Article

Low-Cost Deposition of Antibacterial Ion-Substituted Hydroxyapatite Coatings onto 316L Stainless Steel for Biomedical and Dental Applications

Abdul Samad Khan ^{1,*} and Muhammad Awais ²

¹ Department of Restorative Dental Sciences, College of Dentistry, Imam Abdulrahman Bin Faisal University, P.O. Box 1982, Dammam 31441, Saudi Arabia

² Department of Industrial Engineering, Faculty of Engineering, Taibah University, P.O. Box 344, Medina 41411, Saudi Arabia; myounas@taibahu.edu.sa

* Correspondence: akhan@iau.edu.sa; Tel.: +96-659-4781-075

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Abstract: Substitutions of ions into an apatitic lattice may result in antibacterial properties. In this study, magnesium (Mg)-, zinc (Zn)-, and silicon (Si)-substituted hydroxyapatite (HA) were synthesized using a microwave irradiation technique. Polyvinyl alcohol (PVA) was added during the synthesis of the substituted HA as a binding agent. The synthesized Mg-, Zn-, and Si-substituted HAs were then coated onto a 316L-grade stainless-steel substrate using low-cost electrophoretic deposition (EPD), thereby avoiding exposure to high temperatures. The deposited layer thickness was measured and the structural, phase and morphological analysis were characterized by Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD) and scanning electron microscope (SEM), respectively. The bacterial adhesion of *Staphylococcus aureus* was characterized at 30 min, 2 h and 6 h. The results showed homogeneous, uniform thickness (50–70 µm) of the substrate. FTIR and XRD showed the characteristic spectral peaks of HA, where the presence of Mg, Zn and Si changed the spectral peak intensities. The Mg–HA coating showed the least bacterial adhesion at 30 min and 2 h. In contrast, the Si–HA coating showed the least adhesion at 6 h. EPD showed an effective way to get a uniform coating on bio-grade metal implants, where ionic-substituted HA appeared as alternative coating material compared to conventional HA and showed the least bacterial adhesion.

Keywords: electrophoretic; hydroxyapatite; ion-substitution; coatings; antibacterial; microwave irradiation; dental implants; orthodontic brackets

1. Introduction

The selection of metallic materials for dental and biomedical applications depends on various factors such as corrosion behavior, mechanical properties, cost, fabricability and biocompatibility [1]. Dental instruments, orthodontic brackets, wires, bands, ligatures and maxillofacial screws and implants are manufactured using stainless-steel alloy [2,3]. These metal surfaces are prone to bacterial colonization as biofilms [4]. Therefore, metal surfaces must be coated with antibacterial materials. The antibacterial properties of biomaterials have long relied on surface coating and functionalizing techniques such as bioceramic coatings, covalent attachment of polycationic groups [5,6], impregnating nanoparticles with antimicrobial agents [7–9] and quaternary ammonium, iodide or silver ions [10–13].

Among bioceramics, hydroxyapatite (HA) has been substituted with various ions—including cations and anions [14–16]. The bioactivity, biocompatibility and antibacterial activity of HA can be increased by substituting zinc (Zn), magnesium (Mg), silicon (Si) [17–19]. Several methods for deposition of bioceramics, polymers and composites on metallic implants have been used, these include

dip coating [20], pulse laser deposition [21], electrochemical deposition [22], biomimetic deposition [23], plasma spraying [24] and electrophoretic deposition [25]. Among these processes, plasma spraying has been widely used commercially, however, it has some limitations such as material wastage, high dissolution rate, thickness control and phase change in HA [26,27].

Electrophoretic deposition (EPD) is an economical low-tech method that can be used for coatings [28]. It can be performed at room temperature, providing uniform coating thickness, less material wastage, less manpower, ease for complex shapes. Moreover, the rate of deposition, thickness, deposition particle size can be controlled easily by changing the parameters of suspension and power input [29–33].

Previously, HA particulates were coated on the metal substrates using EPD. However, a major drawback of poor adhesion has been observed [28,33]. It has been reported that differences in thermal expansion can generate cracks on the surface of coated layers or at the interface between the coatings and substrates during cooling after sintering [34]. Generally, sintering of the coatings is required as a post-deposition step however polymeric binders are also used to enhance adhesion to the substrate without carrying out high energy sintering steps and to improve the mechanical strength of coatings [35].

Limited data are available in which Zn-, Mg-, and Si-substituted HA has been coated on a metallic substrate using the EPD technique [36]. Therefore, the present study was designed to synthesize Zn-HA, Mg-HA, and Si-HA using microwave irradiation technique and the aim was to deposit these particles on a bio-grade stainless-steel surface using EPD techniques and characterize the structural and antibacterial activities against *Staphylococcus aureus* in the presence of shear forces. It was hypothesized that these substitutions would help to evade bacterial adhesion and impart antibacterial character to the coated samples.

2. Materials and Methods

2.1. Synthesis of Substituted HA

All analytical-grade materials were used in this study. The precursors for the synthesis of substituted HA include calcium nitrate tetrahydrate $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ (Fluka Honeywell, Charlotte, NC, USA) and diammonium hydrogen phosphate $(\text{NH}_4)_2\text{HPO}_4$ (Sigma Aldrich, St. Louis, MO, USA) used as calcium and phosphorous precursors, respectively. Zinc nitrate hexahydrate $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (Merck, Darmstadt, Germany), magnesium nitrate hexahydrate $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (AppliChem GmbH, Darmstadt, Germany) and tetraethyl orthosilicate (TEOS) (Aldrich, St. Louis, MO, USA) were used as zinc, magnesium and silicon substituent salts, respectively. The $(\text{Ca} + \text{Zn})/\text{P}$, $(\text{Ca} + \text{Mg})/\text{P}$ and $\text{Ca}/(\text{P} + \text{Si})$ ratio was maintained at 1.67 as given in Table 1. Zn-HA, Mg-HA, and Si-HA powders were prepared based on the chemical formulae $\text{Ca}_{10-x} (x\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O} \cdot \text{PO}_4)_6(\text{OH})_2$, $\text{Ca}_{10-x} (x\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O} \cdot \text{PO}_4)_6(\text{OH})_2$ and $\text{Ca}_{10}(\text{PO}_4)_{6-x}(\text{SiO}_4)_x(\text{OH})_{2-x}$, respectively as proposed earlier [37–39].

Table 1. Concentration of precursors used in syntheses of hydroxyapatite (HA), Zn-HA, Mg-HA, and Si-HA.

Groups	$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ Conc. (M)	$(\text{NH}_4)_2\text{HPO}_4$ Conc. (M)	Conc. Of Substituted Salts (M)
HA	0.105	0.062	–
Zn-HA	0.10	0.059	$\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O} = 0.008$
Mg-HA	0.0823	0.063	$\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O} = 0.021$
Si-HA	0.102	0.047	TEOS = 0.018

A pure HA was synthesized initially by using a microwave irradiation technique as described earlier by our group [40]. The synthesis was carried out by dissolving required amounts of $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ and substituting Zn and Mg ions containing salts in deionized water; $(\text{NH}_4)_2\text{HPO}_4$ was separately

dissolved in deionized H₂O. The pH of both the solutions was adjusted above 11 by adding ammonium hydroxide (NH₄OH) as required; (NH₄)₂HPO₄ was added dropwise at 2 mL/min to a solution of Ca(NO₃)₂·4H₂O and substituting ion-containing salt. The pH was continuously monitored and maintained above 11 by adding NH₄OH. Whereby, TEOS was dissolved in (NH₄)₂HPO₄ and added dropwise in Ca(NO₃)₂·4H₂O solution and the same procedure was followed. After addition, the solution was left for 30-min stirring. It was then microwave irradiated in a modified domestic microwave oven (Samsung MW101P, Seoul, Korea) at 1000 W for 10 min (30 s ON: 30 s OFF). The suspension was filtered, washed three times with distilled water and oven-dried at 80 °C for 24 h. The obtained powder was ground to carry out deposition and characterization studies.

2.2. Deposition Procedure Used to Prepare Substituted HA Coatings

The electrophoretic technique was carried out on 1 × 2 cm² stainless-steel 316L substrates (used as cathodes) and carbon anode fixed 1 cm apart. The plates were ground and polished using silicon carbide study (1500, 2000, and 4000), washed and degreased by ultrasonication using methanol (Sigma Aldrich, St Louis, MO, USA). Suspensions for EPD were prepared by adding HA, Zn–HA, Mg–HA, and Si–HA (respectively) in 0.5 wt.% PVA solution. Nitric acid (1.0 M) was added to the solutions until clear solutions were obtained and pH was kept 1–3. Power supply (E Lite 300/300 plus power supply) was regulated at 10 V, suspensions were heated at 80 °C using hotplate and EPD process was carried out for 3 min. During EPD process, current decreased constantly, however, the potential difference across the electrodes remained the same. This happened due to the deposition of HA and substituted HA particles on the electrode and an insulating layer is formed which reduced the electric field influencing electrophoresis for the incoming particles [41]. The as-deposited coatings were air-dried at room temperature for 24 h, later oven-dried at 120 °C for 24 h to evaporate surface absorbed water.

The thickness of coating substrates was measured according to ASTM D6132 [42] using Lendteknet's ultrasonic pulse–echo meter. The consequential coatings were homogenous, uniform with a thickness of 50–70 µm.

2.3. Fourier Transform Infrared Spectroscopy (FTIR)

Fourier-transform infrared spectroscopy (Thermo Nicolet 6700, Thermo Fischer Scientific, Waltham, MA, USA) with photo-acoustic cell at a resolution of 8 cm^{−1} and 256 scan numbers were used for the FTIR data collection of PVA, HA, Zn–HA, Mg–HA, and Si–HA. The spectral range was 4000–400 cm^{−1} and OMNIC software v7.2 was used to evaluate the peak positions.

2.4. X-ray Diffraction (XRD)

The X-ray diffraction (XRD) measurements were recorded using a diffractometer (PANalytical XPERT-PRO model, Malvern, Stuttgart, Germany) operated at 40 kV and 40 mA using Cu-Kα radiation. The detector was scanned over a range of angle 2θ = 20°–80° at a step size of 0.02° to identify the HA phase.

2.5. Scanning Electron Microscopic Analysis

The surface morphology of coated samples was investigated by scanning electron microscope (JEOL JSM6490A, JEOL, Tokyo, Japan) at a voltage of 20 V. Samples were gold-coated using JFC 1500 I sputtering device. Images were taken at various magnifications.

2.6. Preparation of Controlled Discs of Substituted Has for Antibacterial Investigations

Compact discs of HA and substituted Has were prepared to be used as control samples for bacterial adhesion testing using hydraulic press HP142009 (Al-Qamar Machinery, Al-Qamar, Lahore, Pakistan). The powders were subjected to a pressure of 550 psi to prepare discs with 8 × 2 mm², whereby each disc weighed 0.2 ± 0.008 g.

2.7. Bacterial Adhesion Testing

Staphylococcus aureus (American Type Culture Collection, 25,923) was revived from glycerol stock culture on nutrient-agar (N-Agar) and then sub-cultured on tryptone soya broth (TSB) for 24 h at 37 °C in an incubator. The culture dishes were prepared 2 mL of TSB and 20 µL of inoculum. Each type of HA disc and HA coating was subjected to bacterial adhesion testing at 30 min, 2 h and 6 h in triplicate. Shear force was provided by using a shaking incubator (Daihan Scientific, Seoul, Korea) at 120 rpm.

Each disc and plate was taken out at 30 min, 2 h and 6 h in 9 mL 0.9% normal saline solution in 25-mL capped test tubes and sonicated (WiseClean sonicator, PMI-Labortechnik GmbH, Grafstal, Germany) for 2 min at 37 °C to dislodge attached bacteria. Absorbance was measured at 600 nm using a UV-vis spectrophotometer (Bio-Rad Laboratories, Portland, ME, USA).

Each sample material was fixed with 2.5% glutaraldehyde (Merck, Darmstadt, Germany) for 24 h. Sequential dehydration was done step-wise for 30 min in each concentration of alcohol (50%, 60%, 70%, 80%, 90%, and 100%). After this, each sample plate was stained with crystal violet dye (Scharlau, Barcelona, Spain) and counterstained with safranin (K.Y.L Industrial And Manufacturer And Traders Co, Ltd., Tianjin, China) so that bacteria adhering to the coated surface are easily visible. Optika B-600 MET Microscope (Optika, Ponteranica, Italy) was used for microscopic analysis and Optika Pro vision (Optika, Ponteranica, Italy) was used for acquiring digital images at 100X.

Data were analyzed using SPSS Statistics for Windows Software, version 21 (IBM Software, New York, NY, USA). Descriptive statistics were used to calculate the maximum, minimum, mean and standard deviation values. A comparison of two and more than two groups was done by one-way analysis of variance test and followed by post hoc Tukey's test. All the *p* values of less than 0.05 were considered statistically significant.

3. Results

3.1. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR data were collected for the deposited HA, Zn-HA + PVA, Mg-HA + PVA and Si-HA + PVA samples. Comparative spectra with complete range (4000–400 cm^{−1}) and selected range (2000–400 cm^{−1}) are given in Figure 1a,b, respectively.

A broad peak attributed to the H-bonded OH stretch of the PVA group at 3200–3570 cm^{−1} could be seen in Figure 1a(i). The asymmetric and symmetric stretching peaks of C–H were appeared at 2971 and 2910 cm^{−1}, respectively. The spectrum (Figure 1a(ii)) of the control sample (as precipitated HA) showed the deep absorption peaks of PO₄^{3−} at 1083–1035 cm^{−1}. The peak at 963 cm^{−1} was due to the asymmetric stretch of the PO₄^{3−} group. The bending peak of PO₄^{3−} was visible at 564–604 cm^{−1}. There was a prominent CO₃^{2−} band from 1343–1490 cm^{−1} and a bending peak at 827 cm^{−1}. The broad band at 3100–3570 cm^{−1} represented the OH stretching and peak at 1635 and 633 cm^{−1} represented the bending peak of OH.

In Figure 1a(iii–v), bands at 1380–1510 cm^{−1} corresponded to CO₃^{2−} (B type carbonate substitution) and 1630–1650 cm^{−1} attributed to bending peak of hydroxyl group. Intense bands at 964, 1030 and 1100 cm^{−1} corresponded to P–O stretching vibration modes followed by bending peaks at 564 and 602 cm^{−1} in all three spectra. The change in peak intensities was observed with substituted HA groups, whereby P–O, C–O, and O–H peaks were markedly reduced as shown in Figure 2. The presence of Si–O in the apatite lattice was validated by the appearance of band at 830–1220 cm^{−1} [37,42] as seen in Figure 1a(v). Variation in peak positions and assignments was observed with substituted HA as tabulated in Table 2. The PVA spectrum showed the characteristic peaks of PVA, whereby 0.5% of PVA was added to all the substituted HA samples prior to EPD. There exists a slight band shifting which was attributed to the addition of PVA.

Figure 1. (a) Comparative IR spectra showing the characteristic peaks of (i) polyvinyl alcohol (PVA), (ii) HA, (iii) Zn-HA, (iv) Mg-HA and (v) Si-HA. PVA spectrum shows the hydroxyl band ($3570\text{--}3200\text{ cm}^{-1}$), C-H ($2971, 2910\text{ cm}^{-1}$) and C-O-C band ($1150\text{--}950\text{ cm}^{-1}$). Spectral peak of HA appears at $3570\text{--}3100\text{ cm}^{-1}$ (stretching O-H), 1635 cm^{-1} , 633 cm^{-1} (bending O-H), $1490\text{--}1343\text{ cm}^{-1}$ (carbonate), $1083\text{--}1035\text{ cm}^{-1}$, 963 cm^{-1} (stretching P-O) and $604\text{--}564\text{ cm}^{-1}$ (bending O-P-O). Change in peak intensity observes with substituted HA groups, whereby the characteristic peak of Si-O appears in Si-HA at $1220\text{--}830\text{ cm}^{-1}$; (b) selected range of comparative spectra of (i) HA, (ii) Zn-HA, (iii) Mg-HA and (iv) Si-HA.

Figure 2. Peak height analysis showing a change in peak/band intensities after substitution of HA with Zn, Mg and Si. Change in peak intensity exhibits the influence of substituted ions on the HA structure. The significant change in peak intensity observes at O–P–O ($604\text{--}564\text{ cm}^{-1}$), P–O ($1100\text{--}960\text{ cm}^{-1}$), ν_3 C–O (827 cm^{-1}), ν_1 C–O ($1383\text{--}1450\text{ cm}^{-1}$) and O–H (1640 cm^{-1}).

Table 2. FTIR bands and peak assignments as observed in this study.

Peak Assignment	HA	Si-HA	Mg-HA	Zn-HA
Degenerated bending (ν_2) phosphate	470	–	–	–
Triply degenerated bending mode, ν_4 , of the O–P–O bond	564–604	500–650 (band shape) *	500–650 (band shape) *	564, 600
Bending peak of C–O	827	827 **	827 **	835
Asymmetric stretching peak (ν_1) of PO_4^{3-}	963	–	–	941 ***
triply degenerated vibration, ν_3 peak of P–O	1083–1035	1180–920 *	1130–920 *	1050–1110
Stretching mode, ν_1 , of the CO_3	1343–1490	1500–1410	1440	–
Bending peak of O–H	1635 and 633	1640	1640	1642, 625 *
Si–O–Si	–	1220–830, 430–470	–	–
Si in Si-HA	–	876	–	–

* degeneration of the ν_3 PO_4^{3-} domain at 1020 cm^{-1} , ν_4 O–P–O band at $560\text{--}604\text{ cm}^{-1}$ due to the SiO_4^{4-} in the environment of the phosphate ions. ** peak almost diminished. *** additional peak of Zn appears.

3.2. X-ray Diffraction (XRD)

Figure 3 shows the XRD spectra of as-deposited and heat-treated coatings. As expected, all the patterns confirmed to the presence of a major phase of HA, as compared to the standards (ICDD 009-0432 and JCPDS-896438) [43–47]. Figure 3a shows at 31.7 , 32.2 , 32.9 , and 39.9 diffraction peaks at 2θ values which correspond to (211), (122), (300), and (310) planes, respectively. No significant phase changes were observed in the as-deposited and heat-treated coatings however, the sharpness of the peak suggested better crystallinity for the case of HA and Zn-HA (Figure 3b) coatings. XRD pattern of Zn-HA coatings exhibited almost all HA peaks, in addition, a very weak diffraction peaks at 26.16° and 54.36° diffraction angle corresponding (220) and (101) plane, respectively match with the Zn peak (JCPDS-040784). Figure 3c,d shows amorphous phases of HA at $2\theta \sim 31.9$ and no other phases related to silicon oxide (from Si-HA sample) and stanfieldite (from Mg-HA sample) were observed. Although the

XRD patterns of both Si-HA and Mg-HA corresponded to that of HA, however, the diffraction peaks lost intensity, proving a progressive loss of crystallinity [43,44].

Figure 3. XRD spectra of as-deposited and heat-treated (ht) coatings. (a) HA; (b) Zn-HA; (c) Si-HA; (d) Mg-HA.

3.3. Scanning Electron Microscopic Analysis

The SEM images (Figure 4a–d) show the surface morphology of HA, Zn-HA, Mg-HA and Si-HA-coated samples. Uniform distribution of coating was observed in all samples, in which the particle size was in 5–10 μm .

Figure 4. SEM images of (a) HA, (b) Zn-HA, (c) Mg-HA and (d) Si-HA showing the covering of substrate with deposited particles. Particles are in the micron range.

3.4. Antibacterial Adhesion Analysis

The values of absorbance depicted in Table 3 can be correlated with the amount of bacterial adhesion shown at each time interval for both the control discs and coated samples. In the case of control discs, HA and Mg–HA samples showed an increase in absorbance value with the passage of time. In the case of coated samples, initially at 30 min and 2 h, the absorbance value of the silicon sample was higher, but at 6 h the absorbance value was reduced. Absorbance values for HA, Mg–HA and Zn–HA constantly increased from 30 min to 6 h. A statistically significant difference ($p \leq 0.05$) was observed between HA and substituted HA groups. Within substituted HA groups, a statistically significant difference ($p \leq 0.05$) was observed with a time interval.

Table 3. Absorbance mean (SD) values for discs and coated HA, Zn–HA, Mg–HA and Si–HA samples at 30 min, 2 h and 6 h of bacterial adhesion.

Time	Control Discs				Coated Plates			
	HA	Zn–HA	Mg–HA	Si–HA	HA	Zn–HA	Mg–HA	Si–HA
30 min	0.086 (0.008)	0.000	0.015 (0.009)	0.090 (0.006)	0.09 (0.003)	0.019 (0.008)	0.006 (0.000)	0.059 (0.002)
2 h	0.095 (0.002)	0.026 (0.008)	0.017 (0.009)	0.11 (0.006)	0.14 (0.003)	0.063 (0.005)	0.020 (0.001)	0.162 (0.005)
6 h	0.18 (0.002)	0.024 (0.004)	0.042 (0.007)	0.094 (0.005)	0.15 (0.006)	0.081 (0.009)	0.086 (0.002)	0.025 (0.007)

Images of stained biofilms (using crystal violet dye and counterstained using safranin) at 1000× are shown in Figures 5–7. Mg–HA showed the least adhesion with *S. aureus* at all the time intervals in both the control disc and coated plate as shown in Figure 5. Zn–HA however showed (Figure 6) a larger number of bacterial colony adherents on the control disc than the coated plate which contained a defined dispersion of thinly beaded colonies. Si–HA offered (Figure 7) most adhesion with *S. aureus* at 30 min and 2 h with a recession in number at 6 h in both discs and coated plates.

Figure 5. Optical microscopic images of Mg–HA discs at (a) 30 min, (b) 2 h and (c) 6 h and Mg–HA-coated samples at (d) 30 min, (e) 2 h and (f) 6 h of Mg–HA. Arrows indicate the presence of *S. aureus* on substrate surface. Scale bar ~150 μ m.

Figure 6. Optical microscopic images of Zn–HA discs at (a) 30 min, (b) 2 h and (c) 6 h and Zn–HA-coated samples at (d) 30 min, (e) 2 h and (f) 6 h of Mg–HA. Arrows indicate the presence of *S. aureus* on substrate surface. Scale bar ~150 μm .

Figure 7. Optical microscopic images of Si–HA discs at (a) 30 min, (b) 2 h and (c) 6 h and Si–HA-coated samples at (d) 30 min, (e) 2 h and (f) 6 h of Mg–HA. Arrows indicate the presence of *S. aureus* on substrate surface. Scale bar ~150 μm .

4. Discussion

Metallic implants are inert materials and do not interact with the biologic environment. Hence, coating of HA-based materials on metallic implant surfaces can impart a strong affinity for, and chemical bonding with, the host hard tissue [48]. Substituted HA particles were successfully synthesized using microwave irradiation techniques. The microwave irradiation technique is a quick way to achieve nanoparticles on a large scale [49]. The substituted ions perform a vital role in the

biologic responses of HA. The atomic structure of HA allows to substitute almost half of the periodic table elements [50]. Hence, the biologic performance of synthetic HA can be improved and tuned via substitution, doping and grafting of different ions, small functional compounds, peptides and polymers.

A structural and phase analysis of HA and substituted HA was investigated with FTIR and XRD, respectively. It is known that substitution of Mg in HA crystals can reduce the lattice parameters as the ionic radius of Mg^{2+} is smaller than the ionic radius of Ca^{2+} . Its substitution in HA decreases its crystallinity and can destabilize the apatite structure [51,52]. Similar behavior was observed by XRD pattern in this study. The reduction in the IR absorption band of PO_4^{3-} was observed with the Mg substitution and the corresponding bands at 1080 and 650–500 cm^{-1} became broader and intensity was reduced.

Similarly, Zn is substituted at the Ca(II) site in synthetic HA [53]. Its higher substitution in HA decreases crystallinity; therefore, a lower molar percentage was used in this study. It has been reported that 1 wt.% substituted amount of Si in HA was enough to improve bioactivity [54,55]. The appearance of the peak in the XRD spectra at $2\theta = 26.16^\circ$ corresponding to the (220) plane showed the presence of a new phase called parascholzite ($\text{CaZn}_2(\text{PO}_4)_2 \cdot 2\text{H}_2\text{O}$) also known as calcium zinc phosphate in the apatite lattice [56]. The spectral peaks of PO_4^{3-} and OH became broadened and reduced the crystallinity of the apatite. The ν_1 -stretching P–O peak was merged in a band and a new peak appeared at 941 cm^{-1} attributed to zinc ammonium phosphate. The appearance of a weak band at 1410 cm^{-1} (denoted to NH_4^+) also supported this suggestion [57]. The CO_3^{2-} peak presented in pure HA was not observed in the Zn-substituted HA, which is an indication that Zn ions replaced the carbonated group into the HA.

It was found that the substitution of Si into HA ($\text{Ca}_{10}(\text{PO}_4)_{6-x}(\text{SiO}_4)_x(\text{OH})_{2-x}$) changed the hydroxyl stretching bands at 630 and 3570 cm^{-1} . However, both stretching and bending bands positions were unchanged by the silicate substitution. The CO_3^{2-} band also appeared at 1500–1410 cm^{-1} . It is reported that the substitution of PO_4^{3-} by SiO_4^{4-} reduces the number of hydroxyl groups required for charge balance [58]. Furthermore, hydrogen-bonded OH groups attached to the Si were expected to occur, while Si–O–Si bending mode frequencies were observed at 827 and 470 cm^{-1} . Therefore, the intensity of the ν_3 carbonate peak was increased. The results of this study are in accordance with previous studies [59,60].

This study assessed the antibacterial activity of three types of substituted HAs with *S. aureus* in the presence of shear force. For that, an in-depth analysis of the antibacterial activity of each substituted HA was done with the help of a UV-vis spectrophotometer. A time-based study was conducted to investigate an increase or decrease in the number of bacterial colonies on the substrate. It has been observed from previous studies on bacterial adhesion that the time frame from zero to 6 h is critical in bacterial attachment on the substrate [61]. Therefore, in the present study, the antibacterial activity of zinc, magnesium and silicon-substituted HA was conducted at up to 6 h.

In the case of Zn–HA, an initial attachment was deterred however zinc substitution did not seem to affect proliferation rate at 2-h and 6-h incubation time periods. Availability of Si–O seemed to aid in the initial attachment of bacterial colonies onto the coated plates and discs as Si–HA showed a large number of bacterial attachments at 30 min and 2 h in both the discs and coated samples. However, silicon did not aid in further proliferation of *S. aureus*, which led to the recession in their number at 6 h.

It appears that Mg–HA and Zn–HA had a better propensity of deterring initial bacterial attachment. On the other hand, silicon substitution had an antibacterial effect at a 6-h incubation time interval. Bacterial adhesion follows a complex mechanism and depends upon the physicochemical properties of bacterial cells and substrate [62]. It has been reported that *S. aureus* has a dominant electron donor character at intermediate pH (pH 5, pH 7 and pH 9) and shows greater attachment to the acidic and predominantly hydrophobic substrate [63]. Bacterial adhesion depends not only on the hydrophobicity of the bacterial cell wall [64] but also on environmental factors like pH, ionic strength, medium composition and temperature. Overall, Si–HA showed the most adhesion and Mg–HA

showed the least adhesion. Zn–HA had a better antibacterial profile on coated samples in comparison to its disc sample that can be due to the thinner film of Zn–HA. This means that thinner films are better suited for the provision of antibacterial property to the coatings. Furthermore, the inhibitory effect of Zn-substituted HA on the development of bacteria is established and in another study, $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ have shown its impact on the reduction of the numbers of *S. aureus* bacteria [38].

Further studies should be conducted on the same lines with different bacterial strains. Therefore, a much clearer image of the antibacterial profile of these coated materials can be acquired. Moreover, substitution with other elements should also be tried and their antibacterial activities should be studied in detail.

5. Conclusions

The role of each substitution in imparting antibacterial property to HA coatings becomes prominent in this study. Microwave irradiation techniques were successfully used to synthesize HA and substituted HA (Zn–HA, Mg–HA, and Si–HA). EPD—a low-cost deposition technique—was used to coat as-synthesized HA and substituted HA powders on biomedical-grade 316L stainless-steel surface. Spectroscopic and X-ray diffraction analysis confirmed the presence of substituted ions in HA. Uniform coatings were achieved through the EPD process. Magnesium-substituted HA offered better antibacterial activity against *S. aureus* in comparison to zinc and silicon-substituted HA. The bacterial adhesion was influenced by both the bacterial chemistry and the physicochemical properties of the coated substrates. The environmental factors also seemed to play an important role in bacterial attachment. Mg–HA not only showed lesser bacterial adhesion, but also stopped bacterial proliferation. Therefore, out of Zn–HA, Mg–HA and Si–HA, Mg–HA has great prospects as antibacterial, bioactive bioceramic coatings on metallic biomedical and dental implants.

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