Evaluation of fluorohydroxyapatite/strontium coating on titanium implants fabricated by hydrothermal treatment

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Abstract

Titanium and its alloys are considered as appropriate replacements for the irreparable bone. Calcium phosphate coatings are widely used to improve the osteoinduction and osseointegration ability of titanium alloys. To further improve the performance of the calcium phosphate-coated implants, strontium (Sr) was introduced to partially replace the calcium ions. In this study, the effect of Sr ion addition on the fluorohydroxyapatite (FHA)-coated Ti6Al4V alloy was investigated and all the coatings were treated under hydrothermal condition. X-ray diffraction (XRD) and scanning electron microscopy (SEM) were used to investigate the phases and microstructures, respectively. Shear tests were done to evaluate the bond strength of the coating layer. MTT, adhesion, and alkaline phosphatase tests were performed to evaluate the biocompatibility and osteogenic behavior of the samples. Results showed that the average crystallite size for the strontium-doped FHA samples was 48 nm and the bond strength had increased 13.15% in comparison with FHA-coated samples. Analysis of variance showed *p* value for all MTT tests at more than 0.322 and there was not any evidence of cell death after 7 days. The results of the ALP test showed that the increase of the cell activity in Sr samples from day 7 to 14 is three times higher than the FHA ones.

Keywords Fluorohydroxyapatite · Strontium · Hydrothermal · Cell culture · Alkaline phosphatase · Shear · Ti6Al4V alloy

Abbreviations

FHA	Fluorohydroxyapatite
FHA–Sr	Fluorohydroxyapatite/strontium
FA	Fluorapatite
Sr	Strontium
MAA	Micro-arc anodizing
XRD	X-ray diffraction
SEM	Scanning electron microscopy

Introduction

Titanium alloys have been used for a long time as implant materials in orthopedic and dental surgery (Verma 2020). Although Ti and its alloys are successfully used as biomaterials, there is still a need to improve the bone tissue

interactions with these implants. One interesting strategy is to coat Ti with bioactive materials such as hydroxyapatite (Nguyen et al. 2020). Calcium phosphate coatings act as active interface for bone tissue proliferation, due to their similarity to bone minerals. By creating new chemical bonds between human bone and implant surfaces, they can accelerate the process of implant fixation in the bone. However, pure hydroxyapatite coatings have a relatively high decomposition rate under intrinsic conditions. Degradation resistance can be increased by adding fluorine ions in its structure and forming fluorine–hydroxyapatite while maintaining its biocompatibility (Arcos and Vallet-Regi 2020; Bucur et al. 2020; Liu et al. 2020; Wolf-Brandstetter et al. 2020; Yao et al. 2020).

With the replacement of fluoride, instead of hydroxyl ions, fluorapatite with the formula $\text{Ca}_{10}(\text{PO}_4)_6\text{F}_2$ and fluorohydroxyapatite with the formula $\text{Ca}_{10}(\text{PO}_4)_6\text{FOH}$ can be obtained (Feroz and Khan 2020). In the presence of fluoride ions in HA, adhesion and toughness of coating at the interface increased (Feroz and Khan 2020; Jaafar et al. 2020; Kabir et al. 2020; Rezaee et al. 2020). Fluoride exists in human bones and teeth as an essential element against dissolution. Furthermore, fluoride promotes the mineralization and crystallization of calcium phosphate in

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the bone-forming process (Suchanek et al. 2015; Makarova et al. 2020).

There are different methods for coating calcium phosphates on titanium implants, including plasma spraying, thermal spraying, sol–gel and electrophoretic methods. The surface of the calcium phosphate plasma-sprayed coatings may melt at very high temperatures. Due to fuzzy metamorphism, absorbable phases form and decompose in a short time after implantation (Graziani et al. 2017; Bennett et al. 2019). Other disadvantages of this method can be the creation of coatings with a relatively high thickness (30–100 μm) and high crystallinity, which may be susceptible to delamination and degradation after implantation (Zheng et al. 2017; Xia et al. 2018). In comparison, the sol–gel method offers certain advantages such as high homogeneity due to atomic scale mixing, low crystallization temperature, high purity of the product, the ability to achieve a specific chemical composition with a high degree of stoichiometry and applicability of coating complex shapes such as dental implants (Catauro et al. 2016; Ergün and Başpınar 2017; Suwanprateeb et al. 2018).

Calcium phosphate coatings need to be heat treated and their properties can be severely improved using an appropriate surface treatment technique. Among various thermal treatments, hydrothermal treatment is one of the most powerful methods for producing different forms of HA coatings with good surface quality and Ca/P ratio close to the stoichiometric value (Kavitha et al. 2018; Zhang et al. 2018; Ali et al. 2019; Su et al. 2019; Qadir 2020). Some advantages of hydrothermal treatment are good controlling coating structure and composition, relatively low processing temperatures which enables the low residual stresses and formation of crystalline structure. Crystal size would be in the range of nanometer to micrometer in the hydrothermal process that encourages adhesion of bone cells (Tang et al. 2005; Xiong et al. 2010; Li et al. 2019).

The previous works showed the influence of strontium ions on bone formation. Sr^{2+} ions stimulate the proliferation and differentiation of osteoblastic cells while subsequently inhibiting the activity of the osteoclasts. More specifically, Sr activates the calcium-sensing receptors, which agitates bone formation (Zhang et al. 2016; Robinson et al. 2017; Cao et al. 2019; Sedelnikova et al. 2019; Surmenev and Surmeneva 2019). The doses of Sr affect the mineralization procedure. As such, a low amount of Sr leads to an increase in the mineralization of bone. However, a high dose of Sr has a negative effect, using more than 20 $\mu\text{g}/\text{mL}$ Sr disturbing the activity of osteoblasts (Lei et al. 2017; Mao et al. 2017). It is shown that Sr^{2+} ions alter the morphology, crystallinity, and solubility of apatite coatings. In addition, Sr^{2+} affects the early formation of biomimetic coatings on single crystalline rutile substrates (Engstrand et al. 2012; Avci et al. 2017).

To the best of our knowledge, structural and biological evaluation of fluorohydroxyapatite/strontium (FHA–Sr) coatings on the anodized Ti alloys by MAA technique have not been clarified. This work provides dual mechanical arc anodizing (MAA) and sol–gel coating methods for Ti6Al4V surface modification and using the hydrothermal treatment for controlling the composition and structure of the coating layer. Furthermore, an in vitro assessment of strontium-doped fluorohydroxyapatite coatings on Ti6Al4V alloys, treated under hydrothermal condition, has yet to be reported. Therefore, the objective of this study is to prepare fluorohydroxyapatite and fluorohydroxyapatite/strontium coatings with low Sr concentrations and evaluate their structure and morphology by XRD and SEM techniques. The response of osteoblastic cells under in vitro conditions was also investigated by experimental and statistical analysis. In addition, we have used shear test analysis for evaluating interface bond strength of the sol–gel-derived films.

Materials and methods

Ti6Al4V sheets were cut into 15 mm $\times$ 15 mm $\times$ 1 mm. At first, for increasing the corrosion resistance and roughness, the surface of the titanium substrates was anodized in 1 molar sulfuric acid solution at 150 V (MAA process). Calcium nitrate tetrahydrate [$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$] (Merck, Germany), triethyl phosphide (TEP, [$\text{P}(\text{C}_2\text{H}_5\text{O})_3$] (Merck, Germany) and ammonium fluoride [NH_4F] (Merck, Germany) were used as precursors of FHA. In this way, some controlled amounts of TEP and ammonium fluoride were first dissolved in ethanol. After adding distilled water, the solution was stirred vigorously for 24 h at room temperature. A high dose of Sr could suppress the formation of FHA while a low concentration shown to increase bone mineralization and have higher solubility (Wang et al. 2019; Graziani et al. 2017). Therefore, in a separate container, a stoichiometric amount of calcium nitrate and 1.2 mM strontium nitrate (Titrachem, Iran) was dissolved in ethanol with vigorous stirring for 24 h. Then, Ca-containing solution was added slowly to the P containing solution and aged at room temperature for 24 h.

Ti plates were ultrasonically cleaned with acetone, ethanol (70%), and distilled water for 1 min in each step. FHA and FHA–Sr sols were subsequently applied on the pre-cleaned Ti plates by dip coating process at a withdrawal speed of 5 mm/min (Deep coater, Adeeco, Iran). During dip coating process, apatite sols were applied on the plates with an ultrasonic assistance at a constant ultrasonic frequency of 40 kHz (Ultrasonic cleaner, DL-120A, China). The deposited films were initially dried in the oven for about 30 min at 80 °C. Then, they were hydrothermally treated at 190 °C for 7 h.

All coatings were characterized using scanning electron microscopy (SEM, LEO 1450VP) in conjunction with energy-dispersive spectroscopy (EDS). XRD analysis using Cu-K α radiation was conducted to characterize the phases of FHA and FHA–Sr films (XRD, GNR EXPLORER, Italy). The shear bond strength for each sample was evaluated using the Instron universal load testing machine according to ASTM C633-79 in such a way that peak loading force was recorded when the coating was completely peeled away from the surface (Lu et al. 2019). Cell viability of MG63 cells was measured using MTT assay (MTT, Sigma, USA) according to the international standard (ISO 10993). Data were analyzed using one-way ANOVA analysis of variance (IBM SPSS statistics 23) and the statistical difference was considered to be significant at $P < 0.05$. Alkaline phosphatase activity was measured on the coated samples in 7 and 14 culture days and the absorbance was read using a spectrophotometer at 405 nm.

Results and discussion

X-Ray diffraction analysis

XRD patterns of pure FHA and FHA–Sr coatings are shown in Fig. 1. They were hydrothermally treated at 190 °C for 7 h. The major diffraction peaks identified for FHA are observed at 2θ values of 26.4°, 29.5°, 32.1°, and 40.1°. In the FHA–Sr pattern, a broad peak was observed around 31.5° which was related to the overlapping of planes (2 1 1), (1 1 2), and (3 0 0). Figure 1 shows the higher presence of FHA and FHA–Sr than other compounds like hydroxyapatite (HA). As can be

Fig. 1 XRD pattern of FHA and FHA–Sr coatings under hydrothermal treatment at 190 °C/7 h. (filled square: fluorohydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6\text{F}_x\text{OH}_{2-x}$), filled circle: Sr-doped fluorohydroxyapatite ($\text{Ca}_{10-x}\text{Sr}_x(\text{PO}_4)_6(\text{FOH})$), filled diamond: Strontium hydrogen phosphate (SrHPO_4))

seen in Fig. 1 some peak positions in FHA–Sr compared with FHA changed from 26.4° to 26.2°, 29.5° to 27.8° and 40.1° to 39.8°. This displacement is due to the distortion created in the crystal lattice. The substitution of Sr^{2+} for Ca^{2+} may occur because of the high chemical affinity of two cations and represent by x value in the chemical formula of hydroxyapatite compounds, i.e., $\text{Ca}_{10-x}\text{Sr}_x(\text{PO}_4)_6(\text{OH})_2$.

The unit cell of crystalline hydroxyapatite has ten cations: four at the M(1) site (aligned in the column) and six at the M(2) site (arranged at the apexes of equilateral triangles). According to the step kinetics theory and Rietveld Refinement characterization, Sr^{2+} can occupy both M(1) and M(2) sites which leads to an increase in the lattice parameters a and c and lattice distortion due to the larger Sr^{2+} ions compared with Ca^{2+} ions (Pal et al. 2019; Wang et al. 2019). Bigi et al. (2007) showed Sr^{2+} ions preferentially incorporate at M(1) sites of apatite crystals in hydrothermal condition. M(1) sites are larger than M(2) and also have longer M(1)–O bond distances, thereupon less stress and distortion will apply in hydrothermal condition (Boyd et al. 2015).

XRD pattern of the FHA–Sr coating shows significant narrowing compared with the FHA pattern, and more crystallinity of the phases in FHA–Sr than FHA. It was also confirmed by Ehret et al. (2017) and Xia et al. (2010) who detected a significant peak narrowing, increasing intensities, and shifting to slightly lower peak positions by Sr incorporation in the HA lattice structure (Gopi et al. 2014). It is expected this observation is due to the fact that Sr is heavier and contains more electrons than Ca and so leads to more scattering X-rays of the FHA–Sr-coating layer than FHA coating. O'Donnell et al. (2008) showed that lattice parameters (a and c) and so the unit cell volume and density increased linearly with Sr addition, consistent with a larger and heavier ion entering in the apatite lattice.

Suchanek et al. (2015) showed samples that are subjected to hydrothermal treatment had a more crystalline structure than the air-treated samples. The presence of hydroxyl groups in a humid and hot environment accelerates the conversion of amorphous calcium phosphate structures to crystalline structures. This condition allows the coatings to crystallize at low temperatures, which is a very effective way in preventing crystal overgrowth and also or reducing unwanted elements that generally form due to heat treating apatite coatings at temperatures higher than 450 °C. The crystallite size of the coating materials is inversely proportional to peak width according to the Deby–Scherrer Eq. (1) (Liu et al. 2001; Salehi et al. 2015):

$$\Delta(2\theta) = \frac{0.9\lambda}{D\cos(\theta)}, \quad (1)$$

where $\Delta(2\theta)$ represents the full width at half maximum intensity (FWHM), λ is the wavelength for Cu(K α)

($\lambda = 0.15418$ nm), and D is the crystal size in nanometer. As determined from XRD investigations, the FHA coating consists of crystalline FHA with a crystal size of about 35 nm and FHA–Sr coating with a crystal size of 48 nm. In the present study, Sr substitution is equal to 0.18 by considering the $\text{Ca}_{10-x}\text{Sr}_x(\text{PO}_4)_6(\text{FOH})$ formula. This is consistent with the observations of some researchers about substituting larger Sr^{2+} ions for Ca^{2+} ions (Li 2010; Gallo 2011; Wang et al. 2019). This is while Sanyal and Raja (2017) reported that FHA and Sr–Ce–FHA compounds synthesized by the sol–gel method had a crystallite size of 60.5 nm and 65.4 nm, respectively. Shokri et al. (2014) also showed FHA crystallite size that was synthesized by the sol–gel method was 50 nm.

Fig. 2 SEM image of titanium surface after MAA process

Morphology of the coating

SEM micrographs and EDS spectra were used to evaluate the morphologies and the chemical composition of FHA and FHA–Sr samples. To provide the chance of micromechanical interlocking of the coating layer, it was decided to anodize titanium alloy samples before being exposed to the sol–gel process. In Fig. 2, the morphology of the titanium surface after the MAA process is observed. As it is evident, there are a large number of pores on the surface of the sample and there are no cracks on the surface, indicating the uniformity and suitability of MAA operations. Anodizing process is a well-established technique for increasing surface areas on metals before bonding procedures (Tredwin 2009). Micropores on the surface after MAA process encourage apatite deposition and also strengthen the adhesion between the coating layer and the substrate (Sedelnikova et al. 2019). A lot of research works on hollow surfaces have shown improvement in the functions of osteoblasts and enhance the osteointegration response because of surface roughness (Gallo 2011; Huanget al. 2017).

After coating the surface by FHA and FHA–Sr, surface morphology differs from the anodized surface (Fig. 3). Very small polygonal crystals are shown to be precipitated on the hydrothermally treated surface. Usually, there are some surface cracks in the coatings obtained by the sol–gel route for contractions arising from the thermal treatment (Graziani et al. 2018). In the present study, no superficial crack is observed due to the low-temperature hydrothermal treatment. The morphology of the FHA–Sr coating (Fig. 3b) is sphere-like with wide size distribution. Similar morphology was observed by Xia et al. (2010) in the combination of calcium phosphate and strontium. Precipitated FHA–Sr coating under hydrothermal treatment resulted in the rapid formation of a dense and spherical coating on the surface. It is due to intermolecular interaction in the presence of water vapor. The FHA–Sr surface is dense and rough which is

Fig. 3 SEM image of: **a** FHA coating, **b** FHA–Sr coating

favorable for the adhesion and proliferation of cells (Huang and Yoshimura 2020; Xing et al. 2020). These sphere-like particles were composed of plate-like apatites. EDS spectra confirm the existence of biological elements of fluorohydroxyapatite and Sr as dopant element which is compliant with XRD results (Fig. 4).

The dipole–dipole interactions that come from hydrogen bonds are stronger than intermolecular interactions such as Van der Waals forces. It is suggested under hydrothermal conditions multilayer adsorption will occur on the surface which in some parts form accumulated spherical coatings (Li et al. 2019). Moreover, the molecular attraction forces will increase in the presence of moisture and the interparticulate distance will reduce, leading to the dense particulate coating. According to Renaudin et al. (2009) doping of Sr^{2+} into the HA crystal lattice is done at Ca^{2+} sites. At this sites, Sr^{2+} would be surrounded by seven oxygen atoms and one of them is from the OH – group. In this situation, the Sr–OH bond is more elastic and also because of the more ionic radius of Sr^{2+} than Ca^{2+} , a compact surface morphology will be formed.

Shear bond strength

Shear tests were done for the FHA and FHA–Sr-coated samples with the same sample sizes. This test relies on the needed load for removing coating layer on the substrate. The mean bond strengths for FHA and FHA–Sr coatings were 38 MPa and 43 MPa, respectively, according to the Eq. 2 (Jansen and Leon 2009):

$$\sigma = \frac{F}{S}, \quad (2)$$

where σ is the bond strength (MPa), F is the peak force (N), and S is the area of the sample (mm^2). The results show that bond strengths for all the samples are higher than or comparable to those observed for the hydroxyapatite and

fluorohydroxyapatite coatings obtained by sol–gel routes (≈ 10 – 40 MPa) (Wang et al. 2008; Jansen and Leon 2009; Mohseni et al. 2014). For surgical applications, the shear bond strength of apatite coatings and metallic implants should be higher than 15 MPa according to international standard (ISO13779-4) so this is an extremely encouraging result for the biomedical applications of FHA and Sr-doped FHA-coated implants (Ali et al. 2019).

The bonding strength is affected by the combination of both cohesive (within the coating layers) and adhesive (coating to the substrate) strengths of a coating. The adhesive strength is usually evaluated by the surface roughness and the mechanical interlocking between the coating layer and substrates, whereas the cohesive strength is determined by coating properties, such as microstructure and crystallinity (Mohseni et al. 2014; Suwanprateeb et al. 2018). It is proved bonding strength of the FHA coatings is more than HA coatings owing to the formation of chemical bonds at the FHA–Ti interface and also relief of thermal mismatch due to the incorporation of fluoride ions (Jaafar et al. 2020). Some researchers suggested creating a TiO_2 layer because oxidizing the titanium surface can be an effective way for improving the bonding strength of the TiO_2 –FHA bilayer. Titania (TiO_2) is an optimal interlayer due to its high mechanical integrity and its chemical similarity to both FHA and Ti alloy substrates. Furthermore, the greater bond strengths achieved in the present study compared with data found in the literature may be related to good wetting characteristics and intermolecular interaction due to the hydrothermal treatment and also greater diffusion of the fluoride and strontium co-substituted apatite across the interface in the presence of moisture (Wang et al. 2008). According to Renaudin et al. (2009) by doping Sr^{2+} into the HA lattice, Sr^{2+} ions would be surrounded by seven oxygen atoms and the bond angle decreases with the increasing atomic number of the metal ion which leads to a more compact morphology in Sr-doped FHA structures. In this regard, Batra et al. (2013) showed

Fig. 4 EDS analysis of: **a** FHA coating, **b** FHA–Sr coating

covalent bonds in alkaline earth oxides increases from Ca–O bond to Sr–O bond due to increasing involvement of d orbitals (0.406 in Ca–O to 0.715 in Sr–O) which could improve the bond strength of the Sr-doped FHA-coating layer in comparison with FHA coatings. Furthermore, by doing MAA pretreatment, the micromechanical interlocking between the coating layer and titanium alloy surface will increase. Cacciotti (2019) suggested by increasing the oxidizing voltages upper than 50 V due to the reduction of contact angle associated with increment in surface roughness, wettability on Ti6Al4V alloy will improve.

Cytocompatibility evaluation

To confirm the viability of MG63 cells on the anodized, FHA, and FHA–Sr layers, the MTT assay was carried out at 3 and 7 days post-seeding. Figure 5 shows the density of MG63 cells for 3 and 7 days. According to Table 1 after 3 days, the value of P is 0.344 for anodized titanium, 0.322 for the FHA, and 0.444 for the FHA–Sr sample. Based on the MTT results, there were no significant differences in cell density between the anodized titanium and coated samples with FHA and FHA–Sr ($P > 0.05$) after 3 days. After 7 days, there were also no significant differences in cell viability ($P > 0.05$) between anodized titanium, FHA, and FHA–Sr surfaces. Despite the high proliferation rate of MG63 cells on the rough and treated Ti surface at early time points, a slight decrease in MTT response was observed in the test period for the FHA-coated sample but this difference was not significant. Indeed, the MTT assay indicated that three specimens are non-toxic to MG63 cells.

Cell morphology on anodized and apatite-coated titanium surface after 3 days is shown in Fig. 6. A layer with cells observed on the MAA surface that covers almost all the surface-suggesting good spreading. The levels of cell affinity, proliferation, and differentiation depend on the physical and chemical properties of the surface (Gulati et al. 2020).

Fig. 5 Viability percentage of the coated and anodized samples

Table 1 ANOVA results of MTT test for 3 and 7 days

MTT groups		<i>P</i> value	
		3 days	7 days
Control	Anodize	0.344	0.688
Control	FHA	0.322	0.736
Control	FHA–Sr	0.444	0.794
Anodize	FHA	0.961	0.948
Anodize	FHA–Sr	0.846	0.887
FHA	FHA–Sr	0.808	0.939

Fig. 6 SEM images of cells cultured on: **a** MAA surface, **b** FHA surface, **c** FHA–Sr surface. (Pseudopodia assigned in **b** and **c**)

In this sample, although the surface is neutral, the roughness of the surface encourages the reaction between cells and substrate. Based on SEM micrographs, cells on FHA and FHA–Sr surfaces show good health and they were elongated and adhered to the coated surfaces through their pseudopodia. As marked in Fig. 6, MAA and FHA surfaces show dysfunctional characteristics such as premature apoptosis and delayed differentiation characterized by spherical shape cells. Osteoblasts tend to flatten and cover the MAA surface whereas cells on the coated samples show a more differentiated appearance with various long pseudopodia. This difference was probably because of the differences in microtopology and roughness of uncoated and coated samples as reported elsewhere (Kimet al. 2005). Based on Suwanprateeb et al. (2018) research a smooth and rough surface may have similar adhesion forces because cell-material interactions are extremely complex and depend on several mixed parameters. Moreover, since the SEM images are taken after day 3 and also from a few local surfaces, they do not fully reflect the surface capability for cell attachment.

Sr may directly interact with the calcium-sensing receptors in osteoblast cells resulting in increased cell spreading (Lei et al. 2017). It is predicted Sr-modified FHA coating causes more growth and spreading of cells than FHA-coated samples by passing time, due to the presence of more dense and rough areas than the FHA layer. As well as, it was indicated the presence of Sr makes higher surface energy that is a beneficial factor for attachment and spreading cells (Yuan et al. 2017). In the same way, Wei et al. (2009) indicated hydrophilicity of the surfaces significantly affects cell attachment and cell growth. It is predicted in the current study, the surface hydrophilicity and so cell spreading would be improved by incorporating Sr ions, and also it will be further improved by hydrothermal treatment. It is indicated in the literature that Sr^{2+} ions enhance cell proliferation and bone formation when combined with Ca^{2+} in vitro, but a high concentration of strontium could be toxic for cells (Tadier et al. 2012; Chen et al. 2017; Guo et al. 2017). Based on Ehret et al. (2017) research, in vitro studies did not reveal any cytotoxicity of the HA matrices by Sr substitution of less than 50% of Ca.

ALP activity

Figure 7 shows the ALP activity of MG63 cell layers during 7 and 14 days. After 7 days, there was no significant difference between FHA and FHA–Sr samples ($P > 0.05$) (Table 2). ALP activity in FHA–Sr samples was increased at culture day 14 when compared to FHA and control samples ($P < 0.05$). The hydrothermally treated apatite coatings could promote the proliferation and differentiation of osteoblasts by improving surface wettability (Li et al. 2020). Moreover, incorporating Sr into the calcium

Fig. 7 ALP activity of cell layers produced by MG63 during 7 and 14 days

Table 2 ANOVA results of ALP test for 7 and 14 days

ALP groups		P value	
		7 days	14 days
Control	FHA	0.044	0.190
Control	FHA–Sr	0.005	0.000
FHA	FHA–Sr	0.132	0.000

phosphate structures was known to be beneficial to the response of various hard tissue-related cells in vitro.

Sr is a modulator for calcium-sensing receptors, so the presence of ions such as Sr^{2+} improves the proliferation, differentiation, and mineralization processes of osteogenesis (Huet al. 2017; Moghanian et al. 2018; Xie et al. 2018). It is a non-essential element in adult human body, its amount is equal to 0.14 g, and mainly found in the mineral phases of bone. A lower concentrations of Sr improved osteogenic differentiation of mesenchymal stem cells, while higher doses caused cell death (Oryan et al. 2019). Studies showed that incorporation of Sr into HA increased the activity of osteoblasts and inhibited osteoclast proliferation. Ehret et al. (2017) also showed combination of Sr with hydroxyapatite improved osteoblastic differentiation and mineralization process. Like Ca^{2+} , Sr^{2+} is a divalent cation and has been prescribed as an anti-osteoporotic drug that keeps the balance of bone resorption and bone formation. Incorporation of strontium which has more than twice the atomic mass of calcium could play an important role in decreasing the risk of orthopedic fractures and increasing the bone mineral density. However, the mechanisms by which strontium causes its effects on bone have remained unclear (Kanis et al. 2011; Xie et al. 2018; Arcos and Vallet-Regi 2020).

Conclusion

In this study, fluorohydroxyapatite (FHA) and fluorohydroxyapatite/strontium (FHA–Sr) compounds were synthesized using the sol–gel method and coated on Ti6Al4V alloys by dip coating technique. All Ti surfaces were anodized by MAA process before they coated and were hydrothermally treated after the coating process. The morphology of the coatings was changed to dense and sphere-like particles by adding a small amount of Sr^{2+} ions to FHA in the presence of water vapor. Analysis of the phases showed that doping strontium to the primary compound results in a more crystalline structure, displacing the peaks to low-angle positions due to the distortion caused by Sr^{2+} addition and also increasing peak intensities due to heavier Sr^{2+} than Ca^{2+} ions. Shear bond strength for the FHA and FHA–Sr samples was 38 MPa and 43 MPa, respectively. This good adherence of the coating layer can be due to the micromechanical interlocking caused by MAA process, good wetting characteristics, intermolecular interaction due to the hydrothermal treatment and also greater diffusion of F^- and Sr^{2+} at the interface of apatite and Ti alloy. Likewise, more bond strength in FHA–Sr samples could be created as a result of more compact morphology and covalent bonds in the presence of Sr^{2+} ions.

ANOVA results of MTT tests showed that viability percentages remained nearly constant for all samples during 3 and 7 days of culture in comparison with the control sample which indicates they are non-toxic to MG63 cells. Cell adhesion examination showed some spherical shape cells on the MAA and FHA surfaces, whereas only long pseudopodia were observed on FHA–Sr surfaces. According to ALP results for 14 days, ALP activity of FHA–Sr samples increased by 105% compared to day 7, despite for the FHA samples it increased only 43.28% from day 7 to 14. It is estimated that Sr^{2+} ions cause raising in bone mineralization and also they support calcium-sensing receptors when combined with calcium which could promote the proliferation and differentiation of osteoblastic cells.

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Availability of data and materials The data and materials have been properly presented in the main manuscript.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The proposed study complies with all the ethical guidelines.

Consent for publication All the authors have agreed to publish the data in your esteemed Journal.

Consent to participate Informed consent was obtained from all individual participants included in the study.

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