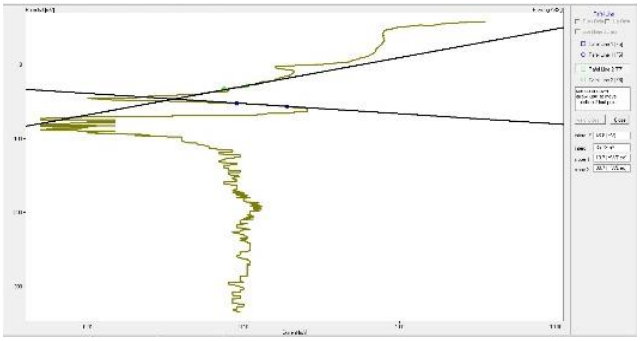
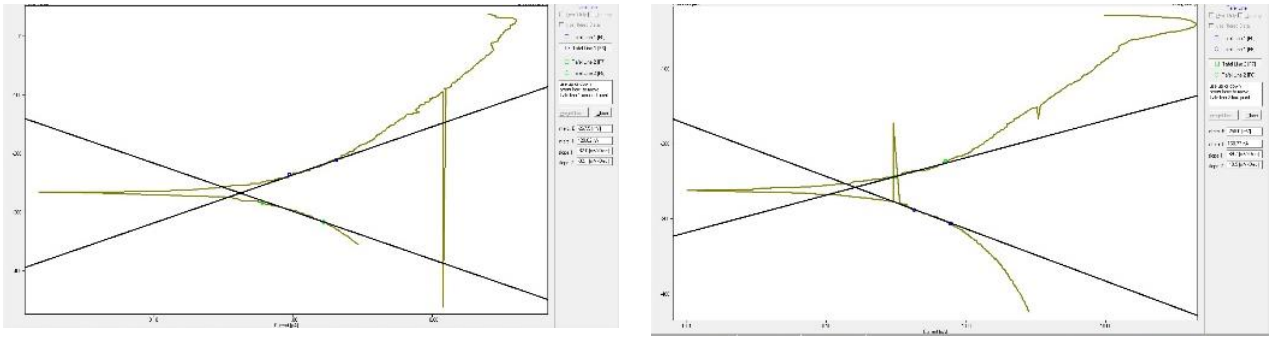


(c)



(d)



(e) (f)

Fig. 6. Linear polarization findings of samples in simulated body fluid solution: (a) Ti without coated by HAP (b) Ti coated by HAP (c) Ti coated by HAP-10% nAg (d) Ti coated by HAP-20 % nAg (e) Ti coated by HAP-30 % nAg (f) Ti coated by HAP-40 % nAg

Table 3 presents the corrosion rates (mpy). The findings suggest that the Hap-nAg coating layer exhibits a consistent and reliable performance. This coating not only enhances corrosion resistance but also alters the surface properties of the materials without impacting the bulk characteristics [28]. In addition, the application of coatings may enhance the ability of the coated specimens to resist corrosion [29-31].

The addition of nano-sized silver particles leads to a reduction in porosity. The assertion is substantiated by the observation that the presence of nano-sized particles leads to a substantial decrease in both porosity and corrosion current density because these little particles have the potential to occupy the pores. The addition of silver particles to the hydroxyapatite solution resulted in a significant decrease in the corrosion current density, reaching its minimum value as seen in Table 3.

Table 3
Corrosion results

Sample composite	Icorr. (nm)	Ecorr. (mV)	Corrosion rate (mpy)	Improvement (%)
Ti without coated	1430	-241.4	0.3357	-
Ti coated by HAP	437.65	-56.7	0.1025	69.4
Ti coated by HAP-10nAg	66.99	-271.6	0.0154	95.4
Ti coated by HAP-20nAg	72.29	-477.8	0.0169	94.9
Ti coated by HAP-30nAg	130.06	-76.2	0.0305	90.9
Ti coated by HAP-40nAg	263.56	-244.3	0.0617	81.6

3.5 Anti-Bacterial Results

It has examined the impact of anti-bacterial properties on Titanium alloy coated with HAP specimens containing varying weight percentages against E. coli culture, as illustrated in Table 4 and Figure 7. When a precise region forms around the disc, it is known as the bacterial inhibition zone. After twenty-four hours of incubation, the coating layer showed a potent anti-bacterial impact. Thus, the inclusion of HAP and nano silver successfully inhibited bacterial attachment on the surface, consequently hindering bacterial proliferation and enhancing anti-bacterial properties. Coated specimens have demonstrated a significant antimicrobial activity attributed to the silver coating's ability to eliminate bacterial strains effectively [32].

Table 4
Anti-bacterial results

Sample composite	Results
Titanium	_
Titanium coated by HAP	_
Titanium coated by HAP-10nAg	_
Titanium coated by HAP-20nAg	+
Titanium coated by HAP-30nAg	+
Titanium coated by HAP-40nAg	_

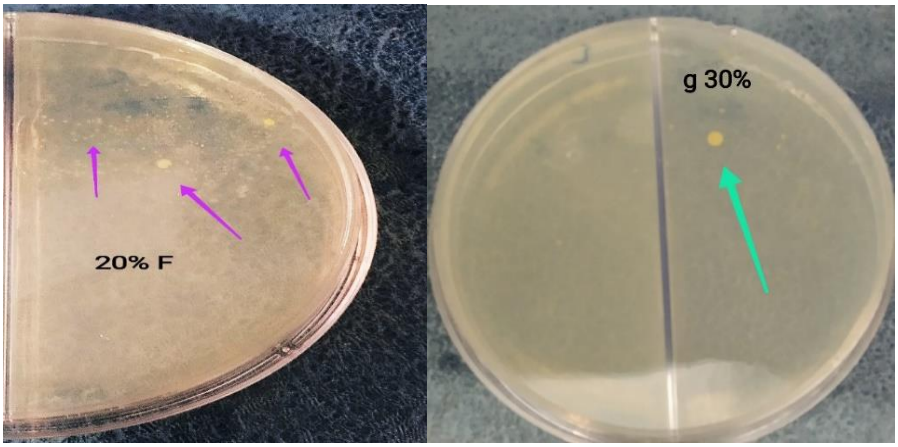


Fig. 7. Anti-bacterial results

4. Conclusions

i. Kind Titanium was effectively coated with bioactive hydroxyapatite utilizing electrophoretic deposition (EPD).
ii. XRD investigation verified HAP coatings' phase purity and stoichiometric properties, whereas SEM revealed a consistent distribution of deposits.
iii. The HAP and HAP-Ag coating morphology is uniform and without cracks when the voltage is set at 30V and the coating period is 30 minutes.
iv. Electrochemical analysis in Simulated body fluid solution showed that samples coated with hydroxyapatite (HAP) had greater corrosion resistance than uncoated Titanium, shown by their nobler Open Circuit Potential (OCP) magnitudes and greater breakdown and protection potentials. The Titanium without a coating was shown to be very prone to corrosion.
v. The HAP coating by Ag has strong antibacterial action versus gram-negative E. coli, according to the antibacterial analysis findings. The antibacterial activity of these coatings rises with the quantity of silver present, indicating their safety and suitability for use in surgical tool applications.

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