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REVIEW ARTICLE

Electrophoretically deposited titanium and its alloys in biomedical engineering: Recent progress and remaining challenges

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Abstract

Over the past decade, titanium implants have gained popularity as the number of performed implantation operations has significantly increased. There are a number of methods for modifying the surface of biomaterials, which are aimed at extending the life of titanium implants. The developments in this field in recent years have required a comprehensive discussion of all the properties of electrophoretically deposited coatings on titanium and its alloys, taking into account their bioactivity. The development that took place in this field in recent years required a comprehensive discussion of all the properties of coatings electrophoretically deposited on titanium and its alloys, with particular emphasis on their bioactivity. Herein, we attempt to assess the influence of the electrophoretic deposition (EPD) process parameters on these coatings' biological and mechanical properties. Particular attention has been addressed to the *in-vitro* and *in-vivo* studies conducted hitherto. We have seen an increased interest in using titanium alloys without the addition of toxic compounds and gaps in the EPD field such as the uncommon endeavors to develop a "Design of experiments" approach as well as the lack of assessment of the surface free energy and detailed topography of electrophoretically deposited coatings. The exact correlation of coating properties with EPD process parameters still seems explicitly not understood, necessitating more future investigations. Ipso facto, the exact mechanism of particle agglomeration and Hamaker's law need to be fathomable.

KEYWORDS
biomaterials, biomedical application, coatings, electrophoretic deposition, implants, titanium alloys

Abbreviations: AO, anodic oxidation; BAG, bioactive glass; BG, bioglass; CNTs, carbon nanotubes; CP-Ti, commercially pure titanium; CR, corrosion rate; CS, chitosan; E_{corr}, corrosion potential; EIS, electrochemical impedance spectroscopy; EPD, electrophoretic deposition; GO, graphene oxide; HA, hydroxyapatite; hBMSCs, human bone marrow stromal cells; hBN, hexagonal boron nitride; hFOB, human fetal osteoblast cells; HNTs, halloysite nanotubes; j_{corr}, corrosion current density; Kao, kaolinite nanoclay; MAO, micro-arc oxidation; MC3T3-E1, osteoblast cells derived from *Mus musculus calvaria*; MG-63, human osteosarcoma cells; MTT, (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide); PE, protection efficiency; peak, material portion; PEEK, polyetheretherketone; PSZ, partially stabilized zirconia; PTFE, polytetrafluoroethylene; R_{pol}, polarization resistance; r-SBF, revised simulated body fluid; S_a, arithmetic average of the 3D roughness; SBF, simulated body fluid; S_{da}, mean dale area; S_{dv}, mean dale volume; S_{ha}, mean hill area; S_{hv}, mean hill volume; S_{mr_1}, peak material portion; S_{mr_2}, valley material portion.

1 | INTRODUCTION

Among the common problems of civilization, there are diseases of the musculoskeletal system, the number of which increases due to a traffic collision, a sedentary lifestyle and an aging society.[1,2] Therefore, scientists focus on finding such biomaterials that would enable long-term replacement of destroyed or damaged bone.[3] Such material must be characterized by high biocompatibility, corrosion resistance in the human body and suitable biomechanical properties.[1,3]

Titanium and its alloys are among the metallic materials and are widely used in implantology.[4] This is not only due to the fact that the chemical compositions of these materials are very diverse, which allows for their appropriate selection in terms of possible allergic reactions.[1] Crucial are their biocompatibility, biomechanical properties and good corrosion resistance (Table 1), which is a consequence of the formation of a stable passive layer on the titanium surface.[3,10] An important parameter of titanium endoprostheses is Young's modulus (Table 1), which should be as close as possible to Young's modulus of the cortical part of the replaced bone.[10] Otherwise, stress shielding may occur, which contributes to the loosening of the implant and ultimately can result in reoperations, which often end in failure.[1,10] Nowadays, titanium and its alloys are used during total joint replacement surgery or as fracture fixation elements.[11] Commercially pure titanium (CP-Ti), depending on the grade, is mainly used as a partial resurfacing of the knee or hip joint (especially as a component of the acetabulum), as well as for (ii) craniofacial reconstruction and (iii) spinal interbody fusion.[10–13] Further, titanium alloys are used for hip and knee arthroplasty, especially as a tibial or femoral component, and also in fracture fixation in a humeral part.[10]

Despite the variety of good properties of titanium materials, there are still concerns with their adequate stability and corrosion resistance in the environment of body fluids, especially in the long-term aspect.[14–16] The release of corrosion products can occasion metallosis, which consequently may lead to the loosening of the implant or deterioration of the implant properties.[14,15] In addition, some endoprostheses are rejected by the recipient at an early postoperative stage due to the occurrence of an allergic reaction.[1] Moreover, the implant should possess adequate microstructure which accelerates the formation of a permanent bond at the tissue-implant interface.[17] Due to the above, methods of surface modification of metallic implants are being sought that would allow to fulfill these requirements, especially in the long-term aspect. The following methods that have already been used can be distinguished: plasma thermal spraying,[18] ion implantation,[19] plasma spraying,[20] magnetron sputtering,[21] physical vapor deposition,[22] chemical vapor deposition,[23] sol–gel,[24] micro-arc oxidation (MAO),[25–28] anodic oxidation (AO)[29–32] and electrophoretic deposition (EPD).[33–36] The last three abovementioned methods belong to the group of electrochemical methods, which are characterized by simplicity and a relatively low price compared to the rest of the mentioned methods. In addition, they facilitate the formation of coatings with disparate morphology, roughness, crystallinity, chemical composition, wettability as well as corrosion and mechanical properties on materials of various shapes and the equipment is inexpensive.[37] All electrochemical methods are characterized by the ability to change the following process parameters: voltage, time and concentration, pH, temperature and composition of the electrolyte/suspension.[7,38–44] However, amid these methods, the EPD has more variables that can be modified. This is primarily due to the fact that in addition to the process parameters, the parameters related to the suspension can also be altered. Such diversity in the possibility of changing parameters gives scientists a wide range of surface modifications, what the authors have shown in this paper. On the other hand, there are some reports where AO and MAO were performed in suspension and thus incorporation, for example hydroxyapatite (HA), into titanium coatings was possible.[45,46] However, these are few reports and the diversity of incorporated compounds is much more modest than that of the EPD. In addition, EPD is characterized by a short process time and high deposition rate compared to other electrochemical methods (Figure 1). Additionally, AO requires in-situ detection techniques to track the course of

TABLE 1 Properties of selected titanium and its alloys compared to properties of human bone and their exemplary application in the biomedical field.[5–9]

Material	Elastic modulus (GPa)	Yield strength (MPa)	Tensile strength (MPa)	Corrosion potential (V)	The influence of elements on the human body
CP-Ti (Grade 1–4)	105	170–480[a]	240–550[a]	−0.3 to 0.2[b]	-
Ti-6Al-4V	112	850–900	895–930	−0.4[b]	V and Al are toxic and can cause Alzheimer's disease, osteomalacia or neuropathy
Ti-6Al-7Nb	110	921	900–1050	0.3[c]	Al is toxic, Nb promotes apatite-formation
Ti-13Nb-13Zr	79–84	900	973–1037	0.2[c]	Nb promotes apatite-formation
Ti-35Nb-5Ta-7Zr	55–66	793	596	-	
Bone	4–40	-	90–140	-	-

[a]Varies according to Grade.
[b]In NaCl (3.5 wt%) solution.
[c]In Ringer's solution.

surface reactions, while MAO is more expensive, not well-commercialized and the design of coating properties is more difficult.[48]

EPD is a bridge between two processes: deposition and electrophoresis.[49] The applied electric field enables the deposition of thick films or bulk components.[50] To date, the following chemical compounds have been deposited on titanium and its alloys: chitosan (CS),[51–53] HA,[52,54–56] polytetrafluoroethylene (PTFE),[57] polyetheretherketone (PEEK),[54,57,58] molybdenum disulfide nanosheets,[54] multi-walled carbon nanotubes,[59] sodium alginate,[60] bioactive glass (BAG),[56,61] copper nanoparticles,[36] iron oxide,[53] graphene oxide (GO),[62] silver nanoparticles,[33] kaolinite nanoclay (Kao),[61] and so forth. Due to the growing number of papers concerning surface modification with the EPD method, it is essential to systematize the achievements and shortcomings so far. In recent years, there have been released review papers that concerned only specific chemical compounds used during the EPD process, for example chitosan-based composite coatings,[63] polymers and proteins,[64] metal oxides,[65] carbon nanotubes[66] or carbon nanomaterials[67] and their content was not entirely focused on the biological aspects of coatings. This literature review focuses on a wide range of compounds used for EPD only in respect of biomedical applications. All properties of the biomaterial

FIGURE 1 Advantages and disadvantages of the electrophoretic deposition method in respect of biomedical applications.[38–41,47] Designed and illustrated by the authors of this work.

coatings were discussed concerning their bioactivity. Particular emphasis was placed on the achievements in the immersion tests, *in-vitro* cell culture assays and *in-vivo* assays, which performance is a first step to the potential use of coated materials in clinical practice. In addition, to fulfill the needs of the reader's group of this work (implantologists, surgeons as well as biomedical, material and mechanical engineers), future outlooks and challenges were discussed.

The methodology of preparing this article was based on several components: topic (titanium and its alloys, electrophoretic deposition), date of publication (chiefly the last 5 years), addresses (scientists engage in similar topics in their research, physicians—implantologists and surgeons), published research results (influence of process parameters on the properties of coatings, notably in terms of their influence on bioactivity).

2 | SHORT DELINEATION OF EPD

EPD facilitates the deposition of coatings on the surface of an object. During this process, particles from a dispersion system are deposited on the surface of the processed material under the influence of an applied electric field (Figure 2).[63] Currently, this method is gaining great popularity in academic and industrial sectors because it has many unique features (Figure 1).[38,50,67–69] Particularly important in the development of this process is the fact that the process can be carried out on materials of any shape (flat, cylindrical, etc.) and size with minimal changes in the construction and position of the electrode as well as the construction of workstation, providing a layer with a superior homogeneity of the microstructure.[47] Therefore, laboratory research conducted with small samples (usually round with a diameter of 10–20 mm or square with a side length of 10–15 mm) can often be successfully scaled for industrial usage, including biomedical fields.[66]

The possibility of changing the process parameters in the case of EPD is much wider than in the case of other electrochemical methods. This is mainly due to the fact that the parameters related to the suspension can also be altered.[50] The parameters incident to the suspension are particle size, as well as conductivity, mobility and zeta potential of suspension.[70,71] The particle size and shape that are used in EPD affect the mobility of the particles in the electrolyte, the zeta potential of the suspension, and the thickness of the deposited coating.[63] Accordingly, they must be of optimal size, shape and weight to remain suspended during the process and ultimately to be completely and uniformly dispersed over the coating.[69,71] Thereupon, they must have optimal properties in order to be able to remain in suspension during the process. The optimal conductivity of the suspension facilitates the mobility of the particles. Highly conductive suspension causes weak particle movement. If the suspension is not very conductive, the particles become electronically charged and become unstable. The optimal conductivity value is different for each system. The conductivity of the suspension can be modified by changing the process parameters: as the temperature and/or concentration of the dispersant decrease, the conductivity of the suspension decreases.[69] The zeta potential is the potential between the dispersant and the layer of fluid that is attached to the surface of a particle. It enables the stability of colloidal systems to be determined and is a key parameter in EPD.[63] The optimal zeta potential influences the interaction between the individual particles of the suspension, and thus the quality of the coatings.[50,72] Zeta potential values usually range from −100 mV to +100 mV.[73]

The parameters related to the process are deposition time, applied voltage, conductivity of substrate and temperature.[69,71] Duration mainly affects the thickness of the deposited coatings. The characteristic of the deposition rate is the time dependence of the deposition rate. It rises linearly at the start of the process and then lowers over time until it reaches a plateau at some point. This happens when the coating is thick enough to break the conductivity.[68,71] The applied electrical voltage also affects the coating, and in general, the greater the voltage, the thicker the coating. However, optimal

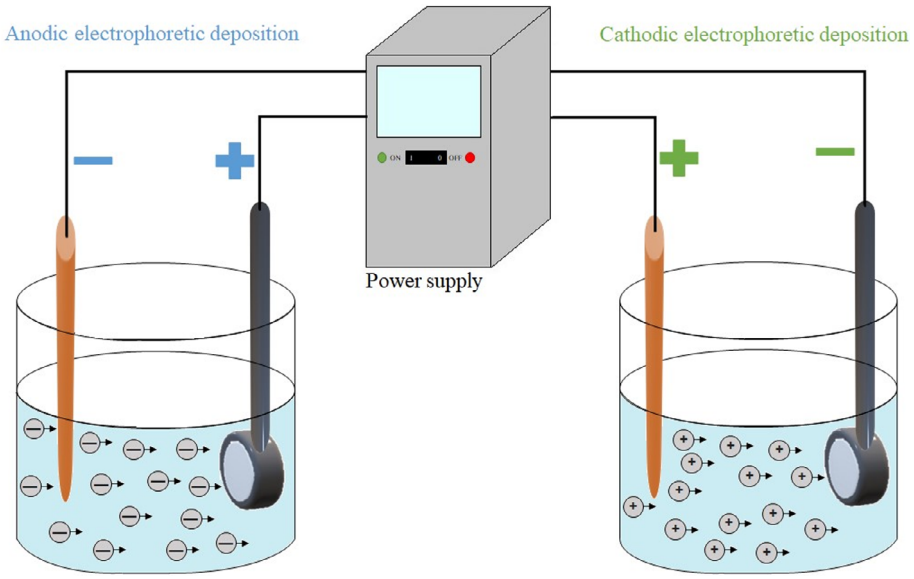

FIGURE 2 Schematic illustration of anodic and cathodic electrophoretic deposition processes. Anode electrophoretic deposition occurs when negatively charged particles are deposited on the positive electrode. Cathodic electrophoretic deposition occurs when positively charged particles are deposited on a negative electrode. Appropriate modification of the surface charge on the particles enables the selection of either of the two deposition modes.

voltage values should be used. This is due to the fact that too low a voltage value does not cause the phenomenon of electrophoresis. Too high voltage value leads to turbulence, which resultantly has a negative effect on the quality of the coating.[68,69,71,72] The quality of the coating also depends on the conductivity of the substrate. A low conductivity value results in a heterogeneous coating and the deposition process is very long.[68] Furthermore, the temperature during the process must be stabilized, because its increase reduces the resistance of the suspension, which consequently affects the velocity of ion migration and reproducibility of the process. It is stated that the ions' mobility increases by about 2.4% for each degree of temperature increase.[74] Therefore, the temperature during the process is usually kept at room temperature (see Section 3 herein), which can be achieved by using a stirring and a cooling/heating system.

3 | PROPERTIES OF COATINGS—ANALYSIS OF ACHIEVEMENTS AND SHORTCOMINGS

3.1 | Morphology

The morphology of the implant should reflect the morphology of the human bone, especially porosity, as it can promote cell adhesion and osseointegration.[75–77] The quality and morphology of the obtained coating and its final parameters depend on many variables.[60] The effect of different voltage values and deposition time on the quality of the obtained coatings on CP-Ti (Table 2) was investigated by Mehana Usmaniya et al.[61] Optimal deposition conditions were obtained at a voltage of 50 V and a time of 5 min because under these conditions the coatings characterized uniform morphology and had no cracks. Heterogeneous coatings were obtained at a lower deposition voltage, while hydrogen evolution was announced at a higher voltage. Apart from the influence of time and voltage, the influence of particles and their sizes on the quality of the deposited coatings can be measured. This issue was raised by Jugowiec et al.[51] where the deposition of sol–gel BAG (0.2–4.5 μm) and chitosan coating on Ti-13Nb-13Zr was practiced. The most homogeneous and continuous composite coatings were obtained at a constant voltage of 10 V for 4 min (Figure 2). Diameter of the sol–gel BAG particles in the coatings ranged from 660 nm to 11.3 μm, which proves that the sol–gel glass particles agglomerate during EPD. Coatings obtained from bioglass (BG) powder (1.6–9.8 μm) and CS were the most homogeneous and continuous at a constant voltage of 6 V for 6 min (Figure 3). The diameter of the BG powder particles in the coatings ranged from 320 nm to 16.5 μm, which confirms the fact that bioglass powder particles agglomerate during EPD. This is crucial because it determines whether thick films or bulk components are obtained on the surface,[50] which defines the surface morphology. Moreover, in all cases, the increase in electrical voltage prompted heterogeneity, what could have been occasioned by the hydrogen evolution.[61] More examples of the influence of EPD conditions on the morphology of the coatings are presented in Table 2.

The processes carried out after EPD also influence the microstructure of the coating, among which can be mentioned initial thermal treatment[78] and target thermal treatment.[55] Incorrectly carried out thermal treatment (the application of too high temperature or too fast process) can cause cracks in the coating.[33] An accurately conducted process improves the homogeneity of coating.[57] Adhesion and migration of cells, as well as the formation of an appropriate bond at the tissue-implant interface, depend on the surface morphology, primarily on its porosity.[75,76] The optimal pore size, which has an affirmative effect on the bioactivity of coatings, is 200–600 μm.[79] Optimizing the surface morphology during the EPD process is elaborated because the particles can agglomerate.[51,61] So far, the exact mechanism of particle agglomeration is under consideration. It is believed that the double-layer distortion model[80] sufficiently describes this phenomenon, but researchers should focus on understanding the precise mechanism. Furthermore, since the morphology of the implant surface has a vast impact on its bioactivity in the human body, it is crucial to modify its surface using respective process parameters. The authors noted that few publications focus on the accurate adjustment of morphology to biomedical requirements without the use of additional post-treatments. We believe that the application of other technology during the EPD process, such as ultrasound treatment, would facilitate obtaining more homogeneous layers. Ultrasound is a widely used auxiliary technology that has already been applied during electroless plating or electrochemical plating. As a result, coatings with increased homogeneity were obtained.[81] The use of such treatment during EPD has been reported sporadically,[62] and it seems to have a particular purpose in the formation of layers on biomaterials, where homogeneity is essential.

3.2 | Thickness

The appropriate coating thickness for implants has not been determined.[82] However, it is substantial that the coating does not delaminate or crack and simultaneously guarantees congenial corrosion protection.[83,84] EPD exhibits an increase in the thickness of the coating (to a certain value what was explained in Chapter 2 herein) with increasing voltage and duration.[33,35] The thickness of the coating is also influenced the particle content of the suspension. In the case of depositing the nanoHA coating on Ti-13Zr-13Nb, it was noticed that an increase in the nanoHA powder content in the suspension gave rise to an increase in the thickness of the coating.[35] This is probably because a larger amount of particles can cause the intensification of the electrochemical process (the coating is filled with particles), and this occasions an increase in the thickness of the coating.[85] The size of the particles in the suspension also affects the thickness of the coating, however, this parameter is not linear what was described by Fajri et al.[55] Natural HA of various sizes (25, 63, 125 μm) was deposited on Ti-29Nb-13Ta-4.6Zr. For these sizes, the following thicknesses were obtained: 121.27, 163.57 and 63.80 μm, respectively. The addition of nanoCu particles to nanoHA powder changes this volatility. As the diameter of the nanoCu particles increases, a decrease in the thickness of the coating is observed.[34] The differences could

TABLE 2 The individual conditions of the electrophoretic deposition process on titanium and its various alloys and the morphology/structure/crystallinity of the obtained coatings.

Material	Suspension	Parameters			Post treatment	Morphology/structure/crystallinity	References
		Temperature	Time (min)	Voltage (V)			
CP-Ti (Grade 1)	40 vol% C_2H_5OH and 60 vol% water + 1 g L^{-1} of BAG—Kao mixture (100% BAG, 80:20 BAG:Kao, 60:40 BAG:Kao or 50:50 BAG:Kao)	Room temperature	1–10	10–100	-	A deposition voltage of 50 V and time of 5 min is optimized (crack-free and uniform coatings). Inhomogeneous coatings and hydrogen evolution were monitored during depositions at lower and higher voltages, respectively. The presence of nanoclay in the coating. Densification of the coatings as the concentration of nanoclay increased in the nanocomposites.	61
Ti-13Nb-13Zr	Particles of BAG (0.5, 0.8, 1, 2, 3 or 4 g L^{-1}) and CS (2 g L^{-1}) in a mixture of distilled water, 50 vol% of C_2H_5OH and 0.5 vol% of CH_3COOH	-	1–8	4–20	-	Microstructure uniformly embedded in an amorphous CS matrix. The deposits of glass particles were nonuniform in thickness. Presence of an amorphous CS phase and amorphous glass phases and also a crystalline phase of HA.	51
	Particles of sol–gel BG (0.5, 0.8, 1, 2, 3 or 4 g L^{-1}) and CS (2 g L^{-1}) in a mixture of distilled water, 50 vol% of C_2H_5OH and 0.5 vol% of CH_3COOH					Microstructure uniformly embedded in an amorphous CS matrix. The deposits of sol–gel bioglass were nonuniform in thickness. The coating was dense and without pores. Presence of an amorphous CS phase and amorphous glass phases.	
Ti-13Nb-13Zr	CS-nanopowder dissolved at a ratio of nc-HA-p 1, 2, 3, 4 and 5 g L^{-1} in 50% C_2H_5OH in distilled water containing 0.5% CH_3COOH	-	1, 2, 3, 4, 5 or 6	8–30	-	Presence of crystalline HA (hexagonal primitive, hp) phase. The nc-HA-p/CS coating microstructure was non-uniform, composed of HA agglomerates of different sizes.	52
	CS-suspension dissolved at a ratio of nc-HA-s 1, 2, 3, 4 and 5 g L^{-1} in 50% C_2H_5OH in distilled water containing 0.5% CH_3COOH					Presence of crystalline HA (hexagonal primitive, hp) phase. The nc-HA-s/chitosan coating microstructure was composed of nanocrystalline HA particles, homogeneously embedded in an amorphous chitosan matrix.	

TABLE 2 (Continued)

Material	Suspension	Parameters			Post treatment	Morphology/structure/crystallinity	References
		Temperature	Time (min)	Voltage (V)			
Ti-13Zr-13Nb	0.1 g, 0.2 g and 0.5 g of HA nanopowder in 100 mL of C_2H_5OH	Room temperature	1	15, 30 or 50	-	For 0.1 and 0.2 g L^{-1} of HA uniform surface; for 0.5 g L^{-1} of HA surfaces always homogenous. Cracks do not appear only on the nanoHA coatings for 0.1 g L^{-1} of HA and 15 V and for 0.1 g L^{-1} of HA and 30 V. Peaks typical of crystalline HA. The amorphous HA was not observed.	35
Ti-13Zr-13Nb	1 g of needle-shaped nano HA and 0.05 g of nanocopper in 1 L of 99.8% pure C_2H_5OH	Room temperature	1 or 2	30	Thermal treatment (vacuum, 800°C for 120 min; cooling in the furnace to room temperature: 200°C h^{-1})	Coatings well adjacent to the Ti-13Zr-13Nb alloy surface, fully crystalline, possessing typical porous structure, and suitable bioactive coatings for load-bearing implants. The presence of crystalline HA.	36
Ti-13Zr-13Nb	0.1 g of HA nanopowder in 100 mL of C_2H_5OH	Room temperature	1	15 or 30	Thermal treatment (vacuum, 800°C for 120 min; cooling in the furnace to room temperature: 200°C h^{-1})	NanoHA coatings (grain agglomerates separated by numerous pores).	33
nanoHA/Ti-13Zr-13Nb	0.01 g of nanoAg powder in 100 mL of C_2H_5OH	Room temperature	5	60	-	The clusters of nanosilver on nanoHA coating; the distribution of nanosilver particles was uniform.	
Ti-29Nb-13Ta-4.6Zr	4 g natural HA (extracted from bovine bones with sizes: 25, 63 or 125 μm) in 100 mL ethanol + HNO_3 to reduce pH to 4	-	5	10	Thermal treatment (heating: 800°C for 660 min; holding: 60 min; annealing: 720 min)	25 μm: microstructure finer, homogeneous, small crack, and distributed more smoothly than others. The best quality of HA coating; 63 μm: coating with good homogeneous and smoothness; 125 μm: an uneven structure. Particles are not deposited evenly. The coating is untidy, have many agglomerations and easily piling up from the substrate.	55

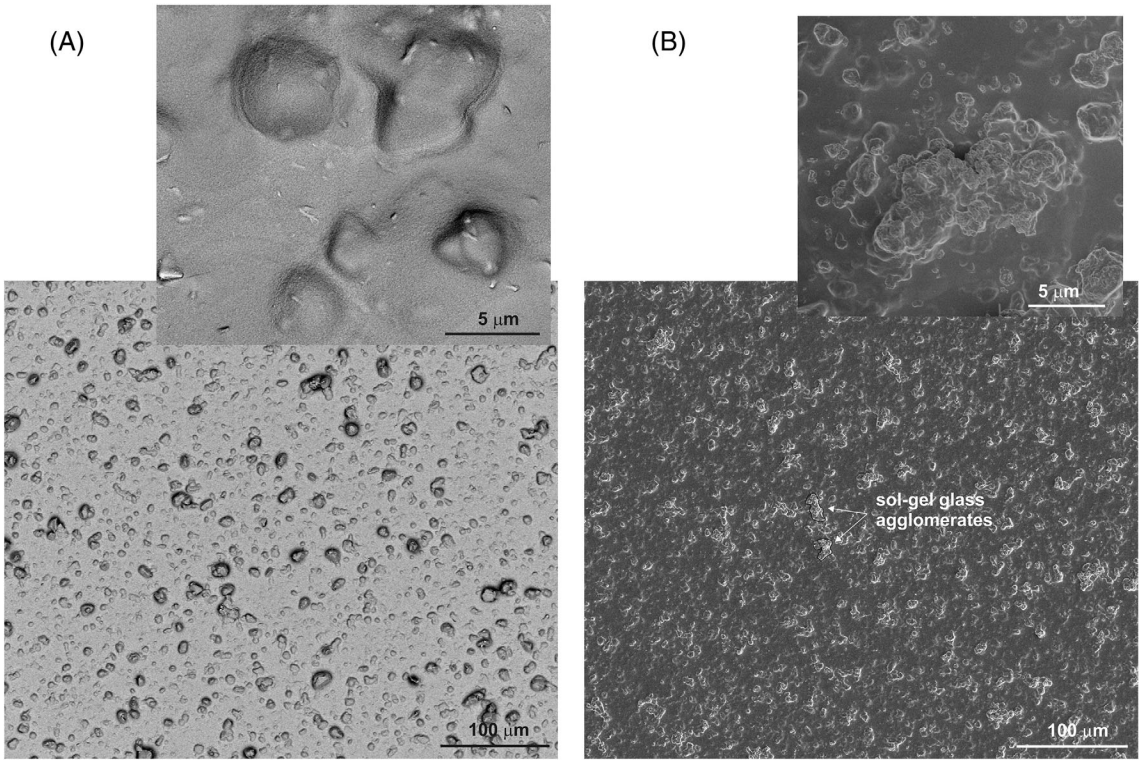

FIGURE 3 Microstructure of the composite coating with BG powder and chitosan deposited at 6 V for 6 min (A) and composite coating with sol–gel BAG and chitosan deposited at 10 V for 4 min (B) on the Ti-13Nb-13Zr. More specifics attributable to the morphology are detailed in Table 2. Reprinted from,[51] with permission from Elsevier (license 5500240381842).

occasion due to the parameters of the particles, that is their chemical composition, mobility or zeta potential.[49,50,54]

Electrophoretically deposited coatings and their thickness are often nonuniform[51,56,86] as the particles during the process can agglomerate.[51] As mentioned above, the better recognition of the mechanism of particle agglomeration and usage of ultrasonic technology could enable an increase in the layers' homogeneity and thus make the thickness of the layers obtained during the EPD process more predictable as well as the process even more repeatable. It was noted that the use of ultrasound during other electrochemical processes enhances the mass transfer process and consequently increases the thickness of the coatings.[81,87] In terms of the above arguments and the fact that the EPD process is characterized by a high deposition rate, the simultaneous application of these two technologies would further reduce the process time. Nevertheless, so far adjustment of the parameters and application of the appropriate post-treatment can enable obtaining coatings that will promote the formation of a strong bond between the tissue-implant interface. We would like to draw attention to the lack of sufficient research on the correlation of the mathematical model for the kinetics of the EPD process known as Hamaker's law with the results obtained in experiments. The law suggested by Hamaker indicates that the amount of material deposited over time during the process is proportional to (i) the electrophoretic mobility of the particles, (ii) the electric field strength, (iii) the electrode surface area and (iv) the mass concentration of particles in suspension.[88] This law is a relatively acceptable tool for EPD process design, especially at low process voltages, and is rarely used in the literature.[69,89] The application of the law at high process voltages is associated with deviations, due to changes in dispersion properties.[66] Therefore, a detailed understanding of aspects of the deposition mechanism is required, which would enable the amelioration of Hamaker's law, facilitating its application within a broader framework of process parameters.

3.3 | Chemical composition and crystallinity

The chemical composition and crystallinity of the coating are other crucial properties that influence the biological features of the coating. The incorporation of appropriate chemical compounds into the coating is a critical problem because it can positively affect the corrosion and tribological properties of the implant, as well as improve its bioactivity and antimicrobial properties.[90–93] EPD enables the deposition of coatings having a chemical composition similar to that of the suspension. Thanks to this, it is possible to deposit hydroxyapatite (which induces implant-tissue bonding)[52,55,59,62,71,78,94] particles with specific properties, for example bactericidal (copper[36] or silver nanoparticles[33] and another as CS[51,86] which is bioactive and germicidal or polymers (e.g., PEEK as well as PTFE[57]) which can improve the mechanical and tribological properties. The formation of composite coatings is also increasingly ubiquitous.[58,60] The Ti-13Zr-13Nb was used as a substrate by Jugowiec et al.[51] (Table 1). Analysis of electrophoretically deposited coatings corroborated the presence of an amorphous CS

phase, amorphous glass phases and crystalline phase of HA, as well as the inherence of Si, O, Ca (about 6 at%) and P (about 5 at%). The presence of calcium and phosphorus in both coatings proceeds from the fact that HA has been deposited on the titanium substrate (the chemical composition of HA includes Ca and P).[95] The positive aspect was the detection of amorphous and crystal phases of HA. In implantology, the presence of crystalline phases is extremely important, as they significantly favor osseointegration (compared to amorphous phases), which promotes cell growth at the tissue-implant interface.[26]

All things considered, by choosing the congruous environment in which the EPD process is conducted, it is possible to obtain coatings with a specific, foreseeable chemical composition.[50,69,72] Additionally, the incorporation of chemical compounds with unique properties can radically change the properties of the coating. However, despite many achievements in the field of incorporating a wide variety of additives into coatings during the EPD process, there are still shortcomings in the incorporation of electrically neutral compounds. Research focusing on the development of biocompatible and natural dispersants and charging agents could contribute to overcoming these issues.[64]

3.4 | Roughness

Titanium implants are currently the basic material used in the production of endoprostheses.[33,34] Due to the surface roughness (Ra), they can be divided into three types[96] minimal (± 0.5 μm), moderate (1.0–2.0 μm) and rough (>2.0 μm). Despite many attempts, the optimal roughness value of the implant, which would enable enhanced cell proliferation and differentiation, has not been established.[82] Nevertheless, it is known that the roughness affects the wettability of the surface, which influences not only osseointegration but also the polarization of macrophages, which are responsible for the appearance of the inflammatory process after implantation surgery.[82,97,98] Rougher implant surfaces (and thus increased surface development) make greater bone-to-implant contact and therefore achieving optimal surface roughness is one of the objectives of EPD coating.[26,35,96] The roughness of coatings obtained on titanium and its alloys can be modified by changing the process parameters.[49,71] This concern has been discepted by Bartmański et al.[35] where for coatings obtained at low concentrations of nanoHA powder in a suspension (0.1 and 0.2 g), the roughness of the coatings decreased with increasing voltage. In the case of a higher concentration of nanoHA (0.5 g) in the suspension, an increase in roughness with increasing voltage was observed. This is because at lower particle concentrations, fewer agglomerates are present on the surface. For all groups of nanoHA coatings, their roughness was higher than that of the uncoated sample Ti-13Zr-13Nb, that could have a positive effect on the osseointegration of the implant with human tissue.[35,53] Other observations were reported by Askari et al.[94] where the suspensions of 0.6 g L^{-1} iodine and 1.2 g HA particles added into 60 mL of isopropanol-acetone with a ratio of 50/50 were used. The surface roughness of the coating was minimal (as opposed to uncoated CP-Ti and sand-blasted CP-Ti. This may be due to the size of the particles used[49,50] which could occasion the formation of a comparatively uniform coating.

In the field of biomedical engineering, optimal roughness values are indiscriminately sought which would contribute to promoting the formation of a permanent bond at the tissue-implant interface and minimize the risk of the formation of a fibrous layer after implantation.[99] For example, Cai et al.[100] observed that the roughness of the titanium surface did not significantly affect the absorption of bovine serum albumin or human plasma fibrinogen. In contrast, some studies state that roughness affects the amount of adsorbed proteins.[101] However, we believe that considering only the arithmetic average of the 3D roughness (S_a) is insufficient to assess the impact of the surface topography on the absorption of proteins, microorganisms' behavior, and so forth. Many scientific publications that are not focused on biomaterials provide a number of other statistical parameters that describe the surface according to ISO 25178 standards.[102] Implementation of some of them in the study of biomaterials may prove crucial for the correct assessment of the influence of surface topography on osseointegration. For example, the parameters S_{ha} (mean hill area), S_{da} (mean dale area), S_{hv} (mean hill volume) and S_{dv} (mean dale volume) could help to accurately assess the topography. In addition, the functional parameters obtained from the areal material ratio curve, that is S_{mr_1} (peak material portion) and S_{mr_2} (valley material portion), are conventionally analyzed to assess the wear-resistance and lubricant retention, respectively. We believe that the study of these parameters in the case of biomaterials would help to better assess abrasion resistance (which is especially important in the case of hip and knee joint endoprostheses), and also enables scrutiny of the quality and quantity of "pockets", which in the case of biomaterials can be considered as convenient places for cell proliferation. Furthermore, the need for the study of surface texture directions should be stressed. This is a study that is standardly performed during topography analysis and is not carried out in the case of biomaterials.[102] Nonetheless, it is required that the layer covering implants will be homogeneous, that is that the topography exhibits isotropy.

3.5 | Mechanical and adhesion properties

Biomechanical and adhesive properties of the implant answer for its long-term lifespan in the human body. Inaptly matched mechanical properties, such as inadequate Young's modulus can cause stress shielding,[1,10] whereas the good adhesive properties of the coating prevent the implant from delaminating or cracking.[83,84] Decrepitude of the coating may lead to corrosion, which consequently may cause metallosis and subsequent loosening of the implant.[14,15] The formation of coatings (both uniform as well as composite and/or hybrid) on durable metal substrates is a way to simultaneously achieve optimal biomechanical and adhesion advantages of the coatings.[70] Currently, a fairly common method of assessing the quality of a coating is nanoindentation, thanks to which both the mechanical properties of the coating can be measured and nanoscratch tests can be performed. Bartmanski et al.[33] specialize in these matters. In their work, they deposited the coating on Ti-13Zr-13Nb at 15 and 30 V (Table 1), then performed a thermal treatment at 800°C for 120 min and finally conducted the above-mentioned studies.

Obtained results are listed in Table 3. It can be seen that the mechanical properties decreased for the applied higher voltage, while the adhesion properties increased for higher voltage. This was also confirmed by Jugowiec et al.[52] Such dependencies probably result from the properties of used HA and the formation of a porous structure on the surface of the titanium alloy. Furthermore, the adhesive properties depend on the value of hardness and Young's modulus.[103] A similar relationship was noticed in the case of the extension of the deposition time for the three-stage treatment (anodizing, EPD, heat treatment)—the extension of the time caused a reduction of mechanical properties and, simultaneously, an improvement in adhesion properties. Extending the duration in electrochemical methods results in a thicker layer, which may occasion better adhesive properties.[84,85] Albeit the tests carried out with the same parameters and the same suspension composition for the two-stage treatment (EPD, thermal treatment) showed that the extension of the treatment time reduces the mechanical and adhesion properties.[36] There are attempts to use EPD along with other surface treatment methods, such as anodizing, as they can have a positive effect on the adhesive properties of the coatings. However, in that case, the properties of the coating change with distance from the substrate and this problem will be presumably further explored.[103] In the case of EPD, a heat treatment is usually necessary in order to obtain satisfactory properties of the coating. Its usage changes Young's modulus, hardness, critical friction, critical load, morphology and crystallinity of the coating.[104]

Obtaining optimal properties is very complicated and it is believed that Young's modulus is a crucial factor in transferring the appropriate mechanical stress from the implant to the surrounding bone. The critical load associated with the adhesion limit should also be increased, as it is essential in orthopedic surgery.[59,78,104] However, the main problem in assessing the adhesion of layers on titanium and its alloys is the lack of manifest international standards.[105] Frequently used scratch tests should be considered only as helpful preliminary examination, as they do not reflect the processes occurring at the interface of implants (especially considering endoprosthesis). We agree with the statement that it is requisite to specify methods or procedures for biomaterials that would facilitate the determination of coated implant biotribology (especially in wet contact—which occurs when the implant is placed in the environment of human body fluid).[105]

3.6 | Corrosion resistance

Titanium and its alloys, thanks to their self-passivation, are refractory to the corrosive effects of many natural environments.[106] Unfortunately, their corrosion resistance significantly deteriorates in the vicious environment of human body fluids, which can lead to metallosis and, consequently, loosening of the implant.[107] The diagram of the corrosion mechanism of titanium materials in the environment of human body fluids is presented in Figure 4. Corrosion resistance of a biomaterial can be degraded or improved after the treatment's application but the objective of the scientists is to ameliorate it. The most widespread method used to assess the corrosion resistance of materials is potentiodynamic polarization measurements performed in solutions simulating the environment of body fluids: SBF, Hank's, 3. 5% NaCl, Ringer's, artificial saliva or 0. 6 M NaCl.[48] Based on this study, it is possible to determine the corrosion current density (j_{corr}), the corrosion potential (E_{corr}) and the polarization resistance (R_{pol}) of samples. These parameters are obtained from the interaction of the Tafel region of a polarization curve.[108,109] Furthermore, the corrosion rate (CR) and protection efficiency (PE) of samples can be determined based on calculated results.[48,110,111]

Pawlowski et al.[48] studied the effect of various surface treatments on the corrosion resistance of titanium and its alloys. In the case of electrophoretic deposition on Ti-13Zr-13Nb, an improvement in corrosion resistance was observed for the CNTs layer with TiO_2 layer (dual-step procedure; the amount of TiO_2 nanoparticles in suspension was 0.15 g and the voltage equaled 50 V). It was observed that the j_{corr} decreased from 4.22 ± 0.2 nA/cm^2 for uncoated titanium alloy to 1.4 ± 0.3 nA/cm^2 for coated titanium alloy. However, increasing the voltage (from 50 to 60 V) as well as increasing the amount of TiO_2 in the suspension (from 0.15 to 0.30 g) significantly deteriorated the corrosion resistance of the material. Another study disclosed that the deposition of hydroxyapatite coatings with nanoHA on Ti-13Zr-13Nb acquires lower corrosion resistance, nonetheless, the addition of nanosilver particles to nanoHA coating reduces the corrosion current density.[33] Jugowiec et al.[52] revealed that the nc-HA-s (ethanol-based colloidal solution of HA)/CS coating improved the corrosion resistance of the Ti-13Nb-13Zr in Ringer's solution at a temperature of 37°C. The properties of hydroxyapatite-based coatings vary as their thickness and porosity affect the corrosion resistance. In porous coatings, the presence of corrosion channels is possible, which may contribute to the formation of local corrosion. The addition of suitable particles, for example nanosilver, causes the pores to "block" and thus reduces the odds of the appearance of corrosive channels.[33,112] Composite coatings are increasingly subjected to tests. Composite sol–gel glass/CS coating on near-β Ti-13Nb-13Zr has been assessed in an immersion test in Ringer's solution at a temperature of 37°C by other researchers.[51] The passive current density was reduced from

TABLE 3 Mechanical and adhesion properties of coatings deposited in suspension consisting of 0.1 g of HA nanopowder in 100 mL of ethanol and then performed a thermal treatment. Reprinted from,[33] with permission from Elsevier (license 5500241455192).

	Mechanical Properties (nanoindentation)		Adhesion Properties (nanoscratch test)	
EPD Voltage (V)	Hardness (GPa)	Young's modulus (GPa)	Critical Friction (mN)	Critical load (mN)
15	0.2245 ± 0.036	41.10 ± 8.91	11.53 ± 2.23	35.83 ± 12.75
30	0.0661 ± 0.008	19.52 ± 1.29	16.03 ± 1.41	66.43 ± 14.09

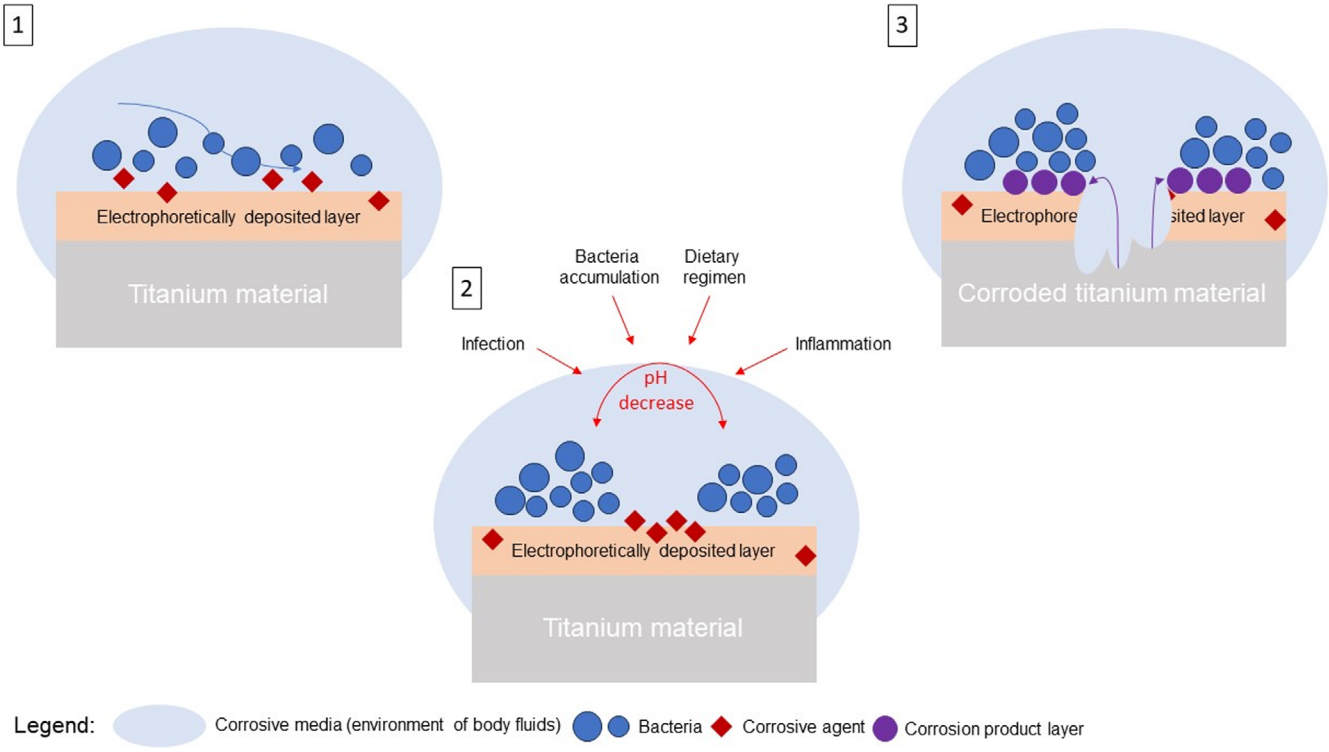

FIGURE 4 The schematic mechanism of electrophoretically deposited titanium material corrosion in the environment of human body fluids. Stage 1: the coated implant is in contact with biological fluids which leads to the adhesion of bacteria on the surface and the diffusion of corrosive agents of human body fluids through the layer. Stage 2: first of all, the formation of biofilm, and thus bacterial metabolism (discharge of lactic acid, formic acid, hydrogen peroxide) fosters the acidification of the microenvironment around the biomaterial. Other contributors to this process are infections, diet, and inflammation. Localized pitting corrosion is formed which leads to the exposure of the titanium surface. Stage 3: local microgalvanic corrosion occurs, where the cathode is the implant surface covered with biofilm, and the anode is exposed titanium material. The reaction of the material with the corrosive media causes the release and accumulation of corrosion products, forming the corrosion product layer.

20 μAcm^{-2} for the uncoated sample to 9 μA cm^{-2} and corrosion potential increased from ≈ −0.43 to −0.28 V. Composite coatings improved the corrosion resistance of the titanium alloy. Mehana Usmaniya et al.[61] scrutinized the corrosion resistance of nanocomposite coatings consisting of a mixture of BAG/Kao on CP-Ti in a simulated body fluid. The charge transfer resistance equaled 9×10^3 and 2.8×10^5 Ω for the uncoated and the deposited substrate, respectively. Confirmation of better corrosion resistance for coated titanium was also received in polarization studies (Figure 5). These properties are probably due to the addition of BAG to the coating, as BAG can impede corrosion in a biological environment.[113] It can be seen from the above tests that it is possible to improve corrosion properties with the use of EPD, however, an appropriate selection of parameters and suspension composition are required.[78,114]

3.7 | Wettability

Surface wettability is a physicochemical parameter that describes a biomaterial. The nature of the coating (hydrophilic or hydrophobic), along with the morphology and surface roughness, have a significant influence on the adhesion of platelets to the implant surface.[60] Hydrophobic coatings have been shown to resist platelet adhesion.[115] Optimum behavior is obtained with coatings with a medium degree of hydrophilicity because the adhesion, proliferation and differentiation of cells to the surface are being watched.[116,117] Moreover, bacteria (including *Staphylococcus aureus*, which frequently causes orthopedic infections) often attach to hydrophobic surfaces.[118,119] This is probably due to the structure of the bacteria—the presence of fimbriae or pili in its outer envelope. These structures are hydrophobic, so they readily link up with hydrophobic surfaces.[17,118] Therefore, the fabrication of a hydrophilic surface can deplete bacterial adhesion and thereby preclude inflammation from occurring.

Increasing the content of nanoHA in the suspension of EPD causes a decrease of wettability values.[35] It was observed by Singh et al.[53] that the contact angle values were significantly decreased for each composite coating (with iron oxide Fe_3O_4, HA and CS) as compared to the Ti-13Nb-13Zr. The lowering of the contact angle in the case of coatings with CS or HA is due to their hydrophilic nature.[120] Generally, the wettability of the surface of the coatings with HA on the Ti-13Zr-13Nb decreases with increasing voltage[33] and with lengthening the deposition time.[36] This may be due to the surface morphology of the considered coatings. Often, increasing the voltage and the time of process result in non-uniform coatings with greater

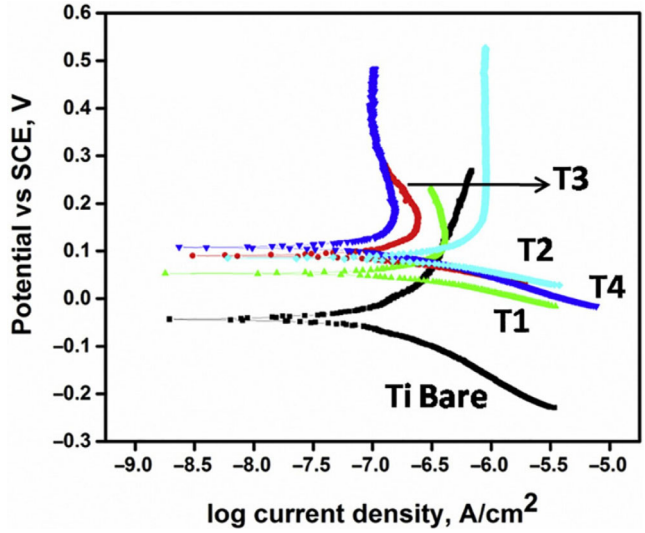

FIGURE 5 Polarization curves for the uncoated CP-Ti and electrophoretically deposited CP-Ti samples in different suspensions: T1–100% BAG, T2–80:20 BAG:Kao, T3–60:40 BAG:Kao and T4–50:50 BAG:Kao. Samples with coatings exhibited better corrosion parameters in SBF electrolyte (reduced corrosion current density compared to the uncoated substrate: 7.9×10^{-7}, 4.4×10^{-7}, 3.3×10^{-7}, 1.7×10^{-7} and 1.5×10^{-7} A/cm^2 for CP-Ti, T1, T2, T3 and T4 sample, respectively; increased corrosion potential compared to the uncoated substrate: −300, 50, 70, 80 and 110 mV for CP-Ti, T1, T2, T3 and T4 sample, respectively). Reprinted from,[61] with permission from Elsevier (license 5500240661009).

roughness, and with increasing roughness, the hydrophilic nature of the coating is enhanced.[121] This may provide better protein adsorption and cell adhesion.[122] Which is conducive to the formation of a stronger bond between the implant surface and the bone tissue.[123] In addition, we would like to draw attention to another feature of the coatings, which is determined on the basis of the contact angle measurement and computer modeling—surface free energy. Its study is important in biomedical applications because it determines the interaction between the implant and human body fluids, thereby affecting the behavior of proteins and the differentiation of osteoblasts.[37] In the case of EPD processes conducted on titanium and its alloys, there is a gap in this area that needs to be filled with the object of a better understanding of the layers' bioactivity.

3.8 | Apatite formation—immersion tests

The study of the possibility of forming apatite on the deposited substrate is one of the tests that enable assessing the bioactivity of the coating in an aggressive environment such as simulated body fluid (SBF) or Hank's solution, which are designed to reflect the composition of human blood, reproducing the aggressive environment of human body fluids.[124–126] In order to properly assess biological activity, samples are placed in a reasonable environment for a specified period of time (e.g., 7 days or 14 days), and then subjected to appropriate tests to determine the morphology and chemical composition of the formed material on the samples. These studies are helpful in predicting *in-vivo* bioactivity.[124,127]

Kwok et al.[127] studied apatite formation after placing a sample in Hank's solution at 37°C for 4 weeks. All coatings on Ti-6Al-4 V coated with HA showed high bioactivity, which was confirmed by XRD tests (the intensity of Ca and P diffraction reflections increased significantly after the immersion test) what can be seen in Figure 6. The uncoated sample of titanium alloy and the uncoated sample of titanium alloy subjected to heat treatment did not show any bioactivity. Similar results were obtained when other scientists[128] placed graphene oxide reinforced CS–HA nanocomposite coatings on CP-Ti in SBF for 5 days to assess their bioactivity. Coatings deposited with HA were coated by flake-like apatite aggregates, whereas no particular changes after assays were noticed on uncoated titanium. Molaei et al.[129] reported that CS–BG–HA–halloysite nanotubes (HNTs) composite coating on titanium and stainless steel 316 showed bioactivity, as after immersion in SBF for 14 days, an increase in carbonated hydroxyapatite was observed. On composite coatings HA-CS-collagen-hexagonal boron nitride (hBN) on Ti-6Al-4 V after 12 weeks of immersion into the revised simulated body fluid (r-SBF) at 37°C, globular clusters with flower-like clusters appeared which were carbonated-apatite structure.[130] It can be noticed that all coatings that were deposited in suspensions containing HA in their compositions displayed bioactivity. It is assumed that HA, when placed in blood plasma, is negatively charged and idiosyncratically attracts Ca^{2+} ions. Then, the positively charged locations interact with the negatively charged PO_4^{3-} ions, thereby causing the formation of bone-like apatite on the surface of the biomaterial.[131] HA improves corrosion resistance and increases the osteoconductivity and osteoinductivity of metallic implants.[132] However, it also exhibits many disadvantages, such as poor mechanical properties or low tensile strength.[132,133] Therefore, coatings are deposited in suspensions containing HA and chemical compounds that have a profitable effect on the properties of the coated biomaterial.

In the examples cited above, the following chemical additions can be distinguished:

- CS—has antimicrobial properties, is biocompatible and non-toxic[79,144]; may have a synergistic effect on the bioactivity of the coating[128]
- BG—is bioactive, however, the addition of dopants may affect their bioactivity and antibacterial activity[129,145]
- HNTs—are biocompatible and have good mechanical properties,[146,147] therefore they can reinforce the coating[130] and may occasion enhanced osteogenic cell differentiation[146]
- collagen—is a component of human bones (acts as a matrix for the crystallization of HA); is biocompatible, bioactive and biodegradable[130,148]
- hBN—is a biocompatible material that shows thermal stability and has high corrosion resistance[130] can shorten the healing time and prevent infection[149]

Scientists are trying to use a wide range of chemical compounds that could potentially increase the osseointegration of the implant

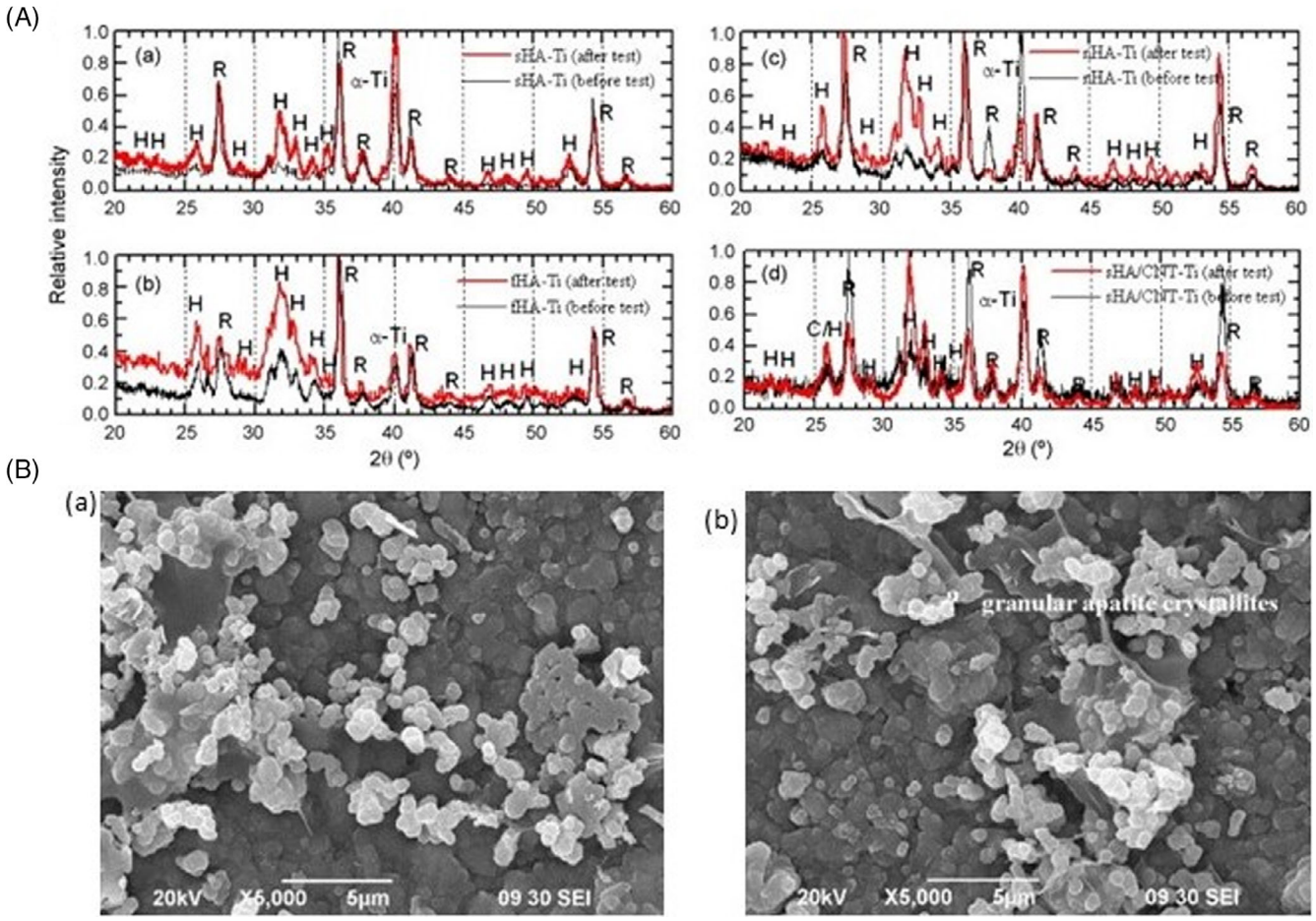

FIGURE 6 (A) XRD patterns for sintered coatings deposited on Ti substrate in suspension with (a) spherical HA (b) flake-shaped HA (c) needle-shaped HA and (d) spherical HA and CNTs before and after immersion test in Hank's solution. H—HA, R—rutile. (B) SEM images of samples deposited in suspension with (a) spherical HA and (b) spherical HA and CNTs after the bioactivity test. Reprinted from,[127] with permission from Elsevier (license5604710158401).

with human tissue, as well as increase the corrosion resistance of the material and adjust the mechanical properties. In addition, compounds with antibacterial properties, such as copper nanoparticles[36] are increasingly used. In Table 4, the selected published data of apatite formation tests of electrophoretically deposited coating on titanium and its alloys are specified.

3.9 | *In-vitro* cell culture assays

Cell culture assays are performed to determine the physicochemical properties as well as the cytotoxic and bactericidal activity of the coating.[150,151] Despite the fact that these tests do not facilitate an inclusive reflection of the conditions prevailing in the human body, their popularity is constantly growing. The main reason for this is their relatively low price as well as the celerity and simplicity that allow for the initial assessment of the reaction of cells with biomaterial.[32,150,152] Such studies are carried out when the mechanical and adhesive properties as well as the morphology, chemical composition and wettability of the coating appear to have promising features in biomedical applications.[32] For this purpose, various cell lines are used, which are presented in Table 5.

Jugowiec et al.[51] analyzed the metabolic activity of osteoblast-like cells MG-63 on composite sol–gel glass-CS coating and composite BG-CS coatings on Ti-13Nb-13Zr. The viability increased with time, but no beneficial effect of sol–gel glass and BG particles on cell adhesion and growth was observed. This is mainly due to the fact that the particles were covered with a thin layer of CS, and unmodified CS did not have a positive effect on cell adhesion and proliferation.[161] Nonetheless, coatings developed in this study were not cytotoxic and can be considered cytocompatible. HA-titanium-CNTs composite coating on NiTi was obtained and cell proliferation was studied using MG-63 osteoblast-like cells by Maleki-Ghaleh et al.[59] After 6 days of immersion in the culture medium, the proliferation of MG-63 on the surface increased more over three times. Promising results were also obtained for the coatings deposited by Asgari et al.[78] on CP-Ti where MTT cell viability assays were applied to study proliferation on two coatings: (1) with HA and (2) with HA, aluminium powder and zirconia powder. The viability after 2 days was ≈100% and ≈117%, respectively, and after 4 days ≈102% and ≈120%, respectively. The addition of zirconia

TABLE 4 The selected published data of apatite formation, *in-vitro* and/or *in-vivo* properties of the electrophoretically deposited coating on titanium and its alloys.

Substrate	Type of coating	Immersion test (environment; conditions; apatite forming ability)	*In-vitro* assay		*In-vivo* assay (model; results)	References
			Cells	Results		
Ti	CS-HA-GO composite coatings	Simulated body fluid; 37°C, 14 days; Yes	MG-63	Increase in cell proliferation and viability compared to the control sample (except for the sample coated in a suspension containing 1.5 g L^{-1} of chitosan). The increase in the concentration of chitosan resulted in a decrease in cell adhesion and their better spreading.	-	134
Ti	CS-BG-HA-HNTs composite coatings	Corrected simulated body fluid; 37°C, 22 days; Yes	-	-	-	129
Ti	Ag-HA composite coatings; Ag-HA-lignin composite coatings	Simulated body fluid; 37°C, 7 days; Yes	-	-	-	126
Ti	CS coating; CS-gelatin composite coatings	-	MG-63	The number of CCK-8 cells varied depending on the concentration of gelatin, however, an increasing trend was observed with increasing culture time and gelatin concentration. Nevertheless, the number of cells during culture remained the highest for uncoated titanium.	-	135
Ti	CS coating; CS-silk fibroin composite coatings	-	MG-63	Cell proliferation was significantly higher for titanium coated with composite coatings than for titanium coated only with chitosan. In addition, the number of cells for composite coatings after 7 days was comparable.	-	136
Ti 99.9%	HA coating; HA-GO coating; HA-CS coating; HA-CS-GO coating	-	BMSCs	The proliferation kinetics, relative ALP activity and calcium deposition on the HA-CS and HA-CS-GO coatings were significantly higher than those on the HA and HA-GO coatings.	Rats; After implantation, bone-like tissues were obtained on all coatings. Bone area ratio and bone-to-implant contact ratio were highest for HA-CS-GO coating, subsequently for HA-CS coating. The results show that osseointegration has occurred.	137

TABLE 4 (Continued)

Substrate	Type of coating	Immersion test (environment; conditions; apatite forming ability)	*In-vitro* assay		*In-vivo* assay (model; results)	References
			Cells	Results		
CP-Ti (Grade 2)	HA coating; CS–HA composite coating; GO–CS–HA composite coatings	Simulated body fluid; 37°C, 5 days; Yes	MG-63	Cell viability decreased after 1 and 3 days and then increased after 5 days of culture. GO–CS–HA coating showed the lowest cell viability compared to the other coatings, but its cytotoxicity was admittable.	-	128
CP-Ti (Grade 2)	CS-gelatin composite coating; CS-gelatin loaded with tetracycline composite coatings	-	MC3T3-E1	The coating with 500 mg tetracycline significantly reduced cell proliferation and exhibited cytotoxicity. The coating with 50 mg tetracycline showed much better proliferation but was worse than the coating without tetracycline.	Rabbits; The coating deposited in suspension with 500 mg tetracycline significantly depleted the number of white blood cells and the post-implantation image featured a trabecular bone structure without Gram-positive staining or microabscesses, as was the case with tetracycline-free coatings.	138
CP-Ti (Grade 2)	$CaSiO_3$ coating; $CaSiO_3$-reduced GO coatings	Simulated body fluid; 37°C, 7 days; Yes	hFOB	The level of cell growth on the $CaSiO_3$ coating was higher, while for the $CaSiO_3$-reduced GO coatings, it was lower compared to the uncoated sample. However, it can be attested that the coatings were not cytotoxic. In addition, the appearance of stress fibers was confirmed in all cells grown on the coatings.	-	139
Ti-6Al-4 V	HA coatings; HA-CNTs composite coating	Hanks' solution; 37°C, 28 days; Yes	-	-	-	127
Ti-6Al-4 V	HA-CS-collagen-hBN composite coatings	Revised simulated body fluid; 37°C, 84 days; Yes	-	-	-	130
Ti-6Al-7Nb	HA coating	-	-	-	Rabbits; On the HA layer on the screw well-developed osteons and Haversian system were noticed. Compared to the uncoated titanium alloy, the sample with HA coating increased the osseointegration of the implant (higher removal mean torque values after 18 weeks after implantation).	140

(Continues)

TABLE 4 (Continued)

Substrate	Type of coating	Immersion test (environment; conditions; apatite forming ability)	*In-vitro* assay		*In-vivo* assay (model; results)	References
			Cells	Results		
Ti-6Al-7Nb	HA coating; Partially stabilized zirconia coating; HA-partially stabilized zirconia composite coating	-	-	-	Rabbits; Compared to the uncoated titanium alloy, the samples with coatings ameliorated the osseointegration of the implant with the bone. The highest removal torque values (after 2, 6 and 18 weeks) were for the implant with composite coating. During histochemical stain studies, all samples displayed higher mature bone formation cellular activity.	141
Ti-13Nb-13Zr	HA coating; Fe_3O_4 coating; HA-Fe_3O_4-CS composite coatings	Ringer's solution; 37°C, 7 days; Yes	-	-	-	53
Ti-13Nb-13Zr	HA-BG-CS composite coating; HA-BG-Fe_3O_4-CS composite coatings	-	MG-63	Cell proliferation increased with the concentration of Fe_3O_4 in the coating	-	142
Ti-29Nb-13Ta-4.6Zr	HA coating	-	-	-	Rats; Compared to the uncoated titanium alloy, the sample with HA coating ameliorated the osseointegration of the implant with the bone (higher removal torque) and depleted inflammation (lower level of inflammatory factor). The HA layer occasioned the growth of new bone tissue (high osteoblast activity and chondrocyte formation). Uncoated titanium alloy was covered mainly by granulation tissue.	143

TABLE 5 Types of cell lines used for *in-vitro* assays on electrophoretically deposited coatings on titanium and its alloy.

Name	Abbreviation	References
Human osteosarcoma cells	MG-63	128,134–136,142
Human fetal osteoblast cells	hFOB	139,153–156
Osteoblast cells derived from *Mus musculus* calvaria	MC3T3-E1	125,157–159
Human bone marrow stromal cells	hBMSCs	137,160

and alumina powders improved the properties of the HA bioactive coatings because of their proliferative properties.[162] In addition, cell adhesion and differentiation are also affected by the nature of the coating (its hydrophilic or hydrophobic nature).[116,117] Table 4 shows more selected published data of *in-vitro* cell culture assays of electrophoretically deposited coating on titanium and its alloys.

The purpose of surface modification of metallic materials is to increase their bioactivity, that is to increase cell differentiation, adhesion and proliferation.[116,117] The increase in the number of cells tested during *in-vitro* assays implicates the absence of cytotoxicity and a prospective application of the biomaterial in implantology.[150,151] It is crucial to simultaneously constrain the growth and adhesion of bacteria at the tissue-implant interface, which depends on the nature of the coating, roughness and the presence of an electrostatic double layer.[118,163,164] Bacteria must overcome energy barriers to be able to attach.[165]

A comprehensive approach to the properties of the biomaterial coatings is essential when assessing their in-vitro properties but obtaining positive results may be a contribution to conducting *in-vivo* assays on various models.[166,167]

3.10 | *In-vivo* assays

In-vivo assays are carried out after obtaining positive results of *in-vitro* evaluations.[166,167] Contemporaneously, *in-vivo* studies are the last type of research that must be performed before clinical trials.[166,167] *In-vivo* assessments facilitate simulating the environment of the human body in which the implant is to be placed.[84,138,167] Only coatings with auspicious properties that have been comprehensively tested in each of the above-discussed aspects (morphology, thickness, chemical composition, crystallinity, roughness, corrosion resistance, wettability, mechanical and tribological properties, *in-vitro* assays) are submitted to such assays. *In-vivo* assays on *Rattus norvegicus* Wistar rats investigated by Nuswantoro et al.[143] showed that HA coating on Ti-29Nb-13Ta-4.6Zr promoted osseointegration and exhibited a higher bond strength (higher removal torque) compared to the uncoated substrate. Moreover, the presence of HA caused the reduction of inflammation. The HA layer displayed high osteoblast activity and chondrocyte formation, whereas uncoated titanium alloy was covered mainly by granulation tissue. Hydroxyapatite facilitates the formation of apatite at the implant-tissue interface so it was possible to obtain promising properties of the biomaterial.[131] Another model—adult New Zealand white rabbits were used for *in-vivo* studies by Alzubaydi et al.[141] Four types of biomaterials were tested (Table 6). The composite coating exhibited prime osseointegration properties as the addition of partially stabilized zirconia (PSZ) could change the morphology and mechanical properties of the coating, which consequently could contribute to better osseointegration of the implant with the tissue.[168,169] More selected published data of *in-vivo* assays of electrophoretically deposited coating on titanium and its alloys are listed in Table 3.

TABLE 6 Statistic of the removal torque values of Ti-6Al-7Nb biomaterial after different distances of time of implantation in rabbits. PSZ—Ti-6Al-7Nb deposited in suspension with PSZ, HA—Ti-6Al-7Nb deposited in suspension with HA, PSZ-HA—Ti-6Al-7Nb deposited in suspension with PSZ and HA.[141] A higher torque removal value indicates better bonding at the tissue-implant interface.

Weeks after implantation		2	6	18
Torque values (N cm)				
Type of sample	Uncoated	≈9	≈18	No precise data
	PSZ	≈11	≈22	≈50
	HA	≈12	≈28	≈55
	PSZ-HA	≈13	≈29	≈62

Abbreviations: HA, hydroxyapatite; PSZ, partially stabilized zirconia.

4 | FINAL REMARKS AND FUTURE OUTLOOKS

This paper reviews the latest advances in electrophoretically deposited coatings on titanium and its alloys. The available literature has been analyzed in detail, highlighting the key properties of the coating and the parameters of the EPD process in respect of the bioactivity of the coating. Despite the many advantages of EPD coatings on titanium and its alloys, challenges remain that should be addressed for further improvement. In the present section, we summarize the final remarks, challenges, and future perspectives of electrophoretically deposited titanium and its alloy in biomedical engineering.

The properties of electrophoretically deposited coatings can be relatively easily adjusted by changing the process parameters, such as the chemical composition of the suspension, applied voltage or deposition time. Therefore, it is extremely important to optimize the process in order to obtain coatings with the desired properties. Currently, optimization is mainly about conducting a lot of time-consuming experiments and finding the right parameters through trial and error. Therefore, it seems crucial to develop an advanced "Design of experiments" approach,[170,171] which would facilitate finding quantitative relationships that would connect the parameters and kinetics of the EPD process with the properties of the coating. Moreover, it is requisite to germinate the exact mechanism of particle agglomeration and Hamaker's law (see Section 3.1. and 3.2 herein, respectively, for more information).

There is a gap in the literature regarding one of the most important features of biologically active coatings—their surface free energy,[37] which mission is to determine the adhesion between the implant and human body fluids.[172] This feature facilitates assessment of the biological response of the implant, that is protein interaction and differentiation of osteoblasts, which in close vicinity to the implant attempt to achieve the lowest possible value of total free energy in the arrangement.[173,174] Therefore, in order to comprehensively assess the biological properties of the electrophoretically deposited coatings on titanium and its alloys, it is crucial to determine the values of surface free energy.

In addition, it is worth considering a more detailed study of the topography of coatings on biomaterials. So far, researchers have focused mainly on the study of the S_a parameter, which in our opinion is not sufficient for a proper analysis of the coating properties. Besides this parameter, there are a number of functional parameters that could be used in the biomedical field. Furthermore, the use of the areal material ratio curve could enable a more precise determination of abrasion resistance of coatings, or the designation of "pockets", which could be defined as a convenient place for cell proliferation. Another useful tool may be the assessment of surface texture directions, which would facilitate the scrutiny of the isotropy (or anisotropy) of the coating. For a more detailed explanation see Section 3.4 herein.

The component that is often present in suspensions is HA, which, as mentioned, despite many advantages, has many disadvantages, including poor mechanical properties. Researchers are trying to reinforce biomaterial coatings by adding other chemical compounds with good mechanical properties to the suspension. However, it seems advisable to combine the EPD method with methods that enable obtaining coatings with high adhesion and good mechanical properties. An example is the combination of the micro-arc oxidation process and EPD, which was already done by researchers a few years ago.[175–177] However, the current development of knowledge regarding micro-arc oxidation is at a more advanced level, therefore it may be possible to better adjust the process parameters to obtain suitable mechanical properties and porosity.

Moreover, titanium and Ti-6Al-4 V were most often electrophoretically deposited materials. First, it seems advisable to discontinue the use of alloys with vanadium, which is toxic in the long term and replace them in all studies with another material such as Ti-13Zr-13Nb. Second, the chemical composition of the substrate also affects the properties of the deposited coatings (e.g., in the case of the Ti-6Al-4 V alloy, a distinct deposition in the vanadium-enriched β phase than in the α phase). Therefore, it seems reasonable to deposit coatings with promising biological properties on another material (e.g., a different titanium alloy).

Currently, mainly composite coatings are being developed, the properties of which may be components of individual chemical compounds but also compounds may have a synergistic effect on each other. The number of studies related to the EPD of composite coatings is expected to increase in the near future. The research will focus mainly on titanium alloys with niobium, molybdenum and/or tantalum and advanced organic–inorganic coatings.

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Conceptualization, Balbina Makurat-Kasprolewicz; formal analysis, Balbina Makurat-Kasprolewicz and Agnieszka Ossowska; investigation, Balbina Makurat-Kasprolewicz; writing—original draft preparation, Balbina Makurat-Kasprolewicz; writing—review and editing, Balbina Makurat-Kasprolewicz and Agnieszka Ossowska; visualization, Balbina Makurat-Kasprolewicz; supervision, Agnieszka Ossowska All authors have read and agreed to the published version of the manuscript.

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