

Potential-controlled electrochemical deposition of hierarchical hydroxyapatite/TiO₂ nanotube coatings for biomedical applications

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ABSTRACT

Hydroxyapatite (HA) coatings on TiO₂ nanotube arrays have attracted considerable interest for improving the bioactivity of titanium implants. However, the influence of electrodeposition potential on coating formation and its correlation with the structural, electrochemical, and biological performance of HA/TiO₂ coatings remains insufficiently understood. In this study, HA coatings were electrodeposited onto anodized TiO₂ nanotube arrays at deposition potentials ranging from 1 to 9 V. The coatings were systematically characterized in terms of morphology, phase composition, surface properties, corrosion resistance, apatite-forming ability, and in vitro cytocompatibility. Low deposition potentials (1–5 V) produced discontinuous HA deposits, whereas an excessive potential (9 V) resulted in non-uniform crystal growth. In contrast, deposition at 7 V generated a homogeneous hierarchical micro/nanostructure with superhydrophilic wettability, improved coating adhesion, enhanced corrosion resistance, rapid apatite formation in simulated body fluid, and superior cell attachment and extracellular matrix protein deposition. These findings demonstrate that deposition potential governs the structure-property relationship of electrochemically deposited HA/TiO₂ coatings and identify 7 V as the optimal condition for achieving balanced physicochemical, electrochemical, and biological properties. The optimized HA/TiO₂ coating shows promising potential for bioactive surface modification of biomedical implants.

1. Introduction

Titanium (Ti) and its alloys are among the most extensively used biomaterials for orthopedic and dental implants because of their excellent mechanical properties, corrosion resistance, and intrinsic biocompatibility [1–3]. Nevertheless, the spontaneous TiO₂ passive film formed on titanium surfaces is biologically inert and does not actively stimulate bone regeneration, often resulting in limited bone-implant integration and the formation of fibrous tissue at the implant interface [4]. Since the long-term clinical success of titanium implants largely depends on rapid and stable osseointegration, considerable research efforts have been devoted to engineering bioactive surface modifications

capable of enhancing the biological interactions between implant surfaces and surrounding tissues. Among the various surface modification strategies, electrochemical anodization has attracted particular attention because it enables the fabrication of highly ordered TiO₂ nanotube (TNT) arrays directly on titanium substrates in a simple and controllable manner [5–9]. These nanotubular architectures provide several advantages for biomedical applications, including increased surface area, enhanced surface energy and nanoscale topographical features that mimic the extracellular matrix (ECM), thereby promoting protein adsorption and cellular adhesion [6–8,10,11]. More importantly, TNT arrays provide abundant heterogeneous nucleation sites for calcium phosphate deposition, facilitating the formation of uniform bioactive

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coatings [10,12,13].

Hydroxyapatite (HA), owing to its chemical composition and crystal structure closely resembling the mineral phase of natural bone, has been extensively investigated as a bioactive coating for titanium implants because of its excellent biocompatibility, osteoconductivity and ability to promote bone-implant integration [12,14–16]. Various techniques have been employed to deposit HA coatings onto titanium-based substrates, including plasma spraying [17], magnetron sputtering [18], sol-gel processing [19], pulsed laser deposition [20], hydrothermal treatment [21] and electrophoretic deposition [22]. Among these approaches, electrodeposition has attracted considerable attention because it can be performed under mild conditions and offers precise control over coating thickness, morphology, and crystallinity through adjustable electrochemical parameters. Moreover, electrodeposition is a simple, cost-effective technique compatible with complex surface geometries, making it particularly suitable for depositing HA onto TNT arrays [13–16,23,24,25].

Previous studies have employed different surface engineering and coating strategies to improve the structural, physicochemical, and biological properties of HA coatings. In 2018, Xia et al. [26] synthesized HA coatings on Ti–6Al–4V substrates using atmospheric plasma spraying followed by hydrothermal treatment. The hydrothermal process increased the coating crystallinity and promoted the formation of HA architectures, which improved surface wettability, degradation resistance, protein adsorption, osteogenic activity, and osseointegration. These findings demonstrated that the structural characteristics and post-deposition treatment of HA coatings can substantially influence their physicochemical and biological properties. However, the study did not address the electrochemical formation of HA or the role of deposition potential in controlling coating development on anodized TiO₂ nanotube arrays. In 2020, Li et al. [14] and in 2022, Dev et al. [16] summarized that previous studies have improved electrodeposited HA coatings mainly on metallic substrates by independently modifying electrochemical parameters (current density, pH, electrolyte composition and deposition mode) or introducing auxiliary strategies such as hydrogen peroxide, pulse deposition and ultrasonic assistance. These studies have tailored individual coating characterizations, including morphology, crystallinity, Ca/P ratio, adhesion, and corrosion resistance. However, these investigations were conducted predominantly on conventional metallic substrates, with the effects of electrochemical parameters examined under different substrate, electrolyte and deposition conditions. Importantly, Dev et al. highlighted the significant role of applied voltage in HA electrodeposition, as demonstrated by Mokabber et al. [27]. In their study, Mokabber et al. employed a two-electrode pulsed electrodeposition configuration, with Ti as the cathode, and investigated applied cathodic cell voltages of –1.4, –1.6, and –1.8 V at different deposition times. The pulse frequency was fixed at 1 Hz, with both the pulse-on and pulse-off times set to 0.5 s. At –1.4 V, a relatively dense and uniform plate-like coating was obtained, whereas increasing the applied voltage to –1.6 and –1.8 V resulted in pronounced coating deterioration, characterized by crack and pore formation and partial or complete coating detachment associated with enhanced H₂ evolution. Consequently, the reported findings remain largely parameter-specific and fragmented, rather than establishing a unified relationship between applied deposition voltage, HA nucleation, crystal growth and hierarchical coating formation. In particular, how systematic variation of the applied voltage regulates HA formation and how the resulting structural evolution governs the physicochemical, electrochemical and biological characteristics of HA coatings on anodized TiO₂ nanotube arrays remain insufficiently understood.

The introduction of anodized TiO₂ nanotube arrays, research progressively shifted from conventional metallic substrates toward nanotubular architectures because of their unique ability to regulate interfacial biological responses and support bioactive coating formation. In 2023, Poddar et al. [28] synthesized TNT arrays with different nanotube diameters on Ti–6Al–4V alloys by electrochemical

anodization and systematically evaluated platelet adhesion, hemocompatibility, and in vitro cell viability. Their results demonstrated that nanotube geometry significantly influenced biological responses, confirming the critical role of nanotopography in regulating cell-material interactions. However, the study focused on optimizing nanotube morphology and did not involve hydroxyapatite deposition. In 2024, Mazare et al. [29] demonstrated that the apatite-forming ability of anodized TiO₂ nanotubes strongly depends on nanotube morphology, diameter, crystallinity and surface functionalization. Using alternate immersion treatment and Ca-functionalization, they showed that pre-loaded HA or Ca species significantly accelerate apatite nucleation in simulated body fluid, particularly on large-diameter nanotubes. However, the study focused on post-functionalization strategies for enhancing bioactivity rather than the electrochemical formation of HA coatings. Consequently, how anodized TiO₂ nanotube architectures regulate hydroxyapatite electrocrystallization under different deposition potentials has not yet been systematically established.

More recently, in 2026, Saedodin et al. [15] prepared HA/TiO₂ composite coatings on 316 stainless steel by electrophoretic deposition and showed that optimization of coating composition and subsequent heat treatment improved coating uniformity, corrosion resistance, wettability, and antibacterial activity. However, this study employed electrophoretic deposition on stainless steel rather than electrochemical HA deposition on anodized TiO₂ nanotube arrays.

Parcharoen et al. [30] demonstrated the feasibility of cathodic potentiostatic HA electrodeposition on anodized TNT arrays at a fixed condition (–2.5 V, 80 °C, 10 min), and evaluated the resulting coatings mainly in terms of morphology, phase composition, elemental composition, adhesion and early cellular response. However, because the deposition potential was kept constant, the potential-dependent evolution of HA electrocrystallization and its relationship with coating physicochemical, electrochemical and biological characteristics could not be established.

Building upon this platform, research has progressively evolved toward multifunctional HA-based coatings through the incorporation of nanoparticles, therapeutic agents, and bioactive metallic ions to simultaneously improve surface properties, corrosion resistance and cytocompatibility [25,31–36]. In parallel, various post-deposition strategies, including hydrothermal treatment, biomimetic mineralization and laser processing have been employed to tailor coating crystallinity, hierarchical architecture and interfacial stability, thereby further enhancing the biological performance and long-term durability of HA/TNT coatings [37,38].

Collectively, previous studies have progressively advanced HA-coated implant surfaces through optimization of coating crystallinity, electrochemical processing, nanotube morphology, surface functionalization, and coating composition. Although applied potential has been recognized as an important electrochemical parameter governing HA electrodeposition, previous investigations have either focused on conventional metallic substrates or employed TiO₂ nanotube arrays primarily to regulate surface architecture, functionalization, and mineralization. However, a systematic experimental investigation of how applied deposition potential influences HA nucleation and crystal growth on anodized TiO₂ nanotube arrays, and how the resulting hierarchical coating architecture influences the physicochemical, electrochemical, and biological characteristics of the coatings, remains lacking. In particular, the relationships between deposition potential, coating architecture, protein adsorption, apatite-forming ability, and cytocompatibility have not yet been systematically established within a unified experimental framework.

To address this knowledge gap, the present study systematically investigates the influence of deposition potential on the electrodeposition of hydroxyapatite coatings onto anodized TiO₂ nanotube arrays over a deposition-potential range of 1–9 V. The resulting coatings were systematically characterized in terms of morphology, phase composition, surface chemistry, wettability, roughness, adhesion strength, corrosion

resistance, apatite-forming ability, fibronectin protein adsorption, and in vitro cytocompatibility. By establishing the relationships between deposition potential, hierarchical coating formation, and the resulting physicochemical, electrochemical, and biological characteristics within a unified experimental framework, this study provides new insight into the structure-property relationships of HA/TiO₂ coatings and offers guidance for the rational design of bioactive titanium implant surfaces.

2. Materials and Methods

2.1. Synthesis of TiO₂ nanotube substrates

Commercially pure titanium (99.9%, Merck) plates with dimensions of $10 \times 10 \times 1 \text{ mm}^3$ were used as substrates. Before anodization, the specimens were ultrasonically cleaned in acetone for 20 min, rinsed with double-distilled water, and dried in a vacuum oven at 40 °C for 2 h. Self-organized TiO₂ nanotube arrays were synthesized by potentiostatic anodization in an ethylene glycol-based electrolyte containing NH₄F and deionized water at 50 V for 60 min [11,39]

2.2. Electrochemical deposition of HA coatings

Hydroxyapatite coatings were electrochemically deposited onto TiO₂ nanotube (TNT) substrates from an aqueous electrolyte containing 0.001 M calcium acetate ($\text{Ca}(\text{CH}_3\text{COO})_2$) and 0.0006 M ammonium dihydrogen phosphate ($\text{NH}_4\text{H}_2\text{PO}_4$). The electrolyte was preheated to 80 °C and continuously stirred throughout the electrodeposition process. Electrodeposition was performed potentiostatically in a two-electrode cell using the TNT substrate as the cathode and a platinum foil as the counter electrode, with an inter-electrode distance of 4 cm for 10 min. The deposition was carried out at applied potentials ranging from 1 to 9 V. After deposition, the specimens were rinsed three times with deionized water and annealed in air at 550 °C for 1 h with a heating rate of 5 °C/min

2.2.1. Materials characterization

Surface morphology and elemental composition were characterized using field-emission scanning electron microscopy equipped with energy-dispersive X-ray spectroscopy (FE-SEM/EDS; JSM-IT800, JEOL, Japan). Elemental compositions and Ca/P molar ratios were obtained

from quantitative EDS point analysis. The specific surface area of the TiO₂ nanotube and HA-coated TiO₂ nanotube coatings was determined by nitrogen adsorption-desorption at 77 K using an ASAP 2060 Accelerated Surface Area and Porosimetry System (Micromeritics, USA). Before analysis, the samples were degassed under vacuum. The specific surface area was calculated using the Brunauer-Emmett-Teller (BET) equation. The crystallographic structure and phase composition were analyzed by X-ray diffraction (XRD; D500, Siemens, Germany) using Cu K α radiation ($\lambda = 1.5406 \text{ \AA}$) operated at 40 kV and 40 mA. Diffraction patterns were indexed using ICDD reference cards for hydroxyapatite (09-0432), anatase TiO₂ (21-1272) and α -Ti (44-1294). The surface chemical composition was analyzed by X-ray photoelectron spectroscopy (XPS). High-resolution spectra were collected for the Ca 2p, P 2p and O 1s core levels to determine the chemical states of the corresponding elements. Surface wettability was evaluated by static water contact angle measurements using the sessile drop method with a contact angle goniometer. The reported values represent the average of at least five independent measurements for each specimen. Three-dimensional surface topography and roughness parameters (S_a , S_q and S_z) were characterized using a digital optical microscope (VHX-7000, Keyence, Japan). Vickers microhardness was measured using a microhardness tester (FM-100, Future-Tech, Japan). Three independent indentations were performed on each specimen and the average value was reported. The adhesion strength of the coatings was evaluated using a PosiTest® AT Pull-Off Adhesion Tester (DeFelsko Corporation, Ogdensburg, NY, USA) in accordance with ASTM D4541. Dollies were bonded to the coating surface using a two-component epoxy adhesive and cured at room temperature for 24 h. Perpendicular tensile loading was then applied until coating failure occurred and the pull-off adhesion strength was recorded in MPa. The corrosion resistance properties of Ti, TiO₂ nanotubes and the HA coating electrodeposited at 7 V were evaluated by potentiodynamic polarization (Tafel plot) measurements in simulated body fluid (SBF, pH $\approx$ 7.0) at 25 °C. Measurements were performed using a conventional three-electrode cell, with the test specimen as the working electrode, a platinum electrode as the counter electrode and a saturated calomel electrode (SCE) as the reference electrode. Tafel plots were recorded over a potential range of -100 to $+100 \text{ mV}$ at a scan rate of 10 mV/s . The corrosion potential (E_{corr}), corrosion current density (I_{corr}) and polarization resistance (R_p) were determined from the polarization curves to evaluate the corrosion

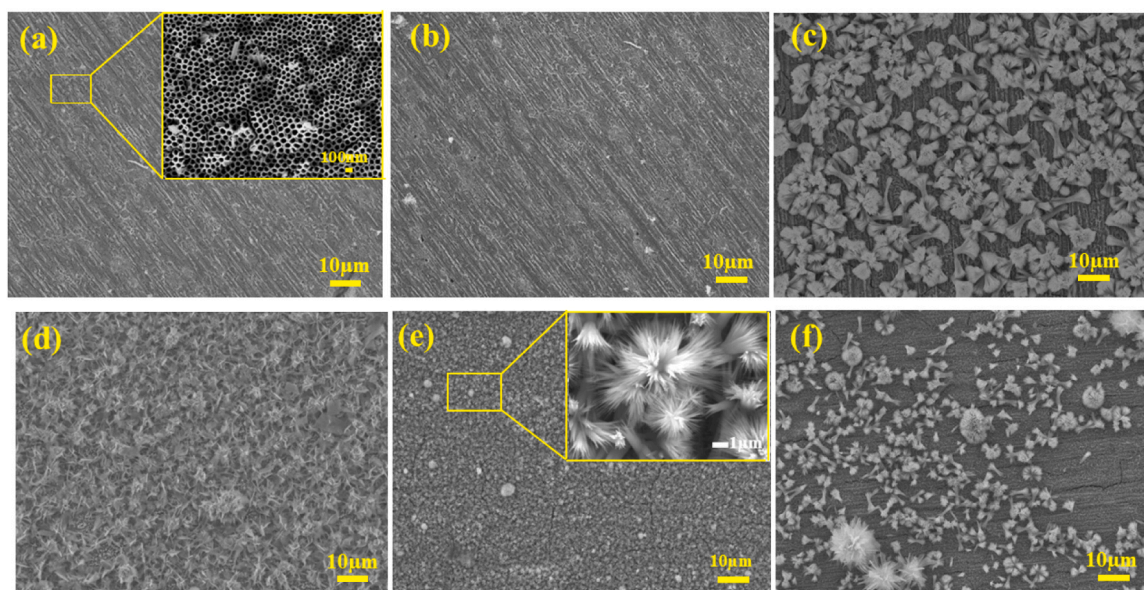


Fig. 1. FE-SEM images of the anodized TiO₂ nanotube arrays and hydroxyapatite coatings electrodeposited at different applied potentials: (a) TiO₂ nanotube arrays, (b) 1 V, (c) 3 V, (d) 5 V, (e) 7 V, and (f) 9 V.

resistance of the different surfaces. All measurements were carried out using an AutoLab PGSTAT302N electrochemical workstation controlled by Nova 2.1.7 software.

2.3. *In vitro* apatite-forming ability

The apatite-forming ability of the coatings was evaluated by immersion in simulated body fluid (SBF) [25]. Each specimen was immersed in 30 mL of SBF at $37 \pm 0.5^\circ\text{C}$ for 7 and 14 days under static conditions using a temperature-controlled laboratory incubator (Memmert, Germany). The surface morphology and elemental composition after immersion were characterized by FE-SEM and EDS, respectively.

2.4. Cell culture, cytocompatibility and fibronectin immunofluorescence

Cell adhesion, proliferation, and fibronectin expression were evaluated using Baby Hamster Kidney cells (BHK-21, ATCC CCL-10) cultured on bare Ti, anodized TiO₂ nanotube (TNT) and HA/TiO₂-coated substrates. Cells were seeded at a density of 2×10^4 cells/mL in Dulbecco's Modified Eagle's Medium (DMEM) onto specimens placed in 24-well culture plates and incubated at 37°C in a humidified atmosphere containing 5% CO₂. Cytocompatibility was evaluated after 48 and 72 h of culture. Following each incubation period, the specimens were rinsed with phosphate-buffered saline (PBS), fixed with 4% paraformaldehyde, and stained with Phalloidin 555 (Invitrogen) for 45 min to visualize the actin cytoskeleton, followed by nuclear counterstaining with DAPI (HiMedia) for 10 min. Fibronectin immunofluorescence analysis was performed on selected specimens after 48 and 96 h of culture. The fixed specimens were incubated with an anti-fibronectin primary antibody (Invitrogen), followed by an Alexa Fluor 488-conjugated secondary antibody (Invitrogen), with PBS washing between each incubation step. Fluorescence images were acquired using a confocal laser scanning microscope (FV3000RS, Olympus, Japan) under identical imaging conditions for all specimens. Cell density was quantified from DAPI-stained CLSM images using ImageJ. Cell nuclei were counted from three representative microscopic fields for each specimen under identical imaging conditions. The average value from three independent specimens ($n = 3$) was expressed as mean $\pm$ standard deviation (SD). Statistical analysis was performed using one-way ANOVA, and differences were considered statistically significant at $p < 0.05$.

3. Results and discussion

3.1. Surface morphology by FE-SEM

Prior to electrodeposition, the anodized TiO₂ nanotube (TNT) substrate (Fig. 1a) exhibited a well-defined and uniformly distributed nanotubular structure, as clearly revealed by the high-magnification FE-SEM image (inset in Fig. 1a), with an average inner diameter of approximately 80–100 nm, confirming the successful formation of the TNT arrays. At 1 V (Fig. 1b), no obvious HA crystallites were observed, suggesting limited HA formation under this condition. Increasing the deposition potential to 3 V (Fig. 1c) resulted in the appearance of discrete HA crystallites with limited surface coverage. At 5 V (Fig. 1d), the density of the deposited crystallites increased, leading to greater surface coverage and a more continuous coating. At 9 V (Fig. 1f), the coating consisted of irregularly distributed aggregates with noticeable size variation and localized agglomeration, reflecting reduced morphological uniformity. Among the investigated conditions, the coating deposited at 7 V (Fig. 1e) exhibited the highest degree of morphological uniformity. At low magnification, the coating displayed a continuous surface with a homogeneous distribution of HA deposits. Higher-magnification SEM images revealed that the deposits consisted of radially oriented needle-like crystallites assembled into flower-like architectures with diameters of approximately 1–3 μm , resulting in a well-defined hierarchical micro/nanostructure. Occasionally,

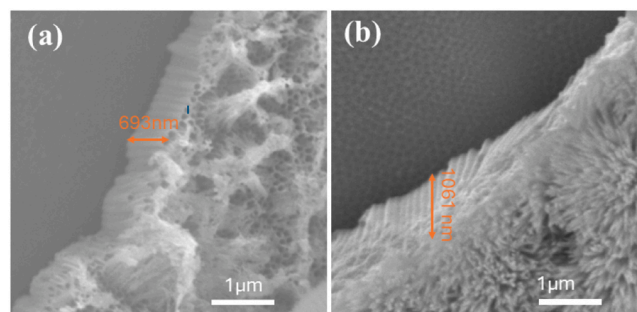


Fig. 2. Cross-sectional SEM micrographs of (a) bare TiO₂ nanotube arrays (693 nm) and (b) HA/TiO₂-7 V nanotube coating (1061 nm).

neighboring flower-like assemblies merged to form larger hierarchical clusters with diameters of approximately 7 μm . These observations indicate that 7 V provided the most favorable deposition conditions for producing a uniform hierarchical HA coating under the present experimental conditions.

The thickness of the synthesized coatings was determined from cross-sectional FE-SEM images (Fig. 2). The TiO₂ nanotube layer exhibited an average thickness of approximately 693 nm, while the total thickness of the HA/TiO₂ coating deposited at 7 V (HA/TiO₂-7 V) increased to approximately 1061 nm, indicating an HA layer thickness of about 368 nm. The cross-sectional images further confirmed the formation of a continuous HA layer on the TiO₂ nanotube arrays. A distinct interface between the HA coating and the underlying nanotube layer was clearly observed, while the TiO₂ nanotubes remained highly uniform with an average inner diameter of approximately 100 nm. These observations demonstrate the successful deposition of a continuous HA layer on the TiO₂ nanotube substrate under the selected electrodeposition condition.

To facilitate the interpretation of the observed coating morphology, Fig. 3 presents a schematic illustration of the proposed formation process of hydroxyapatite on TiO₂ nanotube arrays during electrodeposition, constructed based on the experimental observations in this study and previous reports [13,22,40–42].

Once the initial HA nuclei are electrochemically formed on the TiO₂ nanotube surface (Fig. 3), the subsequent evolution of the HA coating is proposed to be governed by a crystal growth process. Ca²⁺ and phosphate ions in the electrolyte spontaneously assemble into calcium phosphate prenucleation clusters (Posner's clusters, Ca₉(PO₄)₆), which are widely recognized as the fundamental growth units of hydroxyapatite. These Ca-rich growth units preferentially adsorb onto the exposed negatively charged c-plane of the growing HA crystals, whereas the positively charged a,b-planes are less favorable for their adsorption. Meanwhile, phosphate species adsorbed on the a,b-planes may further suppress lateral crystal growth. Following their attachment, the prenucleation clusters continuously incorporate Ca²⁺, PO₄³⁻ and OH⁻ ions from the electrolyte, gradually transforming into a complete HA lattice. Repeated attachment and crystallization of these growth units promote preferential crystal growth along the c-axis, resulting in elongated HA nanorods [16,26]. Compared with the flat Ti substrate, the TiO₂ nanotube arrays are suggested to provide spatially ordered heterogeneous nucleation sites while simultaneously imposing spatial confinement on the early-stage growth of adjacent HA nuclei within the nanotube architecture. Consequently, the basal regions of neighboring nanorods remain tightly bundled, whereas their upper portions continue to elongate beyond the nanotube openings along the preferred c-axis and gradually diverge, ultimately giving rise to the hierarchical flower-like morphology observed in the SEM images.

3.2. Elemental composition and Ca/P stoichiometry by EDS

EDS elemental mapping was used to evaluate the elemental distribution of the deposited coating. As shown in Fig. 4, the Ca and P signals

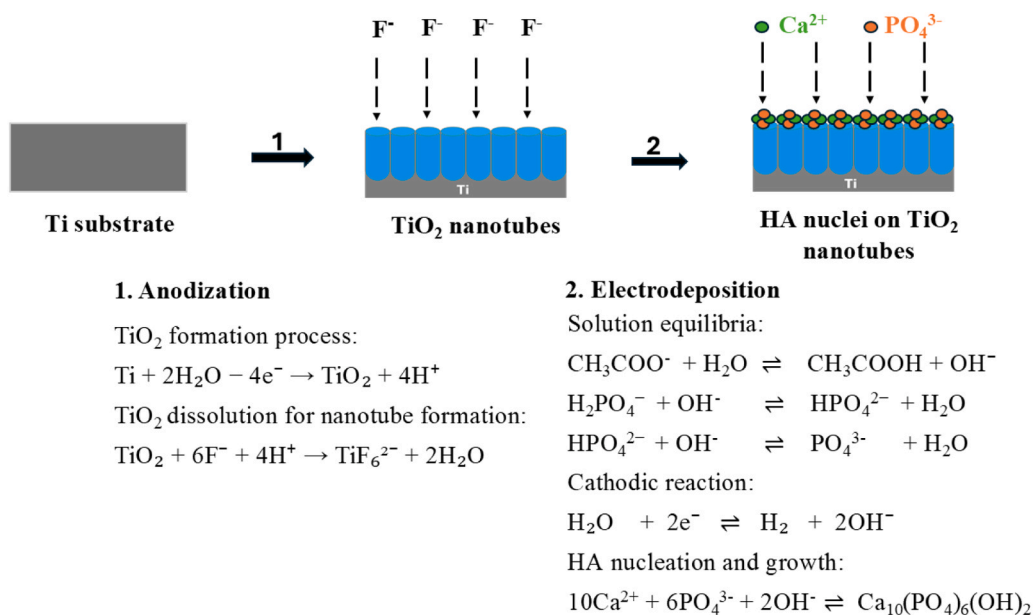


Fig. 3. Proposed formation process of hydroxyapatite on TiO_2 nanotube arrays during electrodeposition.

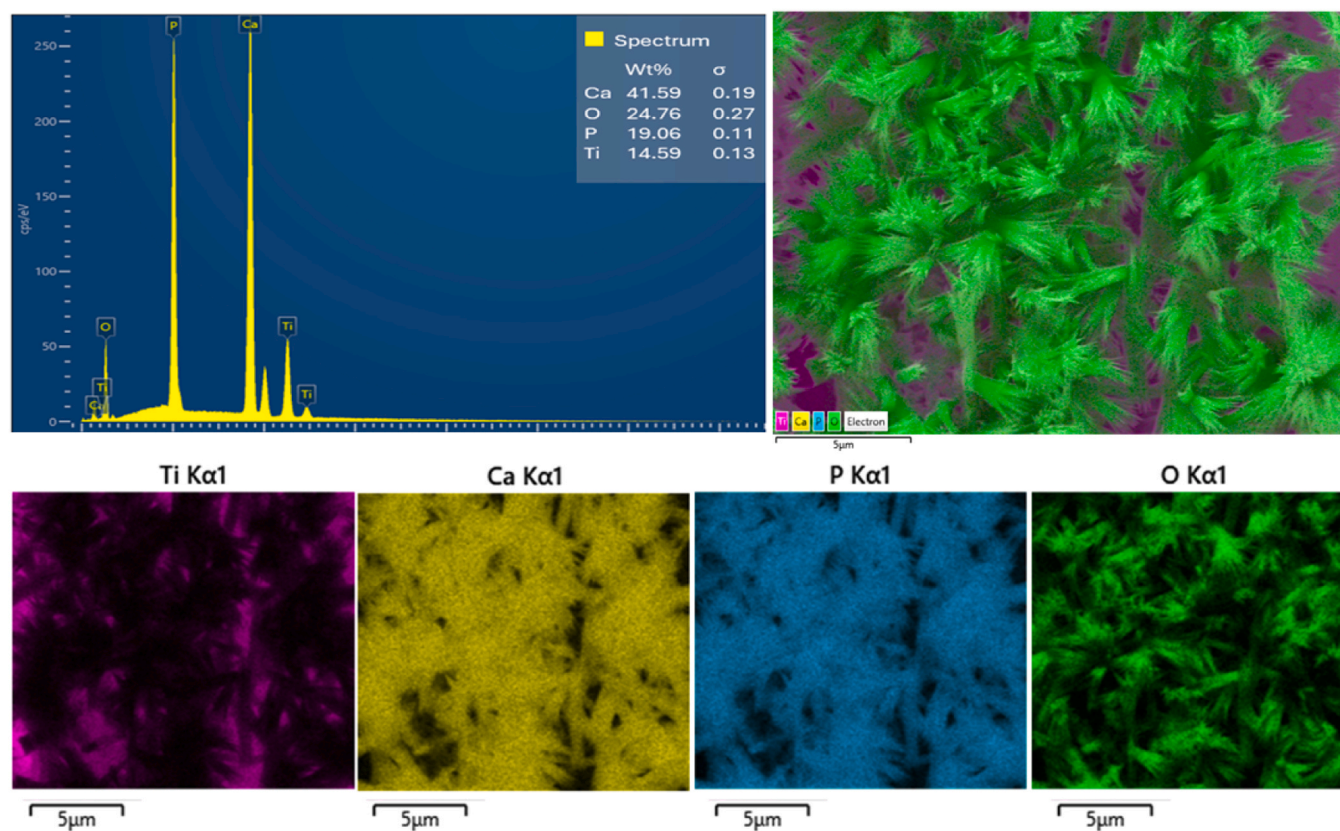


Fig. 4. EDS elemental mapping of HA/ TiO_2 -7 V showing spatial co-localization of Ca and P signals.

exhibited a similar spatial distribution over the entire coating surface, indicating a homogeneous distribution of both elements. The overlapping Ca and P maps were consistent with the formation of a calcium phosphate coating. The crystalline phase and chemical states were further identified by XRD and XPS, respectively.

The quantitative EDS results were summarized in Table 1. The Ca/P ratio depended strongly on the deposition potential. The coatings deposited at 1, 3 and 5 V exhibited Ca/P ratios of 1.43, 1.40 and 1.44,

respectively, all lower than the stoichiometric value of hydroxyapatite (1.67). The coating deposited at 7 V contained the highest Ca (41.59 wt %) and P (19.06 wt %) contents, giving a Ca/P ratio of 1.69. This value is close to the stoichiometric composition of hydroxyapatite and falls within the reported range of cortical bone (1.65–1.71). At 9 V, both the Ca and P contents decreased, and the Ca/P ratio decreased to 1.48. These results agree well with the FE-SEM observations. The coating deposited at 7 V exhibited the most uniform surface morphology together with the

Table 1
EDS analysis of HA/TiO₂ coatings deposited at different potentials.

Deposition Potential (V)	Ca (wt%)	P (wt%)	Ca/P Ratio
1	9.69	5.24	1.43
3	19.28	10.66	1.40
5	11.21	6.05	1.44
7	41.59	19.06	1.69
9	20.80	10.86	1.48

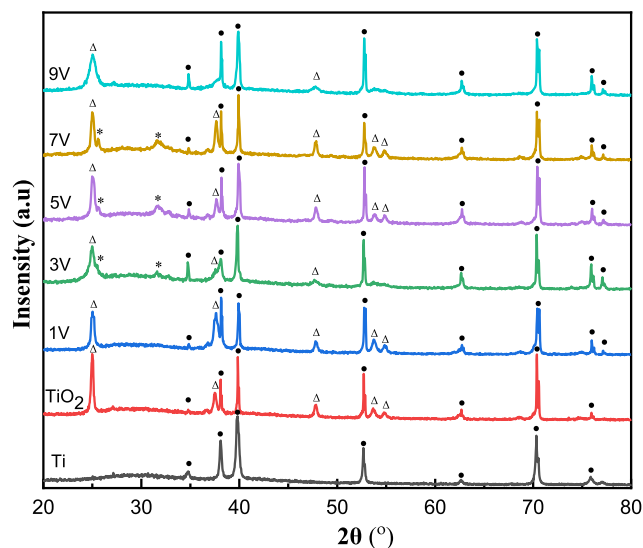


Fig. 5. XRD patterns of Ti, TiO₂, HA/TiO₂ coatings deposited at potentials of 1–9 V. Peak designations: • = α -Ti; Δ = anatase TiO₂; * = HA.

highest Ca and P contents and a near-stoichiometric Ca/P ratio among all investigated samples.

The SEM and EDS observations were further supported by the BET analysis. The specific surface area decreased from 3.87 m²/g for the TiO₂ nanotube coating to 1.66 m²/g after HA deposition. This decrease is likely associated with the partial coverage of the exposed TiO₂ nanotube surface by the deposited HA layer, which reduced the surface area accessible to nitrogen adsorption. Although BET does not directly verify coating coverage, the reduced specific surface area is consistent with the SEM and EDS observations and supports the proposed deposition of HA on the TiO₂ nanotube surface.

3.3. Crystallographic characterization by XRD

Fig. 5 shows the XRD patterns of Ti, TiO₂, HA-coated TiO₂ nanotube layers deposited at different applied potentials (1–9 V). Characteristic reflections at approximately 25.3°, 37.8° and 48.0° were assigned to the (101), (004) and (200) planes of anatase TiO₂ (ICDD 21–1272) [15, 43], confirming that the anodized nanotube layer retained the anatase phase after electrodeposition. In addition, diffraction peaks corresponding to the α -Ti substrate (ICDD 44–1294) [13] were also observed, suggesting that the deposited HA layer remained relatively thin. The formation of hydroxyapatite was strongly dependent on the applied deposition potential. No characteristic HA reflections were detected at 1 and 3 V, suggesting that the electrochemical driving force was insufficient for stable HA nucleation. When the potential increased to 5 V, diffraction peaks located at approximately 25.9° and 31.7° emerged and were indexed to the (002) and (211) planes of crystalline hydroxyapatite (ICDD 09–0432) [13,15,43–45], confirming successful HA formation on the TiO₂ nanotube surface.

At 7 V, all characteristic HA reflections became sharper and more intense, indicating enhanced crystallinity and improved crystal ordering. No secondary calcium phosphate phases, such as β -TCP or

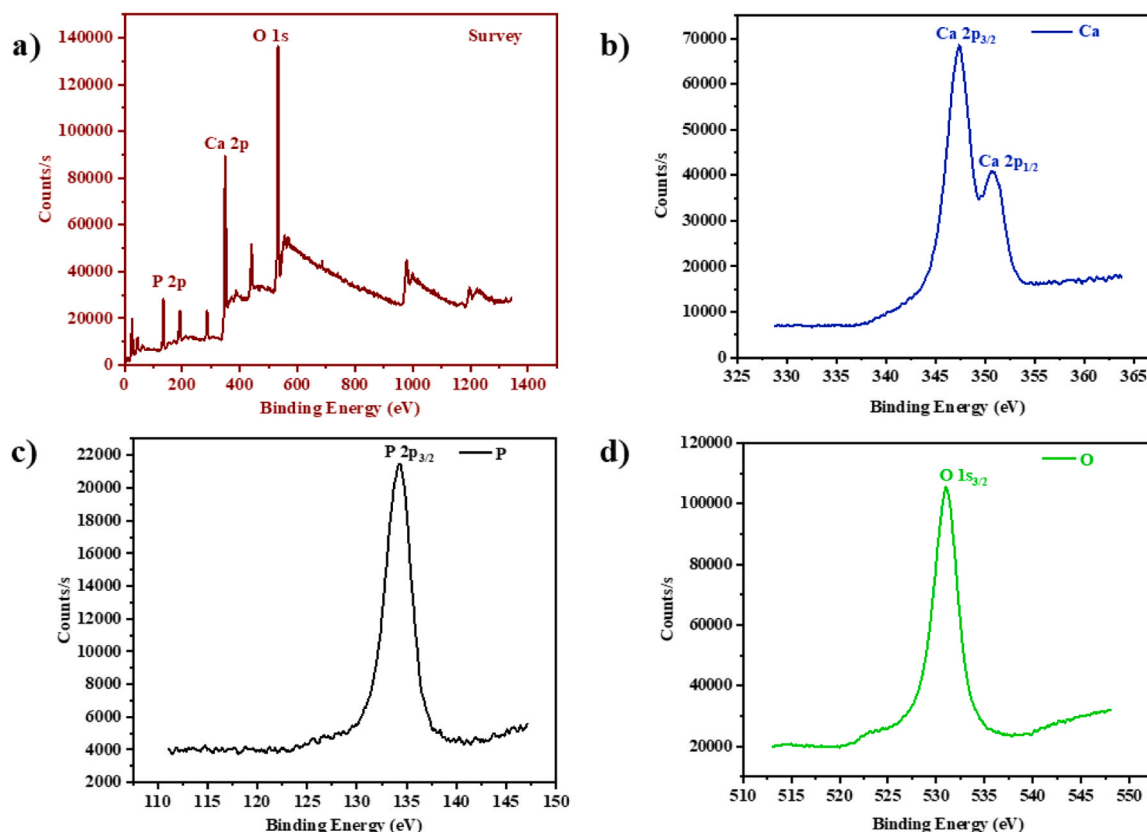


Fig. 6. XPS spectra of the HA/TiO₂ nanotube coating electrodeposited at 7 V: (a) survey spectrum showing Ca, P and O elements, (b) Ca 2p, (c) P 2p and (d) O 1 s.

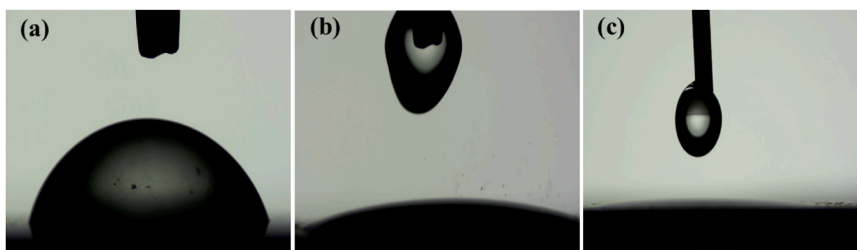


Fig. 7. Representative static water contact angle images for (a) bare Ti substrate, (b) TiO_2 nanotube arrays and (c) HA/TiO_2 -7 V.

CaO , were detected, demonstrating the formation of phase-pure HA under this condition. In contrast, the HA reflections weakened at 9 V, which is likely associated with accelerated hydrogen evolution ($2\text{H}_2\text{O} + 2\text{e}^- \rightarrow \text{H}_2 + 2\text{OH}^-$) at higher potentials. Excessive hydrogen bubble evolution may interfere with ion transport and continuous crystal growth, resulting in reduced crystallinity and the irregular coating morphology observed by FE-SEM. Overall, the XRD results indicate that 7 V provided the most favorable condition for producing highly crystalline and phase-pure HA coatings on TiO_2 nanotubes.

3.4. XPS Analysis

To further elucidate the surface chemical composition of the electrodeposited coating, XPS analysis was performed on the sample prepared under the optimized condition at an applied voltage of 7 V, and the corresponding spectra are presented in Fig. 6. The survey spectrum (Fig. 6a) reveals the presence of Ca, P and O as the predominant surface elements, with no detectable Ti signal, confirming that the deposited layer fully encapsulates the underlying TiO_2 nanotube substrate. Given that the XPS analytical depth is limited to approximately 5–10 nm, the complete attenuation of the Ti 2p signal provides unambiguous evidence that the HA coating is continuous and conformal, with a thickness exceeding this detection limit. The high-resolution Ca 2p spectrum (Fig. 6b) exhibits two spin-orbit components with Ca $2\text{p}_{3/2}$ at 347.0 eV and Ca $2\text{p}_{1/2}$ at 350.6 eV (splitting of 3.6 eV), consistent with calcium in a phosphate bonding environment (Ca-O-P) characteristic of

hydroxyapatite, and clearly distinguishable from CaO (346.3 eV) or $\text{Ca}(\text{OH})_2$ (346.4–346.6 eV). The P 2p spectrum (Fig. 6c) displays a single symmetric peak with P $2\text{p}_{3/2}$ at 133.2 eV, in good agreement with reference values for orthophosphate groups (PO_4^{3-} , 132.8–133.6 eV), ruling out the presence of reduced phosphorus species. The O 1s spectrum (Fig. 6d) displays a principal peak at 531.0 eV attributed to P-O bonds in the phosphate network, with an asymmetric shoulder toward higher binding energies (~ 532.5 eV) arising from hydroxyl groups (O-H) inherent to the HA structure and surface-adsorbed water molecules [29,46,47]. Collectively, the binding energies of all three elements are mutually consistent with the chemical structure of stoichiometric hydroxyapatite, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$.

3.5. Surface properties: wettability, hardness, roughness and adhesion strength

3.5.1. Water contact angle and surface wettability

Surface wettability is an important factor governing the initial interaction between implant materials and the physiological environment [5,48]. Static water contact angle measurements were performed on bare Ti, TiO_2 nanotubes and HA/TiO_2 -7 V (Fig. 7). The bare Ti substrate exhibited a contact angle of approximately 78° , indicating a moderately hydrophilic surface. After anodization, the TiO_2 nanotube coating showed a marked decrease in contact angle to approximately 18° . HA/TiO_2 -7 V exhibited a contact angle approaching 0° , indicating a superhydrophilic surface. The enhanced wettability of the

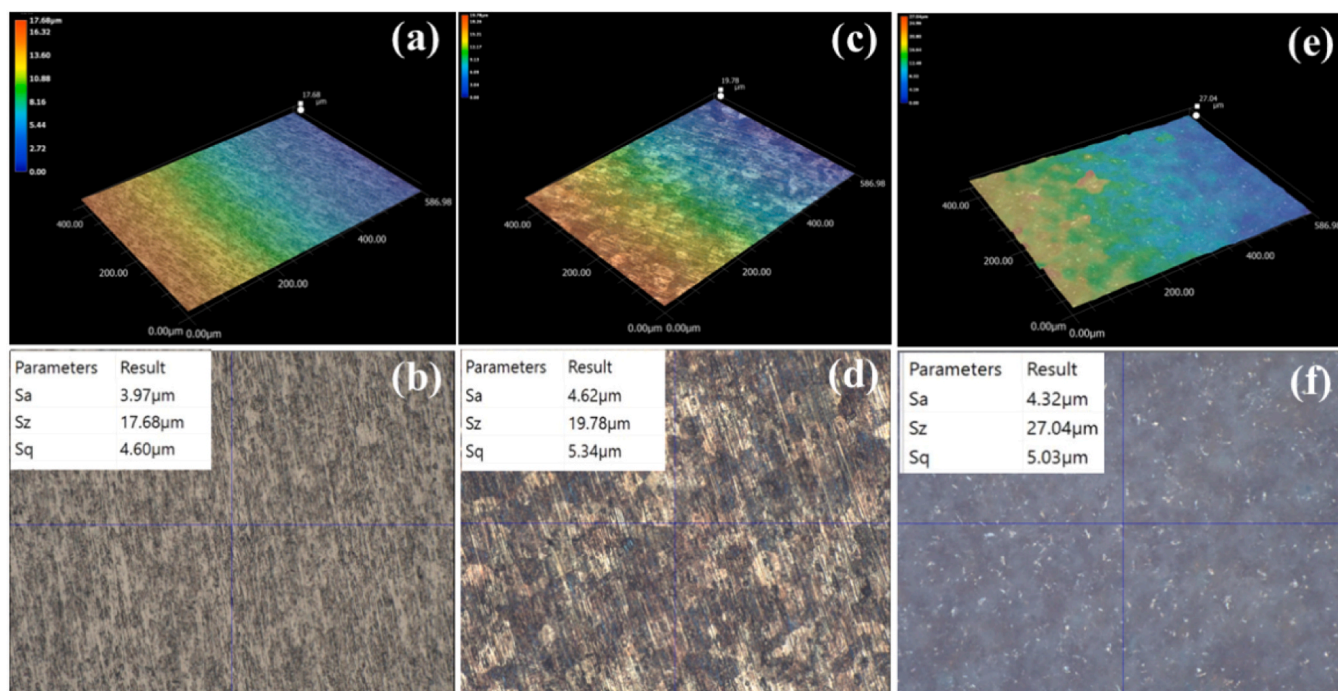


Fig. 8. Surface topography and roughness analysis of (a, b) Ti, (c, d) TiO_2 nanotubes and (e, f) HA/TiO_2 -7 V samples obtained by 3D optical profilometry (VHX).

Table 2Surface roughness parameters of Ti, TiO₂ nanotubes, and HA/TiO₂ –7 V.

Sample	S _a (μm)	S _q (μm)	S _z (μm)	S _q / S _a	S _z / S _a	Surface Character
Ti	3.97	4.60	17.68	1.16	4.45	Anisotropic, polished
TiO ₂	4.62	5.34	19.78	1.16	4.28	Uniform oxide roughening
HA/ TiO ₂ –7 V	4.32	5.03	27.04	1.16	6.26	Hierarchical, heterogeneous

Table 3Vickers hardness (GPa) of Ti, TiO₂ nanotubes, and HA/TiO₂ –7 V.

Samples	Hardness 1 (GPa)	Hardness 2 (GPa)	Hardness 3 (GPa)	Average (GPa)
Ti	1.48	1.49	1.50	1.49 ± 0.01
TiO ₂	2.13	2.18	2.07	2.12 ± 0.06
HA/TiO ₂ –7 V	1.61	1.63	1.57	1.62 ± 0.03

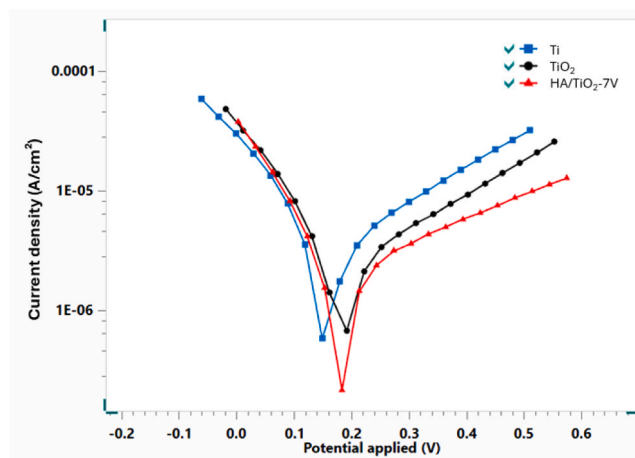
HA/TiO₂–7 V is attributed to both its surface chemistry and hierarchical surface architecture. Hydroxyapatite contains hydroxyl (OH⁻) and phosphate (PO₄³⁻) groups, which readily interact with water molecules through hydrogen bonding, thereby increasing surface wettability [11]. FE-SEM observations showed that the coating consisted of densely packed flower-like assemblies constructed from radially oriented needle-like crystallites. This hierarchical micro/nanostructure increases the effective solid-liquid contact area and promotes rapid spreading of water across the coating surface [4,7]. The combined contribution of the hydrophilic surface chemistry and hierarchical morphology accounts for the near-zero contact angle measured for HA/TiO₂–7 V. The super-hydrophilic surface is expected to facilitate the initial adsorption of proteins and subsequent cell attachment [5,6], which is further supported by the fibronectin adsorption and cytocompatibility results presented in the following sections.

3.5.2. Roughness

Surface roughness was evaluated by three-dimensional optical profilometry (Fig. 8 and Table 2). Unlike atomic force microscopy (AFM), which characterizes the nanoscale topography of localized surface regions, optical profilometry measures the overall surface topography over a much larger sampling area. Therefore, the measured roughness parameters reflect the combined contributions of the titanium substrate, the TiO₂ nanotube layer, and the electrodeposited hydroxyapatite coating, rather than the nanotube architecture alone. The polished Ti substrate exhibited the lowest roughness, whereas anodization increased all roughness parameters, consistent with the formation of the TiO₂ nanotube layer observed by FE-SEM. Following HA deposition at 7 V (HA/TiO₂–7 V), S_a decreased slightly whereas S_z increased markedly. This combination suggests that HA deposition generated localized flower-like HA aggregates, increasing the maximum surface height while only marginally affecting the average surface roughness. This interpretation is consistent with the FE-SEM observations. The resulting hierarchical surface, together with the enhanced wettability, is expected to facilitate protein adsorption and subsequent cell attachment, consistent with previous studies on hierarchical HA coatings [49–51]. This trend was further supported by the enhanced fibronectin adsorption and cytocompatibility observed in the present study.

3.5.3. Hardness

The Vickers microhardness values are summarized in Table 3. Bare Ti exhibited a hardness of 1.49 ± 0.01 GPa. After anodization, the hardness increased to 2.12 ± 0.06 GPa. Following HA electrodeposition at 7 V, the hardness decreased to 1.62 ± 0.03 GPa but remained higher than that of the bare Ti. This result is consistent with the formation of the HA coating observed by FE-SEM. Hardness characterizes only one aspect

**Fig. 9.** The Tafel curves of the experimental samples.**Table 4**

Electrochemical behavior of synthesized samples.

Samples	E _{corr} (V)	i _{corr} (×10 ⁻⁷ A/ cm ²)	R _{corr} (Ωcm ²)	CR (×10 ⁻³ mm/ year)	CP efficient H (%)
Ti	0.1572	28.660	14270	33.303	0
TiO ₂	0.1823	26.245	17413	30.479	8.43
HA/TiO ₂ - 7V	0.1799	25.717	20019	29.883	10.27

of the mechanical behavior of the coating and should not be used alone to predict its performance under physiological loading. Therefore, the interfacial integrity of the HA/TiO₂–7 V and its electrochemical stability were further evaluated by pull-off adhesion testing and electrochemical measurements, respectively. The corresponding results are presented in the following sections.

3.5.4. Adhesion strength

The pull-off adhesion strength of HA/TiO₂–7 V was evaluated using three independent specimens. The measured adhesion strengths were 7.96, 7.41 and 7.27 MPa, giving an average value of 7.55 ± 0.36 MPa. The small variation among the three measurements demonstrates good experimental reproducibility. The average adhesion strength is more than three times higher than the 2.41 MPa reported for HA coatings deposited directly on the Ti substrates, demonstrating the beneficial effect of the TiO₂ nanotube interlayer on coating adhesion [13]. Previous studies have reported pull-off adhesion strengths ranging from 8.87 to 28.7 MPa for HA coatings deposited on TiO₂ nanotube arrays [13]. These differences may be attributed to variations in nanotube dimensions, coating thickness, deposition conditions, and testing procedures. Therefore, direct quantitative comparison should be interpreted with caution. Overall, the results indicate that the TiO₂ nanotube interlayer serves as an effective anchoring interface for electrodeposited HA, resulting in substantially improved interfacial adhesion compared with direct deposition on the Ti substrate.

3.6. Electrochemical behavior

3.6.1. Potentiodynamic polarization

The potentiodynamic polarization curves (Fig. 9) and the corresponding electrochemical parameters obtained by Tafel extrapolation (Table 4) demonstrate that surface modification improved the corrosion resistance of titanium in simulated body fluid (SBF).

The potentiodynamic polarization results showed that both TiO₂ and HA/TiO₂–7 V improved the corrosion resistance of titanium in SBF. The corrosion potential, E_{corr}, shifted from 0.1572 V for the bare Ti to

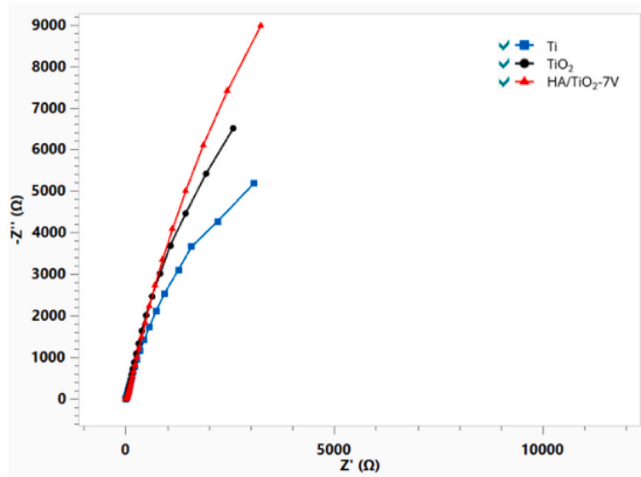


Fig. 10. Nyquist plots of bare Ti, TiO₂ and HA/TiO₂ -7 V samples measured in simulated body fluid.

0.1823 V for the TiO₂ and 0.1799 V for the HA/TiO₂-7 V, indicating a lower thermodynamic tendency toward corrosion after surface modification. Although TiO₂ exhibited a slightly more positive E_{corr} , the overall corrosion performance was determined mainly from i_{corr} , polarization resistance and corrosion rate. The corrosion current density decreased from $28.660 \times 10^{-7} \text{ A/cm}^2$ for the Ti to $26.245 \times 10^{-7} \text{ A/cm}^2$ for the TiO₂ and $25.717 \times 10^{-7} \text{ A/cm}^2$ for the HA/TiO₂-7 V. At the same time, the polarization resistance increased from $14270 \Omega \cdot \text{cm}^2$ to 17413 and $20019 \Omega \cdot \text{cm}^2$, respectively, while the corrosion rate decreased from $33.303 \times 10^{-3} \text{ mm/year}$ for the Ti to $30.479 \times 10^{-3} \text{ mm/year}$ for the TiO₂ and $29.883 \times 10^{-3} \text{ mm/year}$ for the HA/TiO₂-7 V. These results indicate that the TiO₂ layer acted as a protective barrier that suppressed electrochemical dissolution, whereas the HA-containing coating

provided additional protection against electrolyte penetration. Consequently, the corrosion resistance increased in the order $\text{Ti} < \text{TiO}_2 < \text{HA/TiO}_2-7 \text{ V}$, with HA/TiO₂-7 V exhibiting the lowest corrosion current density, the highest polarization resistance and the lowest corrosion rate.

3.6.2. Electrochemical impedance spectroscopy (EIS)

Fig. 10 shows the Nyquist plots of Ti, TiO₂ and HA/TiO₂-7V samples immersed in SBF. All samples exhibit incomplete capacitive semicircles followed by a low-frequency linear tail, indicating that the corrosion behavior is dominated by the passive surface film. The impedance response increases in the order $\text{Ti} < \text{TiO}_2 < \text{HA/TiO}_2-7 \text{ V}$, demonstrating progressively improved corrosion resistance after surface modification. The highest impedance of the HA/TiO₂-7V sample indicates that the HA coating provides the most effective barrier against electrolyte penetration and charge transfer.

The EIS spectra were fitted using the equivalent circuits shown in Fig. 11. Bare Ti exhibited one time constant and was fitted with the $\text{Rs}(\text{Rc}||\text{CPEc})$ circuit, indicating that its electrochemical response is governed mainly by the native oxide film. In contrast, the TiO₂ and the HA/TiO₂-7V samples exhibited two time constants and were fitted using the $\text{Rs}(\text{Rp}||\text{CPEp})-(\text{Rb}||\text{CPEb})$ and $\text{Rs}(\text{R}_1||\text{CPE}_1)-(\text{R}_2||\text{CPE}_2)$ circuits, respectively, reflecting the presence of additional protective surface layers.

As summarized in Table 5, the charge-transfer resistance (R_{ct}) increased from $7.9 \text{ k}\Omega$ for the Ti to $14.1 \text{ k}\Omega$ for the TiO₂ and $20.5 \text{ k}\Omega$ for

Table 5

Equivalent-circuit fitting parameters obtained from the EIS measurements.

Sample	Solution	Layer 1		Layer 2	
		R_s	R_{ct}	R	C
Ti	11.0		$7.9 \text{ k}\Omega$		$173.7 \mu\text{F}$
TiO ₂	12.6		$14.1 \text{ k}\Omega$	31.4Ω	$61.2 \mu\text{F}$
HA/TiO ₂ -7V	16.2		$20.5 \text{ k}\Omega$	70.0Ω	$71.4 \mu\text{F}$

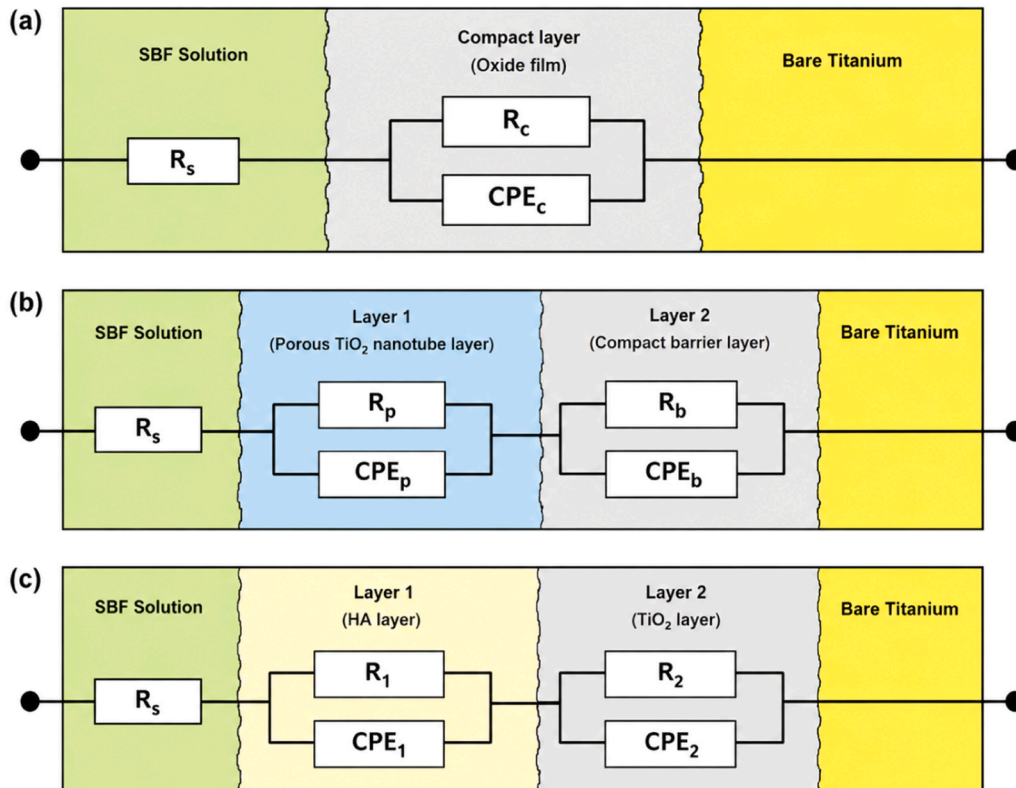


Fig. 11. Equivalent circuit models employed for fitting the EIS spectra of (a) Ti, (b) TiO₂ and (c) HA/TiO₂ -7 V in simulated body fluid (SBF).

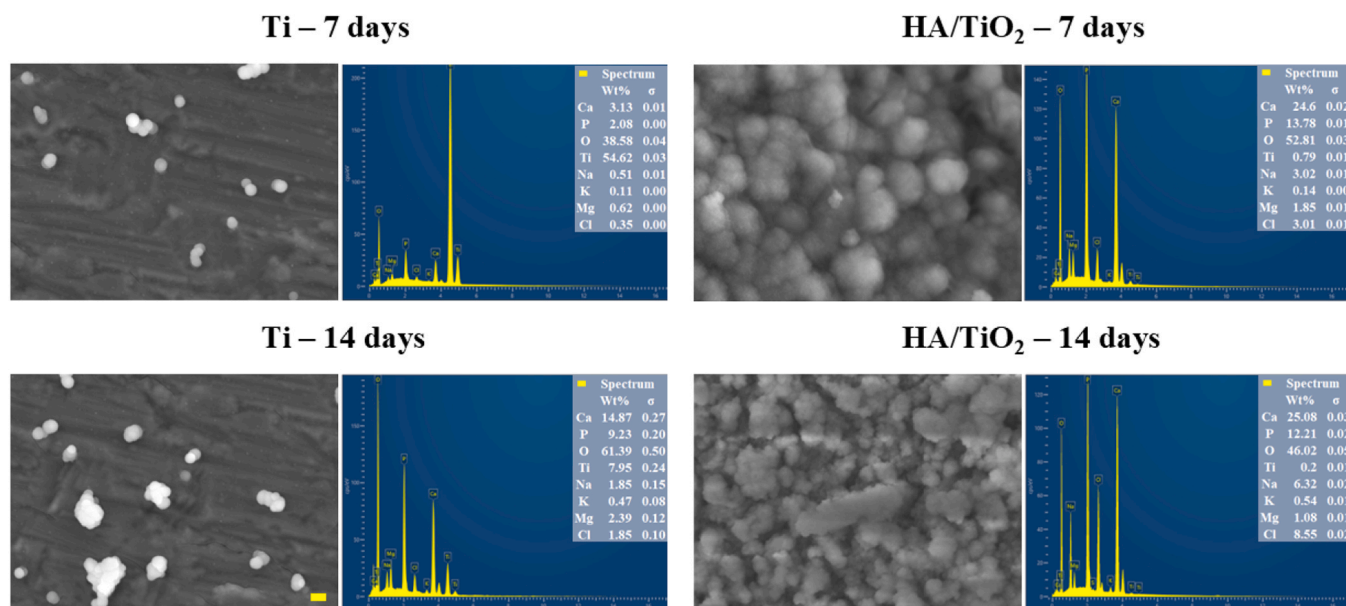


Fig. 12. Surface morphology and EDS analysis of Ti and HA/TiO₂ –7 V after immersion in simulated body fluid (SBF) for 7 and 14 days.

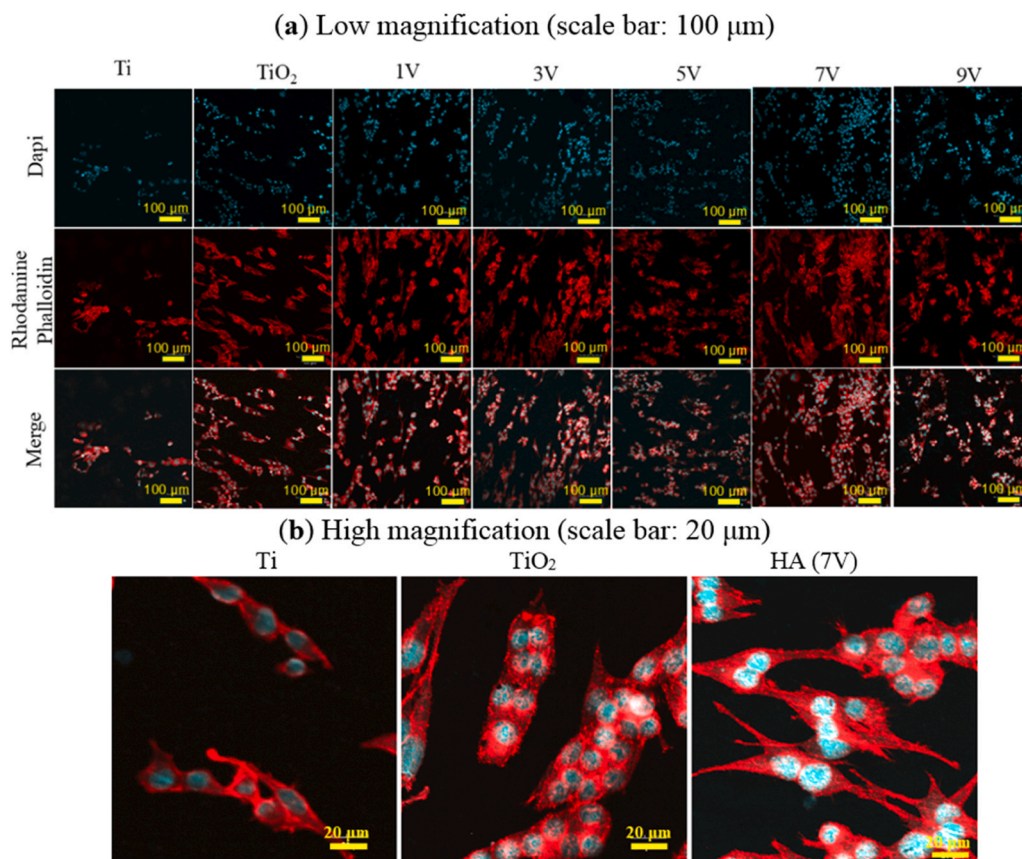


Fig. 13. Fluorescence microscopy images of BHK-21 cells cultured on Ti, TiO₂ nanotubes and HA/TiO₂ coatings deposited at different voltages for 48 h. Cell nuclei were stained with DAPI (cyan) and F-actin was stained with Phalloidin 555 (red).

the HA/TiO₂-7V, while the resistance of the outer layer (R) increased from 31.4 Ω to 70.0 Ω. These results indicate that the TiO₂ nanotube layer and subsequent HA coating effectively suppressed charge transfer and electrolyte penetration, leading to enhanced corrosion resistance. This trend agrees well with the polarization results, confirming the superior protective performance of the HA/TiO₂-7V coating.

3.7. In vitro testing in SBF soaking solution

The apatite-forming ability of bare Ti and HA/TiO₂-7 V was evaluated after immersion in SBF for 7 and 14 days (Fig. 12). After 7 days, only limited calcium phosphate precipitation was observed on the bare Ti, whereas the HA/TiO₂ coating was almost completely covered by

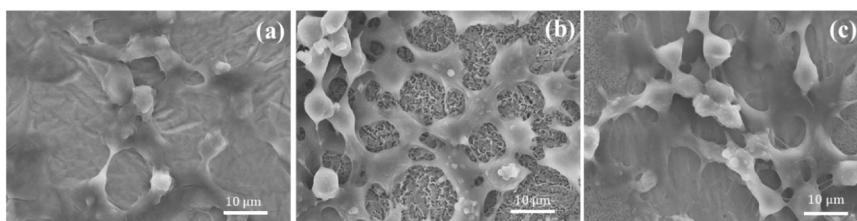


Fig. 14. Representative FE-SEM micrographs of BHK-21 cells on (a) uncoated Ti, (b) anodized TiO₂ nanotube and (c) HA/TiO₂ -7 V following 48 h culture.

newly formed mineral deposits. EDS analysis confirmed the presence of Ca and P on the mineralized surface. After 14 days, mineral deposition on the Ti increased but remained discontinuous. In contrast, the HA/TiO₂-7 V developed a dense and nearly continuous apatite layer composed of closely packed spheroidal particles. The morphological evolution from the initial flower-like coating to compact spheroidal apatite aggregates indicates progressive apatite growth during SBF immersion. These results demonstrate the superior apatite-forming ability of the HA/TiO₂ coating. The enhanced mineralization is consistent with the bioactive HA surface, hierarchical micro/nanostructure and super-hydrophilic surface characteristics identified in the preceding sections, all of which are known to promote apatite nucleation and growth in SBF.

3.8. In Vitro Cytocompatibility and Fibronectin immunofluorescence

The cytocompatibility of all substrates was evaluated by CLSM-based

fluorescence microscopy at 48 h and 72 h post-seeding, with F-actin filaments labeled by phalloidin (red) and nuclei counterstained with DAPI (cyan). Representative micrographs for the 48 h and 72 h time points are presented in Fig. 13 and 15, respectively, corresponding to specimens deposited at 1, 3, 5, 7 and 9 V alongside the uncoated Ti and TiO₂ nanotube controls. A clear voltage-dependent, non-monotonic trend in cellular behavior was observed across both time points, with the 7 V coating consistently demonstrating the most favorable proliferative response among all groups examined.

3.8.1. Cell Morphology and Cytoskeletal Organization at 48 h

The cellular response of BHK-21 cells cultured on Ti, TiO₂ nanotubes and HA/TiO₂ coatings was evaluated by fluorescence microscopy after 48 h of incubation (Fig. 13). The bare Ti exhibited the lowest cell density. After anodization, the TiO₂ nanotube surface supported a higher number of cells than bare Ti. Among the HA-coated samples, the 3 V

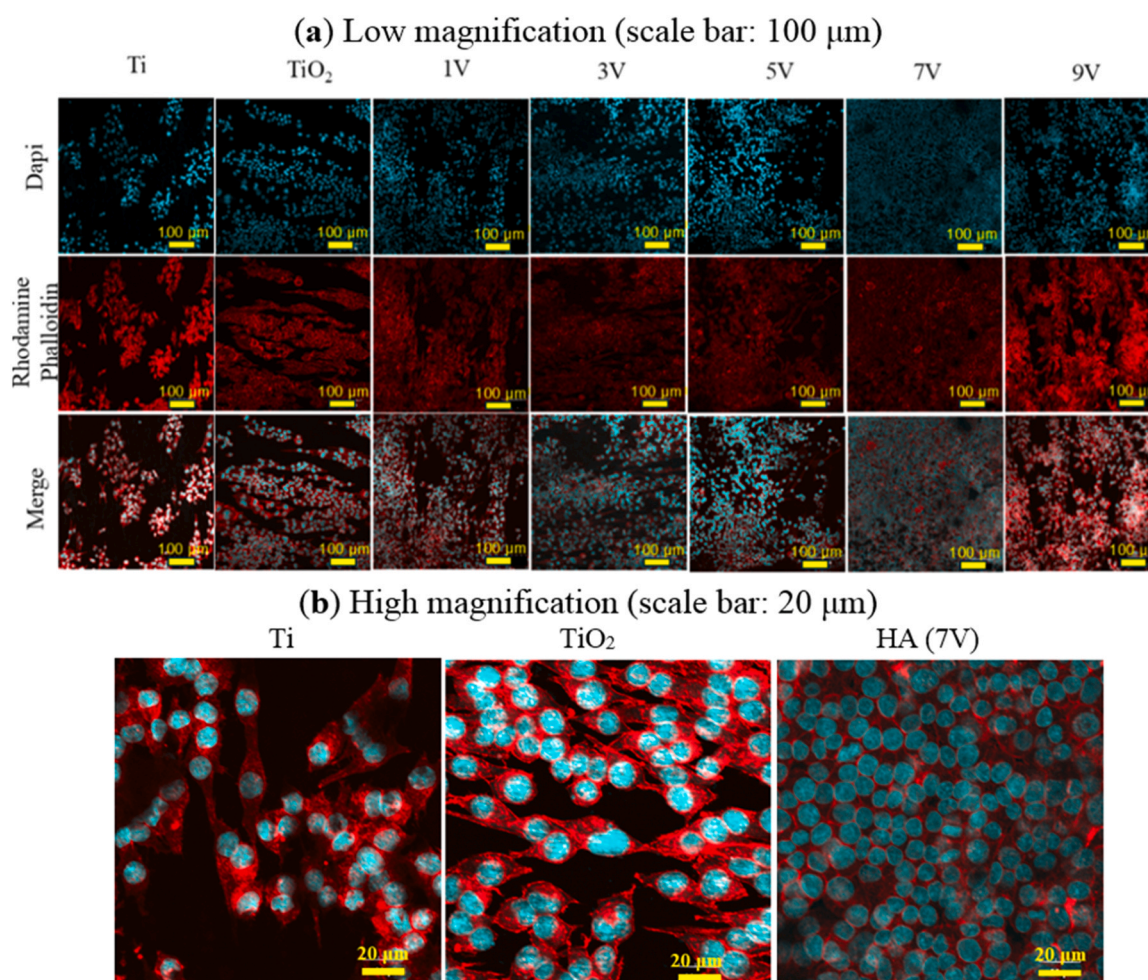


Fig. 15. Fluorescence microscopy images of BHK-21 cells cultured for 72 h on uncoated Ti, anodized TiO₂ nanotube and electrochemically deposited HA coatings at deposition voltages of 1, 3, 5, 7 and 9 V. Cell nuclei were labeled with DAPI (cyan) and filamentous actin (F-actin) was visualized using Phalloidin 555 (red).

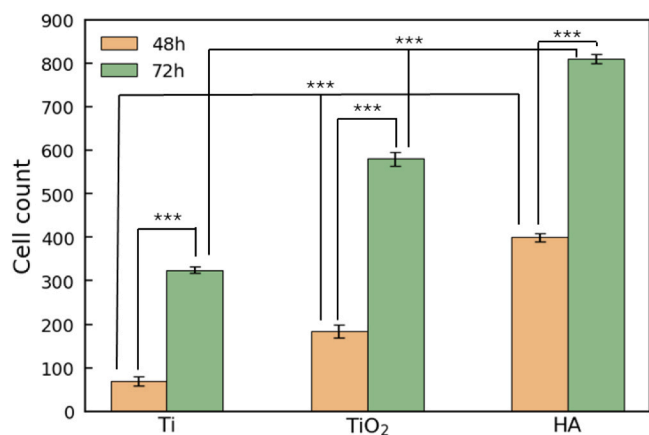


Fig. 16. Quantitative analysis of BHK-21 cell density after 48 and 72 h of culture.

coating showed sparse cell distribution, whereas cell density progressively increased on the 5 V and 7 V coatings. The 7 V coating exhibited the highest cell density and the most uniform cell distribution, while fewer cells were observed on the 9 V coating. Representative high-magnification images showed that cells on the 7 V coating exhibited a more flattened morphology than those on the other surfaces. This trend is consistent with the FE-SEM observations, which demonstrated that the 7 V coating possessed the most homogeneous surface morphology. Overall, the HA/TiO₂ coating deposited at 7 V exhibited the most favorable cellular response among the investigated samples.

Representative SEM images of BHK-21 cells cultured for 48 h on bare Ti, TiO₂ nanotubes, and HA/TiO₂-7 V are shown in Fig. 14. Cells on bare Ti exhibited a predominantly rounded morphology with limited spreading and few cytoplasmic extensions. In contrast, cells cultured on the TiO₂ nanotube surface displayed a more flattened morphology with increased spreading and more numerous filopodia. The HA/TiO₂ coating showed the most extensive cell spreading, with abundant filopodia

extending over the coating surface and close contact between adjacent cells. These observations are in good agreement with the fluorescence microscopy results. The improved cell morphology may be associated with the hierarchical surface structure and the bioactive nature of the HA coating, which have been reported to promote cell spreading and cell-surface interactions on titanium-based biomaterials [52].

3.8.2. Cell Morphology and Cytoskeletal Organization at 72 h

Fluorescence microscopy images obtained after 72 h of culture were shown in Fig. 15. Compared with the 48 h observations, all samples exhibited a progressive increase in cell density. Although the overall trend among the investigated surfaces remained unchanged, the differences became more pronounced after prolonged incubation. HA/TiO₂-7 V showed the greatest increase in cell density, resulting in an almost continuous cell layer covering the surface. In contrast, the 1 V and 3 V coatings still exhibited incomplete surface coverage, while the 9 V coating remained less densely populated than the 7 V coating. These findings confirm that the superior cellular response observed on the 7 V coating at 48 h was maintained throughout the 72 h culture period.

Quantitative analysis of DAPI-stained nuclei (Fig. 16) confirmed the qualitative fluorescence microscopy observations. Cell density was quantified from three representative CLSM fields for each specimen using ImageJ software. At both 48 and 72 h, significant differences were observed among the investigated surfaces (one-way ANOVA, $p < 0.001$). Cell density increased on all surfaces from 48 to 72 h, indicating continuous cell growth during the culture period. The HA/TiO₂ coating deposited at 7 V consistently exhibited the highest cell density, followed by TiO₂ nanotubes, whereas bare Ti showed the lowest values. Although the ranking of the three surfaces remained unchanged, the differences became more pronounced after 72 h, with the HA/TiO₂ coating maintaining a clear advantage over Ti and TiO₂ nanotubes. These quantitative results are in good agreement with the qualitative fluorescence microscopy observations and further demonstrate the superior ability of the HA/TiO₂ coating to support cell growth under the present experimental conditions.

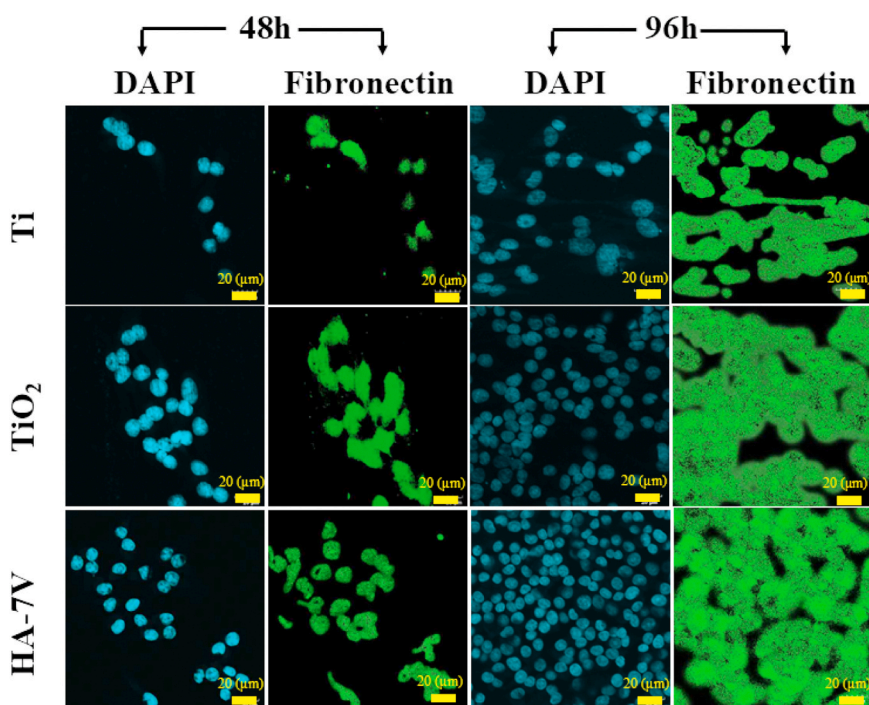


Fig. 17. Representative fibronectin immunofluorescence images of BHK-21 cells cultured on Ti, TiO₂ nanotubes and HA/TiO₂-7 V for 48 and 96 h. DAPI (cyan), fibronectin (green).

3.8.3. Fibronectin immunofluorescence

Fibronectin immunofluorescence staining was performed to evaluate extracellular matrix protein deposition on Ti, TiO₂ nanotubes and HA/TiO₂-7 V after 48 and 96 h of culture (Fig. 17). At 48 h, fibronectin staining was weak and discontinuous on the bare Ti. A stronger and more continuous signal was observed on the TiO₂ nanotube surface, whereas the HA/TiO₂ coating exhibited the most extensive fibronectin distribution. After 96 h, fibronectin expression increased on all surfaces, consistent with the increase in cell density observed by fluorescence microscopy. Nevertheless, distinct differences among the substrates remained. Bare Ti still showed discontinuous fibronectin staining, while the TiO₂ exhibited a more homogeneous distribution. In contrast, the HA/TiO₂ coating displayed the strongest and most uniform fibronectin signal, covering nearly the entire cell-populated region. Because fibronectin is a key extracellular matrix protein involved in cell adhesion, the enhanced fibronectin deposition on the HA/TiO₂ coating provides further evidence of its improved cytocompatibility.

4. Conclusions

Hydroxyapatite coatings were successfully synthesized on TiO₂ nanotube arrays by electrochemical deposition. The deposition potential played a critical role in regulating the coating morphology, composition, and functional properties. Among the investigated conditions, deposition at 7 V produced a homogeneous hierarchical coating with a near-stoichiometric Ca/P ratio (1.69), superhydrophilic wettability, improved interfacial adhesion, enhanced corrosion resistance, and good cytocompatibility. Moreover, the optimized coating significantly promoted bone-like apatite formation during immersion in simulated body fluid (SBF), demonstrating good in vitro bioactivity. Collectively, the results demonstrate that optimizing the deposition potential is an effective strategy for tailoring the surface characteristics and biological performance of HA/TiO₂ coatings. The HA/TiO₂ coating therefore shows promising potential as a bioactive surface modification for biomedical implants.

CRediT authorship contribution statement

Le Thi Tam: Validation, Methodology, Formal analysis. **Hoang Van Vuong:** Resources, Methodology, Data curation. **Nguyen Truong Giang:** Visualization, Validation, Software. **Nguyen Van Toan:** Visualization, Validation, Resources. **Tran Thi Trang:** Writing – original draft, Methodology, Data curation. **Nguyen Thi Nguyet:** Visualization, Data curation, Conceptualization. **Nguyen Thi Thanh Tuyen:** Software, Methodology, Formal analysis. **Le Van Toan:** Validation, Methodology, Data curation. **Vuong-Hung Pham:** Writing – review & editing, Supervision, Project administration. **Le Thi Bang:** Methodology, Formal analysis, Conceptualization. **Ta Quoc Tuan:** Visualization, Software, Resources. **Cao Xuan Thang:** Validation, Methodology, Formal analysis. **Nguyen Ngoc Thang:** Supervision, Formal analysis, Data curation.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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Data availability

Data will be made available on request.

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