Fabrication of Nanohydroxyapatite-Chitosan Coatings by Pulse Electrodeposition Method

Najme Shahverdi1 · Arash Montazeri2 · Alireza Khavandi1 · Hamid Reza Rezaei1 · Fateme Saeedi2

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Abstract
In this study, Mg–2 wt% Zn scaffolds were fabricated by the powder metallurgy method, and the effects of the porosity content on the microstructure, and the mechanical properties of the scaffolds were studied. Nanocomposite coatings were deposited by pulse electrodeposition (PED) method on Mg–2Zn scaffolds and studied using a scanning electron microscope (SEM), X-ray diffraction (XRD), energy-dispersion spectrometer (EDS), and Fourier transform infrared spectroscopy (FT-IR). The coatings morphologies showed that optimal coating was obtained at 40 V pulse voltages, 10 min coating time and nanohydroxyapatite(nHA)/chitosan (CS) ratio = 10. The pulse-peak current density (CD), the pulse duty cycles (DC), pH and temperature were considered 10 mA/cm², 0.2, 7 and 37 °C, respectively. In optimal coating, Ca/P atomic ratio was obtained at 1.57, which is similar to the value of bone hydroxyapatite. The corrosion resistance and thermal stability of optimal coating were examined by potentiodynamic polarization and thermogravimetric analysis (TGA), respectively. The results showed that the corrosion rate of the optimal coating was 0.58 mm/year, which is very low and appropriate in compared to the Mg–2Zn with a corrosion rate of 2.09 mm/year. The TGA results indicated that the significant weight loss is about 23% and 14% for CS and optimal coating, respectively. The in vitro biocompatibility of the optimal coating was evaluated by cell adhesion, cytotoxicity, and alkaline phosphatase (ALP) assays using MG63 cells. The results indicate that the optimal nanocomposite coating was highly biocompatible, making this material more suitable for applications in bone tissue engineering and to repair bone defects caused by sports injuries.

Keywords Scaffold · Chitosan · Nano hydroxyapatite · Pulse electrodeposition

1 Introduction

Mg and its alloys reveal mechanical properties, specifically density and strength, similar to human cortical bone. The essential advantages of using Mg-alloys for bone implants are their biocompatibility, bio-absorbability, the lack of surgical removal, almost the same elastic modulus of bone, osteoconductivity and antibacterial activity [1–8]. Despite the various advantages, using Mg as a biodegradable scaffold has been restricted because of some significant limitations. The principal limit of Mg is poor corrosion resistance in SBF due to its high activity in physiological terms [8–10]. The high corrosion rate of Mg scaffold contributes to a significant diminish in the mechanical strength of the scaffolds, leading to the generation of a comprehensive volume of hydrogen gas and a notable rise in the local pH value of body fluid. Within the most exceedingly bad cases, the evolution of hydrogen gas has two significant risks: 1. There is a high threat of blockage of the bloodstream, causing the death of a patient, 2. the health of the patient is in peril by the local alkalization near the interface of tissue [6]. Therefore, the most important challenge in using Mg scaffold in the body is to control the corrosion/degradation rate. Generally, the microstructure, composition and surface morphology play critical roles in the efficacy of scaffolds, which can improve the corrosion resistance of Mg in body fluid and promote adhesion of favorable cells [9]. Protective coatings are the main strategy to prevent Mg corrosions [11]. Calcium and phosphorus are the principal elements in bone tissues and Ca–P coatings, mainly osteoconductive minerals such as HA and tricalcium phosphate (TCP), and have been
widely used to make new bones and promote osteointegration on biomedical scaffolds [12–14]. HA owns chemicals, like the structure of bone and biological likenesses to the mineral components in human hard tissues (teeth and bones) [15, 16]. Also, it is harmless, osteoconductive, bioactive, and makes bonding with woven bone [4, 17]. However, due to a lack of inherent osteoinductivity, limited formation of new bone occurs after osteoconduction is achieved [16]. So, coating based on nano-size would be a good idea. It was discovered that human bone apatite is composed of nanosized CaP crystals and that nano-hydroxyapatite (nHA) is similar to the inorganic component of human bone. Moreover, in HA nanostructure, protein adsorption, cellular response, nutrient transport, osteoblast adhesion, and cell proliferation would improve by nanometer-scale [18, 19]. Nevertheless, nHA coatings’ weak biological interaction mechanism and poor mechanical strength limit their application [16, 20]. Polymers combined with hydroxyapatite have been recommended to resolve this issue. Biocompatible polymers approved for human clinical applications constitute a favorable option to modify the initial corrosion resistance and cell compatibility on Mg-based materials to meet the healing requirement [9]. Among various polymers [21], CS is a natural alkaline polysaccharide containing excellent compatibility, good-quality antibacterial properties, pharmaceutical functions and hydrophilicity. It can be utilized as a drug delivery carrier and performs wound healing [22]. Moreover, CS could increase extracellular matrix proteins in osteoblasts and chondrocytes to facilitate bone integration [16]. Furthermore, by protonating the pH underneath 6.5, it can charge into a cationic form [21]. The combination of HA and CS presents good hardness, and its composite coating obtains intense biocompatibility. The hydroxyapatite structure reinforced the chitosan matrix and altered the discharge burst impact [23]. This coating’s harsh microtopography increments the contact region of the implant-bone interface and illustrates great biological activity in conditions of osteointegration and osteoconductivity [16, 20]. Hahn et al. [1, 24] prepared a dense and well-adherent HA/CS composite coating on an AZ31 Mg alloy. All the coatings indicated high adhesion strength and better corrosion resistance than the uncoated Mg alloy. Moreover, the biocompatibility of the coated alloy was improved extremely. Zhang et al. indicated that the electrophoretic deposition could obtain the nHA and chitosan. The composite coating had a good protection effect on AZ91D magnesium alloy when the thickness of nHA/chitosan coating was 0.40–0.61 mm and the effect of coating thickness on the degradation rate of AZ91D magnesium alloy in SBF was elaborated [25]. Coatings are mostly fabricated by electrochemical deposition (EPD) [11, 12], electrophoretic deposition, sol–gel, cold spray and micro-arc oxidation [11, 26]. Between these technologies, PED is an interesting method to get nano deposits in which the duration and amount of voltage and current are controlled. In general, EPD has unique characteristics due to the formation of a uniform coating on porous substrates, controlling the thickness, chemical composition of the coating, simple equipment needed, inexpensive and low deposition temperature [1, 27]. However, the electrodeposition process may increase loose and low adhesive coatings due to the non-favorable effect of concentration polarization and the manufacture of hydrogen. This non-adhesive coating will crust during biodegradation and prevent implanted material’s fixation to the host’s tissue. PED has been utilized diverse coatings showing strong bonding capacity to craved tissues, produce three-dimensional platforms, or make platforms bioactive [28]. PED is the overwhelming approach to controlling the reaction rate. Already the gelatin-loaded HA coating on titanium made through PED was formed uniform in structure. The periodic reaction on the electrodes because of the constantly repeated “on–off–on” pulse process in the electrolytic cell implies that when a pulse is used for benefits the diffusion process and diminishes concentration polarization; thus, the deposition rate is increased, and the advanced deposition effect is attained [29]. Current researches have shown increasing interest in the PED of HA/CS composites since this technique owns merits, including easy operation, pureness deposition, efficiently managed particle size, compact coating, reasonable control of the chemical composition, coating thickness, and suppression of the reduction of H₂ [14, 30]. In addition, it is beneficial that the method can be operated at a low temperature [27]. Seyedraoufi et al. [31] investigated the effects of the PED parameters and alkali treatment on the properties of the nHA coating on the Mg–2Zn porous scaffold for bone tissue engineering application. Montazeri et al. [32] investigated the effect of Graphene oxide contents and PED process parameters, including peak current density and duty cycle, on the fabrication of CS/GO nanocomposite coatings with Mg–2Zn scaffold. The results showed that the optimal conditions for fabricating a uniform CS/GO coating on the scaffolds were 2 wt% GO, CD 20 mA/cm², and DC = 0.5. The novelty of the present work is to prepare nHA/CS coatings on Mg–2Zn scaffolds to control coatings morphology by PED parameters. This research used the PED method to fabricate nHA/CS coatings on Mg–2Zn scaffolds. Biocompatible polymers approved for human clinical applications constitute a favorable option to modify the initial corrosion resistance and cell compatibility on Mg-based materials to meet the healing requirement [9].

For this purpose, first, the effects of the porosity content on the microstructure and mechanical properties of the Mg–2Zn fabricated scaffolds were studied. Then, the deposition of nHA/CS nanocomposite coatings on the Mg–2Zn scaffold was carried out by a PED method. The effects of pulse voltage, coating time and nHA/CS ratios were evaluated to determine of the optimal coating conditions.
Additionally, the biological responses of scaffolds were evaluated using MTT assay, ALP production and cell attachment.

2 Material and Methods

2.1 Materials

The Mg powder (purity of 97%, Merck, Germany) and Zn (purity of 99%, Merck, Germany) were used as the raw materials for fabricating of the scaffolds. Urea particles were purchased from Merck; Germany was employed as the space-holder agent. The CS with medium molecular weight (MW = 161,000 g/mol, degree of deacetylation (DD) of 75.6% and viscosity of 1406 m. Pas), isopropanol and p-nitrophenyl phosphate (pNpp) were supplied from Aldrich Chemical Co. (USA). HA nano powder was synthesized according to Feng et al. method [33]. The nHA powder (No. MHAP04) was obtained from the Nanjing Emperor Nano Material Co. (China), and it was synthesized using the sol–gel technique with Ca (NO3)2·4H2O and (CH3O)3P as precursors.

2.2 Methods

2.2.1 Fabrication of Scaffolds

The Mg and Zn powder mixtures were prepared based on 2wt% Zn, and the urea particles were thoroughly added to the former specimens with different volume contents of (5, 10, 15 and 25%). After mixing the Mg and Zn powder materials with urea particles, porous scaffolds were fabricated by powder metallurgy, applying a press pressure of 200 MPa using a cylindrical mold with an inner diameter and height of 1 cm. The heat treatment cycle consisted of two steps: (1) heating to 250 °C at a rate of 1°/min and preserving this temperature for 2 h: (2) heating to 550 °C at a rate of 10°/min and preserving the final temperature for 2 h [31, 34]. Pore sizes and morphologies of the Mg–2Zn porous scaffolds were observed using scan electron microscopies (SEM).

2.2.2 Coating Process

For preparing the electrolyte solution, about 1 g of CS was dissolved in 1 wt% Acetic acid (100 ml) at 1100 rpm for 20 h. The nHA with the mean particle size of 50 nm was poured into a mold containing ethanol and stirred for 1 h with a magnetic mixer. Finally, these two compounds were mixed to obtain an electrolyte solution. The nHA/CS ratio was 1, 5 and 10. After preparing the electrolyte solution, the coating was applied to the scaffolds. The graphite electrodes were attached to the anode pole, and uncoated scaffolds (Mg–2Zn) were attached to the cathode poles and parallel. After the completion of the coating, the fabricated scaffolds were washed with distilled water and then dried.

It is worth noting that the coating process was carried at CD = 10 mA/cm², DC = 0.2, T = 37 °C and pH = 7. The voltages of 20, 40 and 60 V and coating times of 5, 10 and 20 min were selected as pulsed parameters for the coating process.

2.3 Characterization

The compressive strength and modulus of the scaffolds were measured using compression testing of samples with dimensions of Φ 10 mm × 10 mm. The tests were performed with a SANTAM (STM-20, Iran) testing machine at room temperature at a rate of 0.3 mm/s. Each result was taken as the mean value of testing on five samples. In this study, the coatings’ functional groups were determined by the JASCO 4700 FT-IR in the range of 4000–400 cm⁻¹ wave numbers. A Broker-based D8-Advance device detector was utilized for the XRD measurements. The analyses were carried out at all angles of 2θ (from 5° to 45°) with steps of 0.1° and a stop time of 0.1 s per step at room temperature and pressure of 1 atm. This study used an SEM (the TeScan-Mira III model) equipped with an energy dispersive X-ray spectrometer (EDS) to assess the coating morphology. The corrosion behavior was investigated by the A263 EG&G device from Artisan Technology Group, made in the USA, and was performed in SBF at 37° C. Before the test, the samples were kept in solution for 60 min to stabilize the open circuit potential. All polarization tests were performed from −2.1 to −1.1 V with a scan rate of +1 mV/s. The reference electrode was the standard calomel electrode and the platinum auxiliary electrode was used for all electrochemical tests.TGA results were studied by the Shimadzu TGA 209 F1thermogravimetric instrument in the range of 25–450 °C using a heating rate of 10 °C/min under N₂ at a flow rate of 100 ml/min.

2.3.1 Biocompatibility Studies

2.3.1.1 Cytotoxicity Assay The quantitative toxicity MG63 cells culture tests were performed on the scaffolds for 1, 5 and 10 days to study biocompatibility. The human osteosarcoma cell line (MG-63) was obtained from the national cell bank (The Iran Pasteur Institute, Tehran, Iran). After defrosting the cells, they were transferred to a flask containing DMEM (Dulbecco's Modified Eagle Medium) culture medium with 10% FBS. Then the flask was incubated at 37 °C, with 90% humidity and 5% carbon dioxide concentration. It should be noted that the culture medium was changed every 3–4 days. In order to investigate the toxicity of the samples and their effect on cell growth and prolifer-
tion, the extraction process was performed according to ISO 10993-5 standard [35]. To each sterilized sample, 1 mm of culture medium was added for every 3 cm² surface. Then, after 1, 5 and 10 days, the medium was removed and added to the cells. A certain amount of culture medium (DMEM) was also considered a control.

2.3.1.2 MTT Assay To evaluate the cell adhesion, first $1 \times 10^4$ cells with 100 µl of culture medium were poured into each well of the 96-well culture plate and then incubated at 37 °C for 24 h to allow the cells to adhere to the bottom of the plate. Then, the culture medium was removed from the cells as much as possible and 90 µl of the extract of each sample and the extracts diluted with the culture medium along with 10 µl of FBS were added to each culture well. The culture medium was then removed and 100 µl of MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] at a concentration of 0.5 mg/ml was poured into each well and incubated for 4 h. The formazan crystals were perceived and dissolved by adding 100 µl of isopropanol (Sigma, USA) per well. The plates were placed on the shaker at 37 °C for 20 min before measuring the absorbance. The optical density (OD) of the formazan crystals dissolved in isopropanol was measured by using a scanning multi-well spectrophotometer (ELISA reader) (BioTek ELx808, USA) at a wavelength of 570 nm. Finally, the viability of cells was obtained from Eq. 1.

$$\text{Viability\%} = \left( \frac{\text{mean OD of sample}}{\text{mean OD of control}} \right) \times 100$$  

(1)

2.3.1.3 ALP Activity In a culture medium, ALP activity was measured for 1, 5 and 10 days to investigate the osteoblastic activity. Tissue culture polystyrene (TPS) was used as a negative control group for all tests. The samples were treated with lysis buffer containing 1% Triton X-100. The pellets and supernatants of the cell lysates were separated via centrifugation at 12,000 rpm for 10 min at 4 °C, and then the supernatant was used to measure ALP activity. The supernatant was incubated with pNpp liquid at 37 °C in a 96-well plate for 2 h. The pNpp would be hydrolyzed by ALP to p-nitrophenol (pNp). The addition of 100 µL of 0.2 M NaOH was to stop the hydrolysis. The optical density of the pNp was read at 405 nm using a multiwell microplate reader (STAT FAX 2100, USA) [36]. Finally, the amount of total protein was measured using ELISA Kit (Pars Azmoun, Iran) [37].

2.4 Statistical Analysis

The data were expressed as the mean or the standard deviation of one or more individual experiments wherever applicable. Data analysis was performed with Graph Pad Prism 6.0 and Origin Pro 8.0 for various statistical analyses (p < 0.05).

3 Results and Discussion

3.1 The Characterization of nHA and Mg–2 wt% Zn Scaffold

Figure 1 shows the SEM image and the particle size distribution analysis of synthesized nHA powder. As indicated, the nHA structure has a spherical type morphology (Fig. 1a). The particle size distribution result by image analyzer software indicated that the mean size of spherical particles is estimated at about 50 nm (Fig. 1b). Finally, the viability of cells was obtained from Eq. 1.

$\text{Viability\%} = \left( \frac{\text{mean OD of sample}}{\text{mean OD of control}} \right) \times 100$

(1)

Figure 2 illustrates porosity distribution in Mg–2Zn synthesized scaffold by SEM. Additionally, the porosity of the scaffolds was measured by the method of Archimedes [38]. Two types of porosity can be distinguished: (1) continuous macro porosity that is determined based on the percentage and size of porous material; (2) closed microporosity that has formed by the volume contraction of Mg and Zn powders. According to the research of Čapek [39], there are very tiny micro pores even in the scaffolds without porous factors.

Fig. 1  a SEM image and b particle size distribution of synthesized nHA powder
This is caused by the non-uniform powders and not filling the space between powders while pressing the scaffolds.

As indicated in Fig. 2, the pore distribution is similar; the size of the macro pores is between 100 and 200 and the micropores are 1 to 10 µm. Since the optimal size of pores to grow and osteoblast cell proliferation is about 100–500 µm [40]; therefore, these porosities can be suitable for adhesion and cell proliferation as well as blood circulation and vascularization.

Another essential characteristic of a sufficient scaffold is compressive strength. The compressive strength of the scaffold must be such that it can withstand the mechanical load during the body's operation and does not collapse. The percent of porosities, compressive strength and Young's modulus of the fabricated scaffolds are given in Table 1. The compressive strength of scaffolds has decreased by increasing porosity; because of the concentration of stress around the porosities. Gibson has studied the mechanical characteristic of sponge bone [41]. They reported that the compressive strength of bone is 0.2–80 MPa and its Young's modulus is 0.01–2GPa. In the present research, the compressive strength and Young's modulus of the scaffold with 25% porosity were 25 MPa and 1.8 GPa, respectively. It is necessary to state that more than 25% porosity reduces the strength; on the other hand, low porosity prevents the growth of bone cells, and it seems that 25% porosity is suitable for fabricating the scaffolds. Some studies have shown that the porous Mg–Zn specimens with a porosity and pore size of approximately 21–36% vol% and 150–400 mm, respectively, could have enhanced mechanical properties comparable with those of cancellous bone. In addition, the compressive strength and Young’s modulus of the porous samples decreased with an increase in the volume fraction of porosities at Mg–Zn alloys. Seyedraoufi et al. indicated that the best porosity for improvement of biocompatibility and corrosion properties of Mg–2Zn scaffold coated by nHA was obtained at about 20 vol% [34].

### 3.2 The Formation Mechanism of nHA-CS Coating

The weight or thickness of nHA-CS coatings is the function of voltage, coating time and nHA/CS ratio. The weight of the obtained precipitates by PED can be described by the following formula [42]:

\[
W = \frac{C\mu Ut}{d}
\]

(2)

where \(C\) and \(\mu\) indicate the particle concentration and mobility ions, respectively. \((U)\) is the of voltage the deposition process, \((t)\) is deposition time and \((d)\) is the distance between two electrodes. The mobility ions can be expressed by the Smoluchowski equation [25]:

\[
\mu = \frac{\zeta \varepsilon}{4\pi \eta}
\]

(3)

where \(\zeta\) is a Zeta potential and \(\varepsilon\) is the electrolyte constant, \(\eta\) is the electrolyte viscosity. According to Eq. (2), the deposit's thickness increases with deposition time and voltage. In order to create the higher \(\zeta\) during the process of PED, consequently the enhancement of mobility ions, it is possible, according to previous research, nHA particles and CS-NH$_2$ were protonated in the acetic acid aqueous solution and then nanocomposite coating was deposited as the following reactions [25, 42]:

\[
nHA + H^+ \rightarrow nHA - H^+ \quad \text{(Protonated nHA)}
\]

(4)

\[
CS - NH_2 + H_2O^+ \rightarrow CS - NH_3^+ + H_2O \quad \text{(Protonated CS)}
\]

(5)
The PED included two processes: the charged particles moved to the cathode under the electric field force and were reduced at the cathode surface. After H\(^+\) was reduced to H\(_2\) on the surface of the cathode, the OH\(^-\) concentration in the cathode area increased. Under the electric field force, as the positively charged nHA-H\(^+\) and CS-NH\(_3\)^+ moved to the cathode, they neutralized with OH\(^-\), accompanied by the cathodic reduction, and then deposited on the surface of the cathode (Fig. 3). In the pulse current method, the duty cycle (\(\gamma\)) is given by the following equation [32]:

\[
\gamma = \frac{T_{\text{on}}}{T_{\text{on}} + T_{\text{off}}}
\]

(Deposited nanocomposite coating)

During the \(T_{\text{on}}\) period, the deposition process initiates and the concentration of ions and species in the adjacency of the working electrode is reduced. \(T_{\text{off}}\), where the deposition is stopped for a given period, enables the present ions in the bulk electrolyte to move toward the electrode. This may recover the concentration of the electrolyte. Hence, at higher duty cycles, there is a limited time and chance for recovery of the electrolyte concentration, so the nuclei cannot grow sufficiently. The scheme of the deposition mechanism of nanocomposite coating is given in Fig. 3.

3.3 Effects of PED Parameters

3.3.1 Effects of Coating Voltage

Figure 4 indicates the SEM micro-structure and EDS analysis of coated scaffolds at 20, 40 and 60 V pulse voltages with \(t = 10\) min, DC = 0.2, CD = 10 mA/cm\(^2\) and nHA/CS = 5. According to SEM images, coating prepared at 20 V (Fig. 4a) has agglomerated micro-structures with the disordered arrangement of particles within them. While coating prepared at 40 V showed a much homogeneous, denser and lower porosity surface (Fig. 4b). Increasing voltage to 60 V can close the pores and prevent vascularization (Fig. 4c).

Park et al. investigated PED of HA-CS coatings on titanium substrate for the dental implant. The coatings were
composed of 5 to 20% CS by volume. The morphology of Fig. 4b is similar to the HA-CS coating of Park et al. at voltage 4 V, DC = 0.2, CD = 10 mA/cm² and nHA/CS = 5.

According to EDS analysis of the coated scaffolds at different voltages, the nanocomposite coatings show calcium, phosphorous and oxygen related to nHA. Besides, the presence of nitrogen confirms that CS exists in the coating.

When the applied pulse voltage increased from 20 to 40 V, the corresponding Ca/P molar ratio first increased
from 1.348 to 1.479, which was closer to 1.57 (the Ca/P molar ratio of the bone natural), and then decreased to 1.40 at 60 V. Our results about the effect of different pulse voltage on nanocomposite coating microstructures were similar to Jia et al. [43]. As seen among the coatings, the sample prepared at 40 V indicated a homogeneous and denser surface with the Ca/P molar ratio of 1.479.

Figure 5 shows the FT-IR spectra of nHA and nHA/CS composite coating with the optimal voltage. The bands at 1024 and 500–600 cm⁻¹ correspond to the different modes of the phosphate groups in nHA, while the stretching band (approximately between 2900 and 3700 cm⁻¹) is assigned to the OH group in HA [44]. The most vital characteristic of the carbonate ions bands in the apatite is also visible at 1437–1485 cm⁻¹. The band at 3700 cm⁻¹ is assigned to the hydroxyl groups present in CS [45] and the peak at 1059 cm⁻¹ of pure CS, attributed to the NH₂ group, shifts to a lower wavenumber at 1047 cm⁻¹ for the nanocomposite coating. The absorption bands at 1075 cm⁻¹ and 558 cm⁻¹ relate to the crystallinity of CS [46]. Shift of the band at 1059 cm⁻¹ (corresponds to the phosphate group) to 1047 cm⁻¹ (related to the optimal coating) shows the presence of CS polymer and its interaction with the phosphate groups at nHA/CS nanocomposite [44, 47]. It should be noted that the characteristic band of amide I (1550 cm⁻¹) appearing in pure CS, is absent for the nanocomposites. These results also show the formation of ionic or polar interactions between nHA and amino and carboxyl groups of CS [48].

### 3.3.2 Effects of Coating Time

To study the effect of coating time, following optimal conditions were studied for coating: V = 40 V at DC = 0.2, CD = 10 mA/cm², nHA/CS = 5 and t = 5, 10 and 20 min. Figure 6 shows the morphology and EDS analysis of the coated scaffolds at t = 5, 10 and 20 min. At coating time of 5 min, the coating is not uniformly formed on the Mg–2Zn and agglomerates are observed (Fig. 6a). By increasing the coating time to 10 min, more uniform coating deposits on the Mg–2Zn scaffold (Fig. 6b). Finally, increasing in the coating time to 20 min reduces the required porosity for vascularization (Fig. 6c). The increment at the time (20 min) leads to penetration of a more deposited layer to the pores (according to Eq. 2) and then, the pores become closed. The Ca/P molar ratio to applied coating time was shown in the EDS analysis. When the applied coating time increased from 5 to 20 min, the corresponding Ca/P molar ratio increased from 2.69 to 2.91. According to EDS analyses, the coatings show the presence of calcium, phosphorous and oxygen that relate to nHA; nitrogen and carbon can also confirm CS at the nanocomposite coatings.

### 3.3.3 Effects of nHA/CS Ratio

Figures 7 and 8 show the SEM images, EDS and elemental mappings analysis of electrodeposited coatings at V = 40 V, t = 10 min, DC = 0.2, CD = 10 mA/cm² with contents of nHA/CS = 1, 5 and 10 in the deposition electrolyte solution. As indicated, by increasing the concentration of nHA (Fig. 7a–c), the coating indicates denser morphology. According to Fig. 8a, the microstructure includes strings with a 100 nm diameter similar to nano rods-like. Figure 8b presents that by increasing the concentration of nHA, the microstructure of the coating changes considerably. Increasing nHA/CS ratio from 1 to 5 leads to forming agglomerates formed of tiny particles. The size of these particles is 50–100 nm. By increasing nHA/CS to 10, the particles' size reaches 100–200 µm (Fig. 8c). As indicated, the morphology of the particles changes from nano rod-like to nano spherical-like. The comparison of quantitative results of EDS shows that by increasing the concentration of nHA in the coatings, the amount of nitrogen and carbon in CS has decreased. Indeed, the peak intensity of calcium and phosphorous shows a slight increase. The Ca/P ratio with nHA/CS = 1, 5, and 10 were 3.47, 1.48, and 1.57 respectively. The Ca/P ratio of coating with nHA/CS = 10 is closer to the bone natural. Considering the spherical morphology and Ca/P ratio, coating with nHA/CS = 10 ratio seems optimal. EDS elemental mappings of the coating with V = 40 V, t = 10 min, nHA/CS = 10, DC = 0.2 and CD = 10 mA/cm², are shown in Fig. 8d. It can be observed that Ca and P are uniformly distributed in the coating. On the other hand, carbon, nitrogen and oxygen elements may indicate the presence of CS polymer in the coating.
Fig. 6 SEM images and EDS analysis of coated scaffold in different times: a 5, b 10, c 20 min, V = 40 V, DC = 0.2, CD = 10 mA/cm² and nHA/CS = 5
that when the content of CS was increased to 20 volume percent, the coating exhibited spherical and porous morphology, which is beneficial for cell adsorption and growth [27]. Yang et al. showed that HA particles could regulate cellular behavior, and the morphology of the particles had a significant influence on cellular behavior. Although both the microsphere and micro rod particles inhibited the proliferation of the cells at a high dose, the inhibition effect decreased or even HA microspheres performed better than the micro rod particles for promoting osteogenic differentiation of marrow mesenchymal stem cells (mBMSCs) [49].

Kalia et al. investigated the biocompatibility and ultrastructural effects of two differently shaped hydroxyapatite [Ca_{10}(PO_4)_6(OH)_2] nanoparticles (HA NPs), round (aspect ratio 2.12, AR_2) and rice-shaped (aspect ratio 3.79, AR_4). The results showed that AR_2 had a more significant osteogenic effect when compared with AR_4, as measured, suggesting that HA NPs of this shape or aspect ratio may have a positive effect on new bone [50].

The effect of the nHA/CS ratio on the chemical structure and composition of coatings was investigated by FT-IR spectra (Fig. 9a). As indicated, the peak at 1550 cm\(^{-1}\) of pure CS, attributed to the NH\(_2\) group, shifts to a lower wavenumber at 1464 cm\(^{-1}\), 1460 cm\(^{-1}\) and 1457 cm\(^{-1}\), corresponding to different n-HA/CS ratios, respectively. The absorption band at 1075 cm\(^{-1}\) relates to the crystallinity of chitosan [46]. The peak at 1075 cm\(^{-1}\) shifts to 1047 cm\(^{-1}\), 1051 cm\(^{-1}\) and 1062 cm\(^{-1}\) with different nHA/CS ratios, respectively. The peak at 3700 cm\(^{-1}\) of CS, attributed to the OH group, shifts to a lower wavenumber at 3572 cm\(^{-1}\) in the spectra of all nanocomposite coatings. It seems that the hydroxyl ions on the surface of nHA might interact with CS’s plentiful amino and hydroxyl ions by forming hydrogen bonds [51].

The XRD analysis was also used to study constituent phases of three deposited coatings on Mg–2Zn scaffolds (Fig. 9b). As indicated, all coatings have both nHA and CS phases. The important peaks related to nHA are observed at diffraction angles of 2θ = 25.9°, 32.9°, 33.9°, 34.4°, 47° and 52.6° according to JCPDS no. 09-0432 standard [43, 52, 53]. The XRD pattern of the CS exhibits two peaks, one at 14.2°, which corresponds to the hydrated crystalline structure. In contrast, the broadened peak at about 21.4° indicates the existence of an amorphous structure according to JCPDS.
Fig. 8  a–c SEM nanostructures and EDS analysis of the coated scaffolds with a nHA/CS = 1, b nHA/CS = 5 and c nHA/CS = 10 at $V = 40$ V, $DC = 0.2$, $CD = 10$ mA/cm$^2$ and $t = 10$ min, d EDS elemental mappings of the coating with $V = 40$ V, $t = 10$ min, nHA/CS = 10, DC = 0.2 and CD = 10 mA/cm$^2$
no. 039-1894 standard [54–56]. Moreover, the intensity of the nHA diffraction peaks is enhanced with an increasing nHA/CS ratio [57].

### 3.4 The Properties of the Optimal Coating

This study used polarization curves to compare corrosion resistance Mg–2Zn scaffold and optimal coating (see Fig. 10a). Table 2 lists the parameters extracted from the polarization curves of the samples in the SBF solution. Generally, the cathodic polarization curve presents the cathodic transformation of hydrogen by reduction of water, while the anodic curve indicates the dissolution of alloy Mg. As seen, the anodic polarization curves of the two samples have passive areas that prove the presence of the protective layer on the surface. Optimal nanocomposite coating prevents the corrosion of Mg by developing a passive layer. As can be observed, the corrosion potential of the nHA-CS coating is more favorable than that of the Mg–2Zn scaffold and the corrosion current density of the nHA-CS coating was reduced by 97.2%.

Moreover, the corrosion rate (mpy) of the Mg–2Zn scaffold was 2.09 mm/year, which was very high and inappropriate compared to the nHA/CS coating with corrosion rates of 0.58 mm/year. Therefore, nHA-CS coating can significantly improve the corrosion resistance of the Mg–2Zn scaffold. Tian et al. [58] studied the effect of nHA and PLGA (lactic-co-glycolic acid) composite coating on pure Mg. The results indicated that nanocomposite coating leads to more positive corrosion potential and reduces corrosion rate by up to 53%. Moreover, CS coating on sheets of AZ31 alloy led to a corrosion rate diminished from 1.45 to 0.064 mm/year in NaCl solution (3.5 wt%) [59].

Seyedraoufi et al. [31] studied the effect of nHA coating on the corrosion rate of Mg–3Zn scaffold in SBF solution. Their results showed that calcium, phosphate and sulfate ions could deposit on the surface of Mg–3Zn and prevent its

### Table 2: Electrochemical parameters of the obtained from the polarization curves of Mg–2Zn scaffold and optimal nanocomposite coating

| Scaffold      | Corrosion potential (V) | Corrosion current density (A/cm²) | Corrosion rate (mm/year) |
|---------------|------------------------|----------------------------------|--------------------------|
| Mg–2Zn        | −1.64                  | 9.76 × 10⁻⁵                     | 2.09                     |
| Optimal coating | −1.52                  | 2.68 × 10⁻⁶                     | 0.58                     |

![Fig. 9](image1.png)  
**Fig. 9** (a) FT-IR spectra of CS and nanocomposite coatings and (b) XRD pattern of coatings with nHA/CS = 1, 5, 10; V = 40 V; t = 10 min, DC = 0.2 and CD = 10 mA/cm²

![Fig. 10](image2.png)  
**Fig. 10** (a) Polarization curves of Mg–2Zn scaffold with 25% porosity and optimal coating, (b) TGA analysis of nHA, CS and optimal coatings at V = 40 V, DC = 0.2, CD = 10 mA/cm² and t = 10 min
corrosion by forming of a passive layer. Corrosion potential illustrated a significant increase in the coated sample compared to the non-coated sample. The current density of the non-coated and coated samples was \(8 \times 10^{-3}\) and \(1.4 \times 10^{-4}\) A/cm\(^2\), respectively. Therefore, nHA coating has improved the corrosion resistance of Mg–3Zn. In the present research, the current density of the optimal coating is \(2.68 \times 10^{-6}\) A/cm\(^2\) (Table 2), which has a significant reduction compared to the work published by Seyedraoufi et al. It means using nHA/CS nanocomposite coating on Mg–2Zn scaffolds can notably modify the corrosion resistance of Mg–2Zn.

Thermal degradation behaviors of nHA, CS and optimal nanocomposite coating were studied by TGA (Fig. 10b). As expected, nHA does not show any degradation up to 450 °C. There are three stages in the thermal decomposition of CS. The first stage centered at about 70 °C to 100 °C due to the moisture content. The second stage begins at 265 °C and ends at 340 °C, which shows the degradation of leading CS polymer chains [32, 60] and the three-stage is related to the degradation of the lateral chains. The optimal nanocomposite coating starts thermal degradation around 275 °C and ends at 316 °C. The degradation second stage indicates that the significant weight loss is about 23% and 14% for CS and nanocomposite coating, respectively. Moreover, the residue amounts from TGA obtained for CS, nanocomposite coating and nHA are 54%, 60% and 100% at 448 °C, respectively. The results indicated that incorporating of nHA into the CS matrix increases thermal stability [60, 61]. A weight loss of 60% for composite without nHA was observed at 385.3 °C by Roy et al. In comparison the same weight loss occurs at 436.2 °C for 10% nHA, indicating increased thermal stability due to the addition of inorganic ceramic nHA [62]. As indicated in section FT-IR spectra, the interaction of nHA with the CS matrix is the vital factor preventing the slip of CS polymer chains during the heat treatment in the TGA [32].

3.5 In Vitro Cell Behavior

The MTT assay was further used to quantify the proliferation of MG63 cells cultured onto the Mg–2Zn and optimal coating after 1, 5 and 10 days (Fig. 11a). After 1 day, the average cell viability for Mg–2Zn and scaffold with nHA-CS optimal coating were 110 and 139% compared to the control substrate (TPS). Similarly, after 5 days, these scaffolds exhibited 65 and 100% cell viability, respectively. The cells on the nanocomposite coating proliferated better than the Mg–2Zn scaffold, mainly due to the simultaneous effect of nHA and CS coating. Therefore, nHA/CS composite coating can be used as a suitable coating for Mg–2Zn in bone tissue engineering.

The osteoblastic activity of the MG-63 cells seeded on scaffolds was determined by measuring ALP activity in culture media for 1, 5 and 10 days. It is observed that it was significantly higher for all scaffolds in comparison to the control sample (TPS) after 1 day. However, by increasing the incubation time, ALP expression was decreased. Meanwhile, the relative ALP activity of the nHA/CS
optimal coating was significantly greater than the Mg–2Zn scaffold. The highest value of relative ALP activity was observed for the nHA/CS optimal coating at 1 day. Dhiyya et al. demonstrated that the presence of nHA in the Zn-CS/nHA/β-GP hydrogel increased swelling, protein adsorption, and exogenous biomineralization.

Moreover, adding nHA to the hydrogel develops osteoblast differentiation under osteogenic conditions in vitro and accelerates bone formation in vivo to the depositions of apatite and collagen [63]. Suo et al. investigated the enhancement of osseointegration using HA, CS and GO/CS/HA composite coatings on titanium fabricated by electrophoretic deposition. The results show that HA and CS coating considerably enhanced the bone marrow stromal cells (BMSCs) interactions in vitro. Moreover, this GO/CS/HA coating could increase osseointegration in vivo. As a result, GO/CS/HA may have potential applications in dental implants [16].

The cell morphology of the MG63 cultured cells on Mg–2Zn and nHA/CS optimal coating was illustrated after 5 h in Fig. 11c and d. The spindle-shaped cells could be seen on the Mg–2Zn and nHA/CS optimal coating, but the MG63 cells on nHA/CS surface exhibited better stretch with more Filopodia extensions (Zones with red circles) compared with the Mg–2Zn scaffold. The existence of multiple Filopodia extended on coating provides strong evidence that the cells are well attached to the nHA/CS optimal coating. Therefore, the nHA/CS coating helps the initial attachment, proliferation and growth of MG63 cells.

4 Conclusions

The nHA/CS nanocomposite coating with the PED method was obtained to improve the biocompatibility of porous Mg–2Zn scaffolds. The results showed that changing PED parameters influence the morphology of the coating. Optimal coating conditions in PED process obtained V = 40 V, T = 10 min, nHA/CS = 10 at DC = 0.2 and CD = 10 A/cm². The ratio of calcium to phosphorus in the optimal coating composition was 1.57. The study of the effect of optimal coating on Mg–Zn corrosion resistance with 25% porosity indicated that corrosion rate (mm/year) decreased by 72.2%. In terms of thermal stability, the nanocomposite coating has shown better thermal resistance than CS polymer. In vitro biocompatibility assay using a human osteosarcoma cell line (MG-63 osteoblast) revealed that the nHA-CS optimal nanocomposite coating can provide adequate support for cell growth, proliferation and differentiation of MG-63 cells during in vitro cytological examination. Finally, the research results suggest that the PED technique provides an alternative method for manufacturing the nHA-CS optimal nanocomposite coating on Mg–2Zn alloy. This coating may be applied in the field of scaffolds to repair bone defects caused by driving accidents and sports injuries.

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Declarations

Conflict of interest The authors declare no conflict of interest.

References

1. M. Leigheb, M. Veneziano, R. Tortia, M. Bosetti, A. Cochis, L. Rimondini, F.A. Grassi, Acta Biomedica Atenei Parmensis 92, e2021025 (2021). https://doi.org/10.23750/abm.v92iS3.11757
2. D. Dziuba, A. Meyer-Lindenberg, J.M. Seitz, H. Waizy, N. Angrisani, J. Reifenrath, Acta Biomater. 9, 8548 (2013). https://doi.org/10.1016/j.actbio.2012.08.028
3. J.-W. Lee, H.-S. Han, K.-J. Han, J. Park, H. Jeon, M.-R. Ok, H.-K. Seok, J.-P. Ahn, K.E. Lee, D.-H. Lee, S.-J. Yang, S.-Y. Cho, P.-R. Cha, H. Kwon, T.-H. Nam, J.H.L. Han, H.-J. Rho, K.-S. Lee, Y.-C. Kim, D. Mantovani, Proc. Natl. Acad. Sci. 113, 716 (2016). https://doi.org/10.1073/pnas.1518238113
4. L. Li, M. Zhang, Y. Li, J. Zhao, L. Qin, Y. Lai, Regen. Biomater. 4, 129 (2017). https://doi.org/10.1093/rb/rbx004
5. Y. Song, D. Shan, R. Chen, F. Zhang, E.-H. Han, Mater. Sci. Eng. C 29, 1039 (2009). https://doi.org/10.1016/j.msec.2008.08.026
6. M. Jamesh, S. Kumar, T.S.N. Sankara Narayanan, J. Coat. Technol. 16, 2442 (2012). https://doi.org/10.1016/j.actbio.2012.08.028
7. J.-W. Lee, H.-S. Han, K.-J. Han, J. Park, H. Jeon, M.-R. Ok, H.-K. Seok, J.-P. Ahn, K.E. Lee, D.-H. Lee, S.-J. Yang, S.-Y. Cho, P.-R. Cha, H. Kwon, T.-H. Nam, J.H.L. Han, H.-J. Rho, K.-S. Lee, Y.-C. Kim, D. Mantovani, Proc. Natl. Acad. Sci. 113, 716 (2016). https://doi.org/10.1073/pnas.1518238113
8. L. Suo, N. Jiang, Y. Wang, P. Wang, J. Chen, X. Pei, J. Wang, Q. Wan, J. Biomed. Mater. Res. B 107, 635 (2019). https://doi.org/10.1002/jbm.b.34156
9. H. X. Wang, S.K. Guan, X. Wang, C.X. Ren, Acta Biomater. 6, 1743 (2010). https://doi.org/10.1016/j.actbio.2009.12.009
10. J. Hu, Y. Zhou, L. Huang, J. Liu, H. Lu, BMC Musculoskelet. Disord. 15, 114 (2014). https://doi.org/10.1186/1471-2474-15-114
11. L. Suo, N. Jiang, Y. Wang, P. Wang, J. Chen, X. Pei, J. Wang, Q. Wan, J. Biomed. Mater. Res. B 107, 635 (2019). https://doi.org/10.1002/jbm.b.34156
12. F. Sharifianjazi, A. Esmaeilkhani, M. Moradi, A. Pakseresh, M.S. Asl, H. Karimi-Maleh, H.W. Jang, M. Shokouhimehr, R.S. Varma, Mater. Sci. Eng., B, 264, 114950 (2021). https://doi.org/10.1016/j.mseb.2020.114950
