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Original Research

An in vitro evaluation of novel NHA/zircon plasma coating on 316L stainless steel dental implant

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Abstract

The surface characteristics of an implant that influence the speed and strength of osseointegration include crystal structure and bioactivity. The aim of this study was to evaluate the bioactivity of a novel natural hydroxyapatite/zircon (NHA/zircon) nanobiocomposite coating on 316L stainless steel (SS) dental implants soaking in simulated body fluid. A novel NHA/zircon nanobiocomposite was fabricated with 0 (control), 5, 10, and 15 wt% of zircon in NHA using ball mill for 1 h. The composite mixture was coated on SS implants using a plasma spray method. Scanning electron microscopy (SEM) was used to evaluate surface morphology, and X-ray diffraction (XRD) was used to analyze phase composition and crystallinity (X_c). Further, calcium ion release was measured to evaluate the coated nanobiocomposite samples. The prepared NHA/zircon coating had a nanoscale morphological structure with a mean crystallite size of 30–40 nm in diameter and a bone-like composition, which is similar to that of the biological apatite of a bone. For the prepared NHA powder, high bioactivity was observed owing to the formation of apatite crystals on its surface. Both minimum crystallinity (X_c=41.1%) and maximum bioactivity occurred in the sample containing 10 wt% of zircon because of minimum X_c and maximum biodegradation of the coating sample.

Keywords: Dental implant surface; Bioactivity; Hydroxyapatite; Simulated body fluid; Zircon

1. Introduction

Dental implants have become an established treatment method since their introduction over 40 years ago. According to Albrektsson et al., the quality of the implant surface is a major factor that influences wound healing at the implantation site and subsequently affects osseointegration [1]. Surface-modified implants have the advantages of (a) providing better stability between the bone and the implant, established by a greater contact area, during the healing process, (b) providing a surface configuration that may retain the blood clot, and (c) stimulating bone formation and healing process [2].

The quality of the implant surface can be improved with numerous methods; one of which involves depositing bioactive materials onto the surface of the dental implant to induce osteoconductivity. Hydroxyapatite, [$Ca_{10}(PO_4)_6(OH)_2$: HA], is a calcium phosphate bioceramic material with a chemical composition almost identical to that of mineral components of a bone. HA-coated implants have often been used in load-bearing applications owing to their capability of bonding directly to the bone and improving new bone formation necessary for implant osseointegration [3–6]. Moreover, they have excellent bioactivity and are osteoconductive allowing bone cells to grow on their surfaces. Moreover, bioactive materials, such as HA, can integrate well with living bone

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tissues by spontaneously forming a biologically active bone-like apatite layer on their surfaces. HA products are extensively used as implantable ceramics for hard tissue reconstitution. Despite the advantages of HA, its surface degrades and in some instances separates; therefore, coatings with bioactive additives have been increasingly promoted, which also incidentally present better and faster osseointegration. Such additives can improve final coating characteristics such as bioactivity, thermal stability, and mechanical properties. Different materials have been added to HA to improve the final coating characteristics such as bioactivity [7]. Several studies have been conducted to evaluate additives such as zirconia (ZrO_2) and ZrO_2–Al_2O_3 in dental implant coatings; therefore, we tried to evaluate the bioactivity of zircon ($ZrSiO_4$) added to HA coating, as a new coating, in simulated body fluid (SBF) solution. This solution has been used in several in vitro studies to evaluate the changes on the surface of a bioactive glass ceramic [8]. SBF is an inorganic solution whose composition is similar to that of human blood plasma without organic components.

Zircon—the mineral name for naturally occurring zirconium silicate—is a tetragonal mineral consisting of a silicate of zirconium. The silicate group of biomaterials have the ability to release silicate ions at a definite concentration, which helps in the growth and differentiation of osteoblasts [9] thus leading to new bone formation. Therefore, this characteristic of zircon can be advantageous for surface coating of dental implants.

The plasma spraying technique is the most commonly used method for the application of HA coatings. It is a thermal spraying procedure in which powder particles are melted in a high-temperature plasma flame and propelled toward a substrate material to form a coating. The advantages of this process include high coating adhesion strength and high deposition rate, which allow rapid formation of coatings [10]. The aim of this study is to evaluate the bioactivity and morphological properties of a novel natural HA/zircon (NHA/zircon) nanobiocomposite coating on 316L stainless steel (SS) dental implants submersed in SBF solution.

2. Material and methods

2.1. Preparation of samples

This research was an experimental in vitro study. Bovine bones were boiled for 12 h to remove the attached flesh and fat. Thereafter, the bones were dried for 2 h at 110 °C to remove moisture. To prevent blackening with soot during heating, the bones were cut into small pieces of 10 mm thickness and heated at 500 °C (bone ash) for 2 h in air to allow evaporation of organic substances. The resulting black bone ash was heated for 3 h at 800 °C. This synthesis is called thermal decomposition of bone resource to create NHA. In this work, 316L SS was used as the substrate. The components of 316L SS were examined using elemental analysis, and the elemental composition is shown in Table 1. Specimens of dimensions $50 \times 2\ mm^2$ (diameter × thickness) were cut with the CNC wire-cutting EDM Machine (DK7732F, China suppliers). The samples were polished with a 100–1200 grit silicon carbide (SiC) paper. To produce a scratch-free surface, final polishing was performed using 1000 grit SiC papers. The specimens were cleaned with acetone and thoroughly washed with distilled water. Subsequently, the specimens were surface treated by grit blasting to obtain the desired surface roughness for better adhesion of the coating to the substrate. After the surface treatment process, the specimens were cleaned with distilled water using an ultrasonic cleaner. NHA/zircon nanobiocomposite was fabricated with 0 (control), 5, 10, and 15 wt% of zircon in NHA and coated on the surface of the SS samples using the plasma spray technique. The parameters used in plasma of powders are shown in Table 2.

2.2. Characterization procedure

Phase structure analysis was performed using X-ray diffraction (XRD) analysis with a Philips X'Pert Pro MPD diffractometer using Cu Kα radiation ($\lambda_1 = 0.15418$ nm) over a 2θ range of 20–80°. The obtained experimental patterns were compared with the standard patterns compiled by the Joint Committee on Powder Diffraction and Standards (JCDPS), which involved Card no. 09-432 for HA. The crystalline size and crystallinity (X_c) of the prepared powders were determined using XRD patterns and the modified Scherrer equation [11]. In the current study, X_c was measured according to the procedure described by Karamian et al. [12]. Scanning electron microscopy (SEM) analysis evaluations were performed using a Philips XL30 (The Netherlands) to investigate the surface morphology. The samples were spray coated with Au in high vacuum at an accelerating voltage of 25 kV. The Ca/P ratio was determined using energy-dispersive X-ray spectroscopy (EDX) microanalysis (FEI Quanta 200 ESEM equipped with an EDX EDS device).

2.3. In vitro bioactivity assessment

$NHA/ZrSiO_4$ nanobiocomposite powders with different $ZrSiO_4$ contents were prepared and coated using the plasma spray method. In vitro bioactivity of the nanopowders was investigated by soaking the samples in SBF solution. The SBF

Table 1
Composition of 316L SS materials (wt%).

Element	Cr	Ni	Mo	Mn	P	C	S	Si	Fe
Composition of 316L SS	18.00	12.00	2.50	1.80	0.04	0.02	0.02	0.15	Balance
From ASTM	16–18	12–14	2.00	2.00	0.075	0.02	0.03	1.00	Balance

Table 2
Parameters used in plasma of powders.

Flow rate of carrier gas (SCFH)	Spray voltage (V)	Spray current (A)	Flow rate of primary gas (SCFH)
50	50	500	120

Table 3
Comparison of nominal ion concentrations in SBF and human blood plasma.

Ion	Ion concentrations (mmol)	
	SBF	Human blood plasma
Na^+	142.0	142.0
K^+	5.0	5.0
Mg^{2+}	1.5	1.5
Ca^{2+}	2.5	2.5
Cl^-	147.8	103.0
HCO_3^-	4.2	27.0
HPO_4^{2-}	1.0	1.0
SO_4^{2-}	0.5	0.5
pH	7.4	7.2–7.4

solution was prepared according to the procedure described by Kokubo and Takadama [8]. The coated samples were soaked in an SBF solution of 7.4 pH at 37 °C for 1, 3, 7, 14, and 21 days at a solid/liquid ratio of 1 mg/mL without refreshing the soaking medium. Elemental analysis of the prepared SBF is shown in Table 3. After soaking for the predetermined time period, the samples were rinsed with deionized water and dried in an oven at 110 °C for 1 h. The weight loss of the samples was calculated with the following equation:

$$\text{Weight loss percentage } (\text{WL}\%) = (W - W_0)/W_0 \times 100,$$

where W and W_0 are the primary and secondary weights, respectively.

The released calcium ions were measured over 1, 2, and 3 weeks to evaluate the coated nanobiocomposite samples. The calcium ion concentrations of the solutions were determined using ICP-OES; an average of 5 measurements was taken for each sample, and the typical standard deviation was 0.05–0.2 ppm. To verify the accuracy of the experimental results, the bioactivity measurements were performed two times.

3. Results

3.1. XRD results analysis

Fig. 1(a) shows the XRD patterns of the sintered NHA 0% zircon coating. It illustrates that the only existing phase is for HA. Fig. 1(b) shows the XRD patterns of the coating containing a mixture of NHA and 10% zircon that is composited for 1 h. The presence of 10% of zircon did not create a new phase, which is less than the detection extent of the XRD technique 0.05. However, the presence of zircon resulted in a decrease in peak intensity and a slight increase in peak width of the HA owing to the presence of Si and Zr. Therefore, although the presence of 10 wt% of zircon in NHA did not create a new phase, it was effective, as discussed. Moreover, the peaks exhibit a slight shift and a bit decrease of crystallinity, X_c. These findings obtained using XRD data of NHA 10% zircon are compared with those of HA coating.

3.2. SEM evaluation and EDX analysis

SEM observations of the NHA 0% and 10% zircon coatings after soaking in SBF for 1 and 2 weeks are illustrated in Fig. 2. In the samples, agglomerated spherical and semi-spherical nanoparticles of HA crystals having different dimensions were found. These microphotographs have been taken from different locations on the NHA/zircon samples. The NHA 0% zircon sample has higher density than the NHA 10% zircon. These micrographs show the formation and growth of apatite crystals on the surface of the NHA 10% coating after soaking in the SBF solution. The micro-chemical analysis of the NHA 10% zircon sample was determined using EDX (Fig. 3).

3.3. Bioactivity evaluation of NHA/zircon coating

Bioactivity of the NHA 10% zircon coating was measured two times to verify the accuracy of the findings and prevent bias occurrence. X_c (41.1%) of this sample was the minimum, and it was equivalent to the maximum crystalline size (32 nm) (Table 4).

The results indicate the calcium released after 1, 2, and 3 weeks and weight losses after 1, 3, 7, 14, and 21 days of soaking the samples in the SBF solution (Tables 5 and 6). Analytical values are based on the mean and standard deviation (SD) of three replicates. In all the cases, SD was less than 2%. Tables 5 and 6 summarize the findings of bioactivity analyses. Table 5 illustrates that the calcium release increased significantly after 2 weeks and slightly after 3 weeks in the sample containing 10% zircon. After 2 weeks, weight loss was the most for the NHA 10% zircon sample. Fig. 4(a) indicates that calcium release increases in the sample containing 10% zircon and is the highest among all the samples. Fig. 4(b) shows a comparison of calcium release and pH variation. As the release of calcium increases after 1 week, pH decreases. Among the samples, the NHA 10% zircon sample had the most weight losses, as shown in Table 6.

As can be seen in Fig. 2, the growth of apatite layer for nanocomposite coatings with different amounts of zircon differed markedly from another. It is obvious to see that surface layer grows thicker with increase in zircon amounts. These bright topographic changes recommend that chemical ingrowths of calcium phosphate types occurred; in fact, it shows the bioactive potential of the prepared coatings. Fig. 1(a) shows SEM photomicrographs of NHA 10% zircon coating sample. The SEM photomicrograph reveals a relatively uniform distribution of the zircon nanoparticles in the NHA matrix. Fig. 2 (a)–(d) shows SEM photomicrographs of NHA and NHA 10% zircon coating samples after 1 and 2 weeks of immersion time in SBF solution for comparison. It can be seen from Fig. 2(a) that the corrosion has occurred in NHA coated sample without

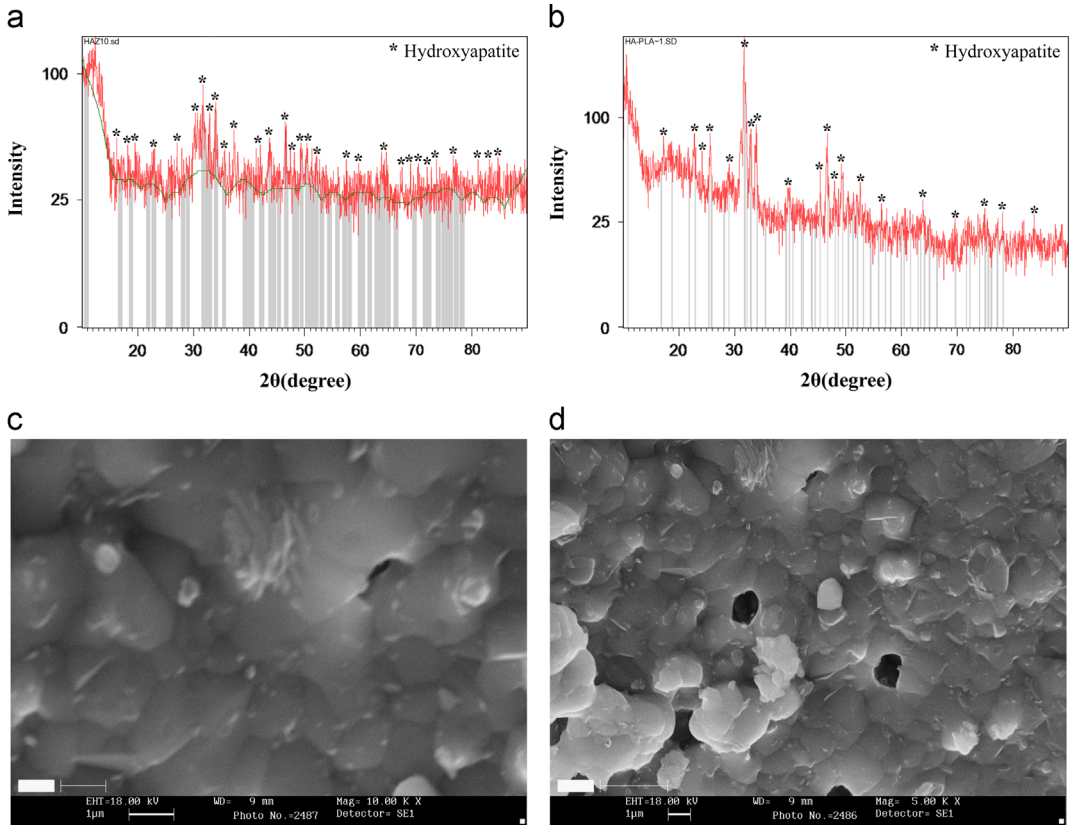


Fig. 1. XRD patterns of (a) NHA 0% zircon and (b) NHA 10% zircon coatings on 316L SS. SEM micrographs of (c) NHA 0% zircon and (d) NHA 10% zircon coatings on 316L SS (see the glassy surface).

formation of white particles on the corroded surfaces after 1 week. Fig. 2(d) shows the formation of white particles in cauliflower shape on the entire surface of the NHA 10% zircon coating sample and grain boundaries that has been shown by an arrow. Thus, the addition of zircon nanoparticles to NHA as reinforcement can accelerate the formation of white particles on the surface of composite samples.

4. Discussion

In the present study, the bioactivity of NHA/zircon coatings having different amounts of zircon (0, 5, 10, and 15 wt%) soaked in SBF solution was evaluated using XRD and SEM analyses. These coatings were fabricated on 316L SS cores. 316L SS may corrode inside the body under certain circumstances in a highly stressed and oxygen-depleted region, such as contacts under the screws of a bone fracture plate. Therefore, 316L SS is suitable only in temporary implant devices such as fracture plates, screws, hip nails, and dental implants. Surface modification methods such as anodization, passivation, and glow-discharge nitrogen implantation are widely used to improve corrosion resistance, wear resistance, and adhesion of 316L SS [13]. 316L SS and Ti alloys are useful in dentistry because of their durability and stability. In this study, the characteristics of SS were improved and evaluated by adding a new type of coating (NHA/zircon) to promote coating/substrate interface.

Zircon is a tetragonal mineral, consisting of a silicate of zirconium. Silicate biomaterials have the ability to release silicate ions at a definite concentration, which helps in the growth and differentiation of osteoblasts [14], thus leading to bone formation. Therefore, in this study, this characteristic of zircon that can be used for surface coating of dental implants was investigated.

Tiny agglomerated apatite particles can be formed on the surface of the sample containing NHA 10% zircon. Shown in the SEM observations (Fig. 2(b)), the grown layer is sometimes referred to as a bone-like apatite because its XRD pattern (Fig. 1(b)) is similar to that of a bone apatite with broad peaks at 2θ angles of HA, which indicates a superfine grain of apatite crystallite.

According to Kokubo et al. [15–17], in vitro immersion of bioactive materials in SBF produces in vivo surface-structure changes in materials such as bioactive glass ceramic. In vitro bioresorbability and bioactivity of NHA depend on its crystallite size. In the current study, X_c was measured according to the procedure described by Karamian et al. [12]. As can be observed from the X_c results (Table 4), NHA 10% zircon has the least X_c and the most amorphous structure. The prepared NHA/zircon powder showed a high bioactivity similar to that of a biological apatite. In fact, HA dissolution increases as X_c decreases (Table 4). Maximum bioactivity occurred in the sample containing 10% zircon because of higher biodegradation. Therefore, the increase in concentrations of Ca^{2+} and

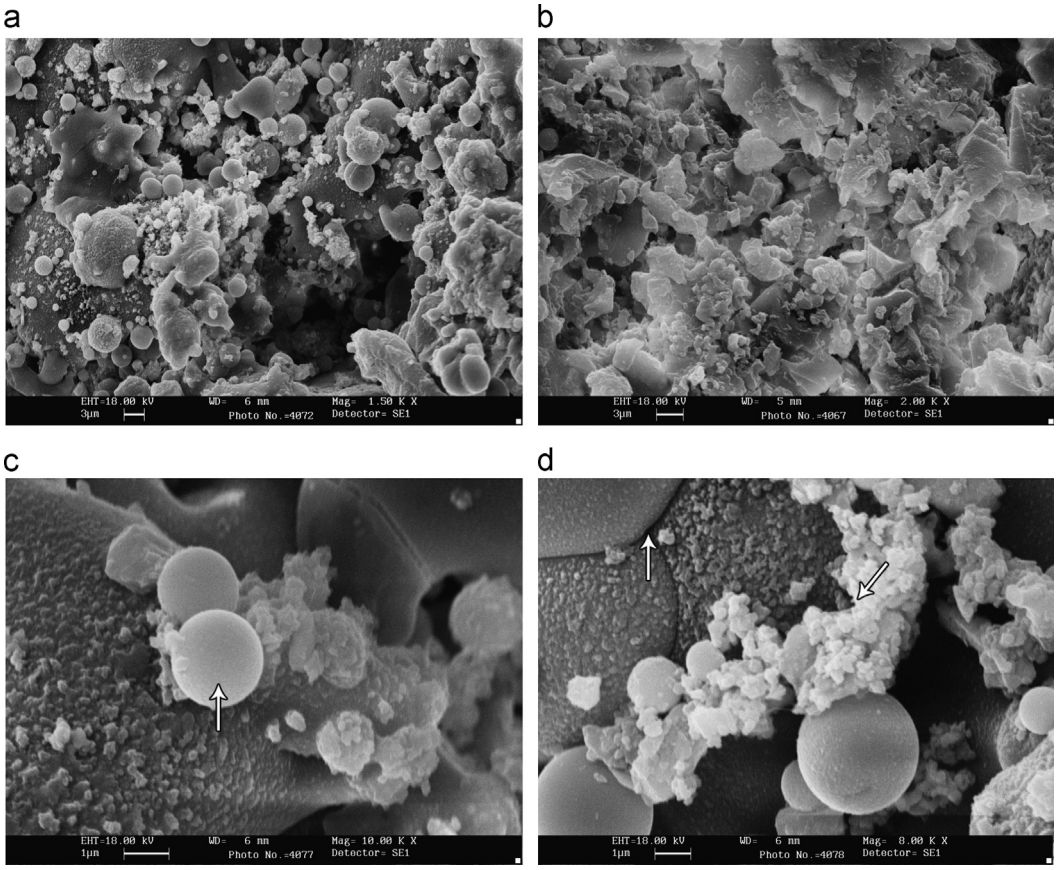
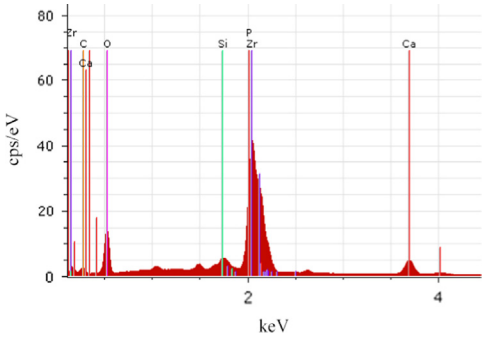


Fig. 2. Micrographs of (a) NHA 0% zircon and (b) NHA 10% zircon coatings after soaking in SBF for 1 week and (c) NHA 0% zircon (spherical particles, ↑) and (d) NHA 10% zircon (grain boundary, ↑ and cauliflower, ↙) coatings after soaking in SBF for 2 weeks.

a



b

Element	Series	[wt. %]	[Norm. wt. %]	[Norm. wt. %]
Carbon	K-series	3.022725991	4.85890391	12.25858833
Oxygen	K-series	20.56386552	33.05554221	62.60685682
Silicon	K-series	0.563041002	0.905064546	0.976514491
Calcium	K-series	5.628165193	9.047036995	6.840402184
Zirconium	L-series	32.43224074	52.13345234	17.31763817
	Sum:	62.21003845	100	100

Fig. 3. (a) EDX spectrum of the NHA 10% zircon sample and (b) microchemical composition determined using EDX.

PO_4^{3-} ions resulted in an increase in local supersaturation, which is beneficial for the nucleation and growth of new apatite crystals. Moreover, as more samples were dissolved, more nucleation sites were created. A higher precipitation rate

Table 4
Values of crystallographic parameters of HA phase in all the samples.

Sample	Crystallinity (X_c %)	Crystallite size ±1 (nm)
NHA 0% zircon	44.8	25.5
NHA 5% zircon	43.2	28
NHA 10% zircon	41.1	32
NHA 15% zircon	42.9	28.2

can be obtained in samples with higher dissolution [18]. The dissolution and precipitation behavior of apatites are the primary factors governing their bioactivity [18]. SEM observation (Fig. 2(b)) shows an amorphous structure and glassy surface for the NHA 10% zircon coating, leading to more biodegradation after soaking for 14 days. This micrograph shows the formation and growth of apatite crystals on the surface of NHA 10% zircon coating after soaking in the SBF solution. In fact, the higher the zircon up to 10 wt%, the faster the apatite crystals grow, which confirms the above interpretations.

Fig. 4(a) shows the rate of release of calcium ions with different amounts of zircon from the NHA into SBF, and the most bioactivity occurs in the sample containing 10% zircon. The most weight loss and Ca^{2+} release in the SBF solution

Table 5
Calcium release of samples in SBF for different soaking times.

Sample	Ca^{2+} release in SBF after 1 week (ppm)	Ca^{2+} release in SBF after 2 weeks (ppm)	Ca^{2+} release in SBF after 3 weeks (ppm)
NHA 0% zircon	36	78	82
NHA 5% zircon	40	90	93
NHA 10% zircon	62	115	118
NHA 15% zircon	48	96	98

Table 6
Percent weight loss (WL%) of samples in SBF for different soaking times.

Sample	WL% of samples in SBF after 1 day (mg)	WL% of samples in SBF after 3 days (mg)	WL% of samples in SBF after 1 week (mg)	WL% of samples in SBF after 2 weeks (mg)	WL% of samples in SBF after 3 weeks (mg)
NHA 0% zircon	0	0.11	0.2	0	0
NHA 5% zircon	0	0.10	0.22	0.06	0.03
NHA 10% zircon	0.11	0.35	0.6	0.15	0.13
NHA 15% zircon	0.02	0.12	0.25	0.12	0.09

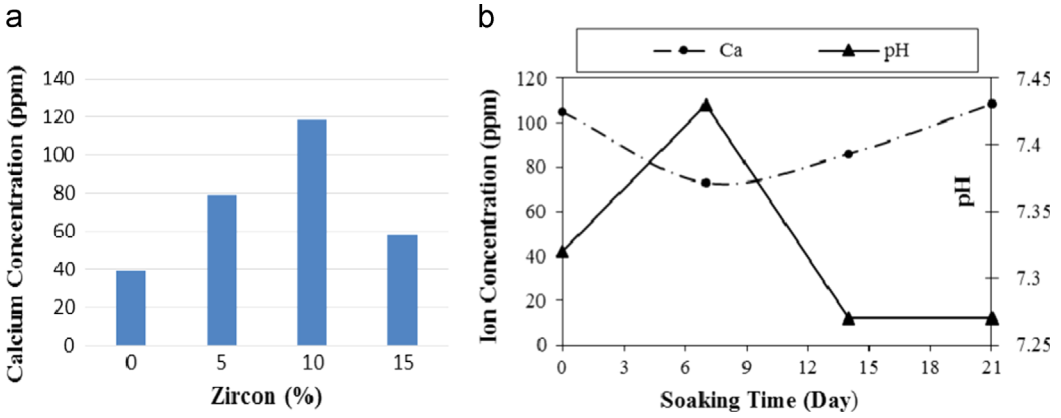


Fig. 4. (a) Ca^{2+} ion concentration of the samples with different percentages of zircon nanobiocomposite and (b) total comparison range of pH and Ca^{2+} ion concentration after soaking in SBF solution for 1, 3, 7, 14, and 21 days in all the samples.

(HA dissolution) occurred in the sample containing 10% zircon. The bioactivity of the nanobiocomposites improved with increasing amounts of zircon reinforcement, which particularly affects biological properties. In fact, the presence of the silicate phase produces glassy components, leading to an increase in the adherence of the apatite coating base to the adjacent SS layer. In addition, increasing zircon in some amounts may improve the homogeneity of the composite, as is the case in the NHA 10% zircon sample. However, over insertion of silicate phase into the composite leads to an increase in porosity, brittleness, and fracture strength, as in the NHA 15% zircon sample.

As shown in Table 5, calcium release increases dramatically from the first week to the second week. Although this increase has not continued into the third week, it was minimal because after the first week, only dissolution occurred in the samples. However, after the second week and probably in the third week, dissolution as well as apatite formation occurred thus leading to the control of weight loss (Table 6) in the samples.

There is a consensus regarding the osteoconductivity [19,20] and biocompatibility [21,22] of HA. The biocompatibility [23,24], osteoconductivity, and osseointegrity of zirconia have been discussed in previous studies [25]. In addition, zircon has a high biological and surface chemical property. Accordingly, a combination of these characteristics has been found in the prepared NHA 10% zircon coating.

To summarize, the proposed novel NHA/zircon coating is suitable for dental applications because of its good bioactivity. However, considering the novelty of the NHA/zircon coating and all the limitations of the current work, more research is needed to validate the present study. Further, the NHA/zircon coating can be evaluated in future studies by applying on titanium implants because of the common use of these implants in dentistry today.

5. Conclusions

HA biodegradation and bioactivity increased in the sample containing 10% zircon. Moreover, maximum number of Ca^{2+}

ions was also released in this sample. The presence of chloride ions in the SBF (153 ppm) induces an increase in HA dissolution.

The presence of zircon decreased NHA powder agglomeration. Zircon in combination with HA can improve osseointegration of an implant. HA/zircon nanobiocomposite implant coating possesses good bioactivity and is suitable for hard tissue formation.

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