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RESEARCH PAPER

Hydroxyapatite-Hardystonite nanocomposite scaffolds prepared by the replacing the polyurethane polymeric sponge technique for tissue engineering applications

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ABSTRACT

Objective (s): Silicate bioceramics containing Zn and Ca like hardystonite (Hr) with chemical formula Ca2ZnSi2O7 has attracted the attention of researchers in biomedical field due to its remarkable biological and mechanical properties. The new generation of bioceramics can applied in bone tissue engineering to substitute with infected bone. However, these zirconium-silicate bioceramics have proper features; they can be used as additive with calcium phosphates (CaPs) like hydroxyapatite (HA). These composite may enhance the weak properties of pure HA powders.

Materials and Methods: In the current study, the Hr powder with sol-gel method was synthesized. Then, hydroxyapatite-hardystonite (HA/Hr) scaffolds with various amount of Hr in HA (0, 5, 10, and 15 wt. %) were prepared by the replacing the polyurethane polymeric sponge technique. Then, the ceramic scaffolds were sintered at 1150°C for 3 hours. The result of the scaffold nanocomposite specimens were tested for compressive strength and bioactivity behaviour. The phase and morphology of the specimens were analysis with X-ray Diffraction (XRD) and Scan Electron Microscope (SEM) techniques.

Results: The bioactivity, sinterability and compressive strength (CS) properties of HA-Hr scaffold nanocomposite has been developed by addition of 15 wt. % Hr in HA matrix. The results showed the scaffold nanocomposite containing 15 wt. % Hr have the highest value of CS which is equal 1.21 MPa. Our data indicate that Hr ceramics possess proper mechanical properties as compared to pure HA, and may possess good biocompatibility in biological analysis.

Conclusion: The study indicates that scaffold containing 10 wt. % Hr had proper bioactivity behaviour which leads the scaffolds surface to form bone-like apatite on the surface of scaffolds.

Keywords: *Hydroxyapatite, Hardystonite, Nanocomposite, Scaffolds, Tissue Engineering*

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INTRODUCTION

Bone tissue engineering is a field that helps the biomaterials engineer to design artificial bone in case of fracture bones caused by accident and tumors [1]. Using bone auto grafts, allograft, and xenografts from human and animal source help to heal the tissue as one of common tissue engineering technique. Recently various types of calcium phosphates (CaPs) have been introduced to apply in tissue engineering application [2]. However; these CaPs have some weak properties like improper mechanical (brittleness) and chemical stability in their structure [3]. One of the most important characteristic of has like porosity and cells transformation of artificial bone play a significant role in the bones regeneration of implanted tissue. The scaffolds porosity is influenced by the architecture of scaffold and materials characteristic in the range of 100 μm. The repair of bone defects consider as a major clinical challenge all around the world. One of the known

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CaPs is hydroxyapatite (HA) which has a potential ability to substitute with human's bones [4-5]. Sadeghzade et al [4]. fabricated pure hardystonite ($Ca_2ZnSi_2O_7$) nanopowder by combustion synthesis method. Their result indicated that mean particle size and the maximum crystallite size of the produced hardystonite nanopowder were almost 40 nm, respectively. Nevertheless, CaPs have some drawback like safety refusal and tissues may have some necrosis. Several bioceramics add to HA to enhance its properties like TiO_2 [1, 3], ZrO_2 [6-7], diopside [8-11], baghdadite [12, 20], hardystonite [13, 28] and etc. Zinc as one of the crucial trace element that also plays an important role in bone metabolism [4, 13]. Ceramics containing Zn salt have good property as a substituted tricalcium phosphate (TCP) and enhance the range of apatite formation in the implants [14, 19]. An hardystonite is a mineral containing Ca, Zn, and Si, with a melting temperature of 1425 °C and a density of 3.40 g/cm3, and so far has no important industrial applications [13-14]. While Zn-incorporated to calcium silicate (e.g. Hr) with chemical formula $Ca_2ZnSi_2O_7$ can have better mechanical and strength properties [14, 28-29]. Recently, the new generation of silicate bioceramics like akermanite [15], diopside [8-11] hardystonite [11, 20], baghdadite [12], nanoclay [11, 18] and bredigite [11, 28] has been explained by researchers [13-18, 28]. In another study, Karamian et al [21]. investigated hydroxyapatite-baghdadite scaffold nanocomposite coated by PCL/Bioglass with polyurethane polymeric sponge technique [20]. They showed that addition of baghdadite can enhance the HA mechanical and biological properties dramatically. In this study HA-Hr nanocomposite scaffolds has been fabricated. A series of tests like bioactivity, and mechanical testing were carried out to analyse their physico-chemical and biological properties to HA–Hr scaffolds. The aim of the current project was to prepare nanostructured HA/Hr scaffolds nanocomposite by replica method using polyurethane foam. The mechanical and biological properties of scaffold nanocomposite were discussed and the optimum sample were selected for bone tissue engineering applications.

MATERIALS AND METHODS

Hydroxyapatite preparation

In this study, bovine bones were used for base matrix ceramic. The bovine bone/natural HA was boiled for 10-12 h to remove flesh and fat according to the previous study [2]. The bones were dried at 110°C for several hours to remove moisture. To prevent blackening with soot during heating, the bones are cut into small pieces of about 10 mm thick and heated at 500 °C (bone ash) for 2 h in air to allow for evaporation of organic substances. The resulting black bone ash was heated for 3 h at 850°C. This synthesis is called thermal decomposition of bone resource to create natural HA, NHA as described in our previous work [2-3].

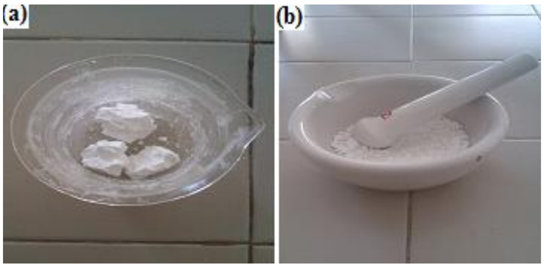

Fig. 1. Natural Hydroxyapatite powder using in the scaffolds nanocomposite a) agglomerated HA, and b) HA powder after high energy ball milling

Hardystonite preparation

Hardystonite powders were prepared by the sol–gel process using tetraethyl orthosilicate (TEOS), zinc nitrate hexahydrate and calcium nitrate tetrahydrate as raw materials. Briefly, the Hr fabricated according to the previous work [22, 29]. The TEOS was mixed with water and 2 M HNO_3 (mol ratio: TEOS/H_2O/HNO_3=1:8:0.16) and hydrolyzed for 30 min under stirring. Then, the $Zn(NO_3)_2 \cdot 6H_2O$ and $Ca(NO_3)_2 \cdot 4H_2O$ were added into the mixture (mol ratio: TEOS/$Zn(NO_3)_2 \cdot 6H_2O$/$Ca(NO_3)_2 \cdot 4H_2O$ = 2:1:2), and reactants were stirred for 5 h at room temperature. After the reaction, the solution was maintained at 60°C for 1 day and dried at 120°C for 2 days to obtain the dry gel. The dry gel was ground and sieved to 250-mesh, transferred into a corundum crucible and calcined at 1100 and 1200°C for 3 h, respectively.

Hydroxyapatite-hardystonite nanocomposite preparation

The prepared HA composite with Hr with (0 wt.%, 5 wt.%, 10 wt.%, and 15 wt.%). Then the produced nanocomposite powders was mechanically activated through high energy ball milling for 60 min to gain pure and homogenised nanocomposite distribution of additive in matrix. To evaluate sinterability, green bodies of hardystonite of about 10 mm×12 mm in size were prepared and fired from 1250°C to 1350°C. The sintered hardystonite ceramics were analyzed by XRD and SEM technique. Fig 1 indicate the agglomerated HA before ball milling and after high energy ball milling. The agglomerated HA particles indicated that after some hours purified and The BPR was 10 and the speed of vial was 600 rpm. The nanocomposite powder then pressed under 60 MPa pressure and the obtained samples were sintered through two step sintering (TSS) process, as shown in Fig. 2.

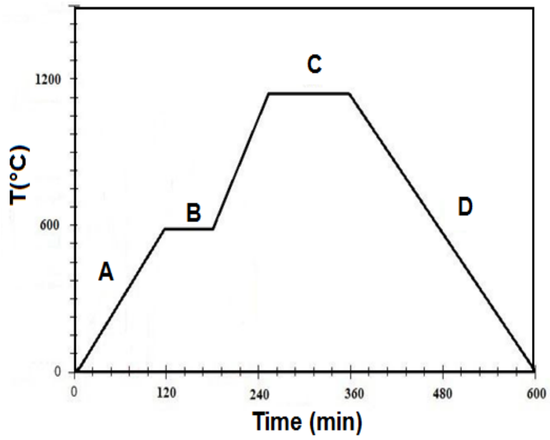

Fig. 2. The Two Step Sintering (TSS) process to develop the materials strength

Polyurethane polymeric sponge technique

To prepare HA-Hr scaffold nanocomposite the replica method using polyurethane foam was utilized. The scaffolds nanocomposite were combined with different concentration of Hr, 0, 5, 10, 15 wt. %. In addition, 1 wt. % sodium tripolyphosphate (STPP) and 1 wt. % Carboxymethyl cellulose were used as a dispersing and binder agent, respectively, during preparation of the ceramic slurry containing HA- Hr. Finally, the scaffolds were sintered at 1350°C for 2 h with the heating and cooling rate of 10°C/min.

Compressive strength test

Compressive strength was determined by crushing cylindrical specimens of dimensions 10 × 20 mm (diameter thickness) using a computer-controlled universal testing machine (Instron 8874, UK) with a ramp rate of 0.5 mm min^{-1}. The ASTM standard No. C0020–00R05 is used to evaluate the cold crushing strength of the produced samples. The samples were tested and replicated for three time in both biological and mechanical investigations.

Bioactivity test

In order to evaluate the apatite formation ability of the HA-Hr scaffold nanocomposites, simulated body fluid (SBF) was used which is described by Kokubo et al. [23]. Cylindrical scaffolds nanocomposite with dimension of 10×12 (diameter × height) were soaked in 12 cc SBF in the falcon tube for 28 days and kept in benmary bath with circulatory water at 37°C.

Chemical changes test

The pH of the solution was measured every two days by a pH meter. In addition, changes in element concentration of SBF were also measured by inductively coupled plasma (ICP) method (Equipment Model, and Zaies).

Materials characterization

X-Ray diffraction (XRD) analysis

X-ray diffraction (XRD) is carried out for structural identification of the pure NHA and NHA-Hr containing various amount of Hr in HA matrix. XRD patterns are recorded with a Bruker AXS Germany make X-ray diffractometer, having CuKα (= 1.5405 Å) incident radiation. The XRD peaks are recorded in 2 theta range of 20–60°. The phase characterization of the NHA and NHA/Hr 20 wt.% nanocomposite was done X-ray diffractometer (X' pert Philips) with CuKα radiation (λ = 0.154 nm) (IAUN, Isfahan, Iran). The XRD traces were recorded in the 2θ range of 10–90°.

Scan electron microscopy (SEM) analysis

The surface morphology and microstructure of the samples before and after incubation is visualized by means of Scanning Electron Microscopy (JSM/JEOL-6360). All the samples are coated with thin film of gold (Au) to reduce charging of the sample. Simultaneously the elemental compositions of the samples are analysed using Energy Dispersive Spectroscopy (EDX). Also, the EDX analysis was utilized for more precise comparison of the amount of apatite formation in the sintered samples soaked in the SBF for certain period of time.

Thermogravimetric analysis (TGA)

Thermogravimetric analysis or thermal gravimetric analysis (TGA) is a method of thermal

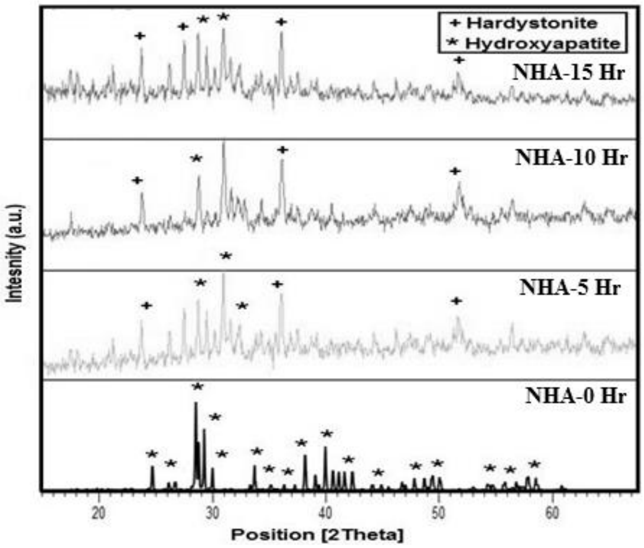

Fig. 3. XRD patterns of Hr powder using in the scaffolds

analysis in which changes in physical and chemical properties of materials are measured as a function of increasing temperature (with constant heating rate), or as a function of time (with constant temperature and/or constant mass loss).

RESULTS AND DISCUSSIONS

XRD Results

Fig 3 shows the XRD patterns of NHA powder. In the current work, the sample just heated at 850ºC to fabricate NHA powders. As depicted in Fig 3-a all the peaks belong to HA phase and there is not any extra phase as impurities. Fig 3(b-d) represented the XRD pattern of nanostructure HA/Hr nanocomposite powder containing 0, 5, 10, 15 wt. %. Hr in NHA matrix. As it is seen, there are both phase's peaks, HA phase and Hr, in the XRD pattern. Also, in the other word, Fig 3 depicts the XRD patterns related to the complete synthesis of Hr at 1150-1200°C for more than 3 hours. As can be seen in this pattern, there is no impurity in Hr product.The XRD pattern of HA compared with the nanocomposite HA/Hr. The comparison indicated that as the Hr reinforcement added to HA, the XRD peaks tend to increase and the intensity reduce. Also, it is worth mentioning that in the sample containing 15 wt.% Hr the Hr peaks appear between 52-55°. In the sample with 15 wt.% Hr the sharp peak belongs to Hr appears between 25-30°. The XRD pattern indicated that as the Hr increase the materials crystallinity decrease.

Fig. 3 shows the XRD pattern of the ceramics showing only the pattern of Hr. Fig. 3 shows the XRD patterns of the Hr powders prepared by this method. A strong Hr peak at about 31° 2θ was obvious in the XRD patterns of the powders calcined at 1100 and 1200°C, which indicated that the main resultant was Hr. The composite particles were prepared by blending HA and Hr particles in PCL solution. The ceramic particles were rarely visible on PCL fibers when observed under SEM. Recent advances in thermal spraying have revealed the ability to create oriented single crystals. The properties of these materials depend on the orientation of the crystals. Testing on such fine crystals is challenging; an easier approach would be to conduct testing on large single crystal [24-27].

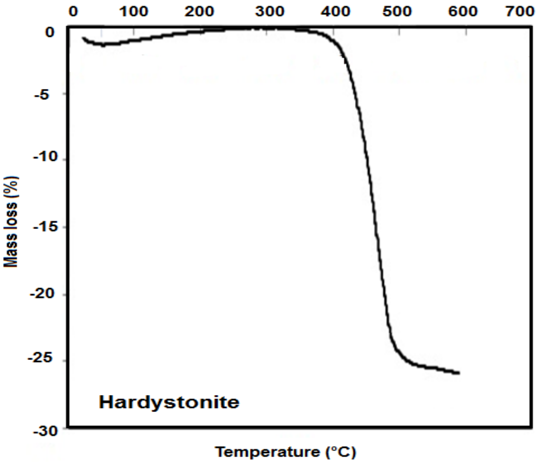

Fig. 4. TGA analysis of different Hr in air atmosphere

TGA Results

The bioceramic used in the present research convert, after heating in air, into highly reactive amorphous silica (SiO_2). Their thermal decomposition behaviour in air was investigated using TGA analysis (see Fig. 4). The data indicate that the ceramic conversion occurs in the 350–650 °C, and is associated with a weight loss that ranges from 10 to 45%, depending on the specific silicone precursor. Another factors for lower modulus of HA scaffolds are the bigger size and lower surface charge of HA particles that led to agglomeration and reduction of mechanical property. The exact reason behind this is uncertain as all these mechanical properties exhibited by scaffolds are complex interplay between native properties, % crystallinity, inclusions, defects, fibre diameter, fibre arrangement etc., some of which may be resolved by individual fibre study which is beyond the scope of this study. The TGA results indicated that Hr loss its weight after 400°C for more than 20% because of water loss in Hr structure.

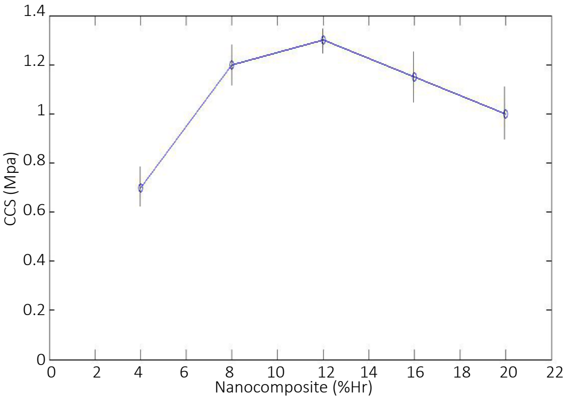

Fig. 5. Compressive strength of samples containing various amount of Hr in HA powder

Compressive strength of scaffold nanocomposite

As it can be seen in Fig 5, as hardystonite amount increased the compressive strength and mechanical properties of the scaffold nanocomposite increased in all sample in the range of 0.8 to 1.2 MPa.

As it is shown as Hr amount increased the compressive strength of the scaffolds samples increased. The maximum useful increase in compressive strength can be for the sample with 10 wt. % Hr.

SEM Results

Chemical changes of scaffold nanocomposite

The surface morphology of the sintered Hr ceramics is shown in Fig.6. It can be seen that most Hr particles were sintered and some micropores were evident (Fig. 6a), The SEM indicated that the sintered Hr ceramics were not completely dense. The high magnification SEM micrograph shows that

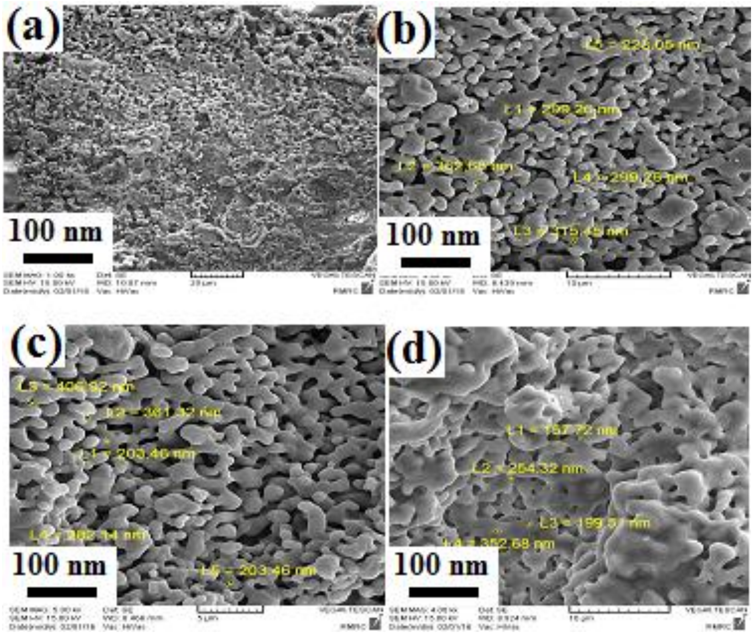

Fig. 6. SEM micrographs of HA/Hr scaffolds with different concentration of (a) 0 wt. % (b) 5 wt. % (c) 10 wt. % and (d) 15 wt.% Hr

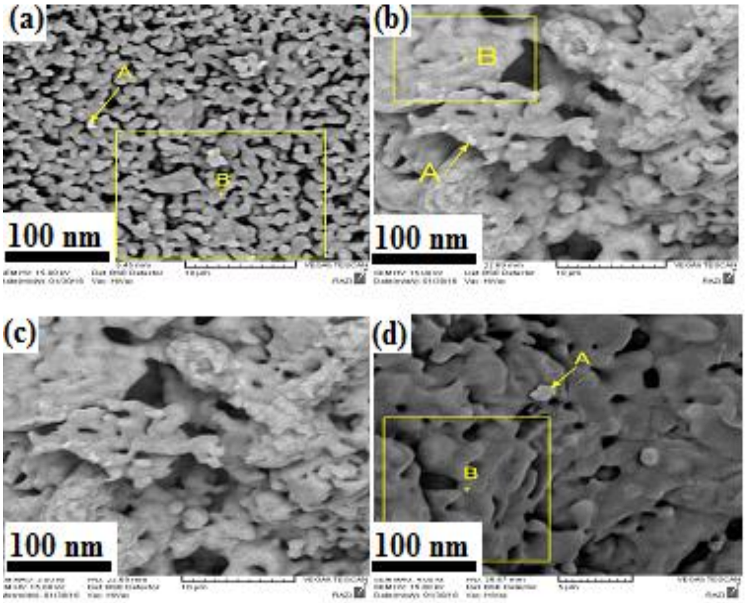

Fig. 7. SEM micrographs of HA/Hr scaffolds after soaking in SBF for 28 days with different concentration of (a) 0 wt. % (b) 5 wt. % (c) 10 wt. % and (d) 15 wt.% Hr

the grain size of Hr ceramics was about 1–5 µm (Fig. 6b). Hr is a mineral, and the naturally occurring Hr was often not pure and associated with other minerals. To our knowledge, there was no report about chemical synthesis of pure Hr powders. Here we report a study on chemical synthesis of pure Hr powders by sol–gel method. The sintering process of scaffold nanocomposite NHA-Hr containing 0, 5, 10, 15 wt. % has been shown in Fig 6 (a-d). The SEM illustrated that as the amount of Hr increased the scaffold surface tends to be more glassy and amorphous.As the result of bioactivity evaluations shows the pH for the sample with Hr has a dramatic increase which is due to high degradation rate of HA (CaPs). The increase in Ca^{2+} amount (alkaline ion phase) cause the increase in pH value. After two weeks the Calcium Ion saturated in the solution and start to precipitated on the samples surfaces. Fig 7 illustrates the SEM micrographs of the scaffolds after soaking for 28 days in SBF solution. In higher magnification, plenty of bright precipitations (cauliflower) were observed on the surface of the scaffolds. As it is seen, by increasing Hr amount in the HA/Hr nanocomposite samples, Fig (7-d) is the comparison with the HA sample that shows increasing density of bright precipitations. It means that bioactivity has been increased by increasing of concentration of Hr and the ability to form and precipitate bone-like apatite as the bright precipitations increased (Fig 7 (a-c)).

EDX point analysis has been done on the point A which shows in the SEM micrographs. It indicates that the bright precipitations are composition of Ca and P, Ca-P compounds. In fact, nucleation of the bright precipitations carries out on the Hr particles. Also, the result proved that Si^{+4} ions have very important role at the bright precipitations formation. Therefore, Si^{+4} ions leads to the formation of silanol (…Si–OH^-) group in the surface layer, a pH increase, and finally the production of a negatively charged surface with the functional group (Si–O). The prepared bio-layer on the surface of the samples leads to use the Calcium ion and a reduction in pH amount. As the HA amount in the nanocomposite increases the solution have more alkaline potential and increased pH. No difference in ALP activity was seen with the increase in concentration of either HA or HS. However, ALP activity of HS scaffolds significantly increased as compared to HA containing scaffolds, indicating the positive role of Zn and Si in bone metabolism. Our results also well support the role of these ions in bone metabolism; however does not give detailed insight into the mechanism of action of Zn and Si towards osteoblastic differentiation. To elucidate the mechanism of Zn and Si in osteoblastic differentiation, various studies are being carried out but are not yet fully characterized. The EDX analysis of samples with 10% and 15% Hr (points A, B in Fig. 8c, d) are shown in Fig. 8.

As can be seen, the surface that the apatite layer has not been formed on it (point B), has a higher Ca concentration than the surface of apatite layer precipitated (point A). This confirms what was said in this study about the growth of the apatite layer. On the other hand, the samples with higher amount of Hr, have a more Si than the samples with a lower amount of Hr (see EDX analysis of point A for both percentage 10 wt.% and15 wt.% of Hr). This is also confirms that with increasing the percentage of Hr up to 15, the Si-OH nucleation sites also increases lead to ease of formation of apatite layers. To study the degradation profile, scaffolds were immersed in PBS solution for two months at 37°C and the percentage mass loss of the scaffolds were measured at day 7, 28, 42 and 60. Fig. 8a clearly indicates that up to 28 days all scaffolds showed very less % mass loss. This happened due to presence of HA. Hr containing scaffolds led to faster degradation compared to HA containing scaffolds due to presence of Si whose superior bioactivity is well documented in literature.

On the other hand, Si ions in the Hr can form silanol groups on the surface of the scaffold (see Fig. 8). The formation of silanol groups induces a negative charge on the surface. Subsequently, the existing Ca^{2+} ions in the SBF can be adsorbed on the surface and consequently result in the deposition of calcium ions and the formation of an appetite layer on the surface of the scaffolds.

Chemical changes evaluation

Fig 9 shows the results of the ICP, elemental chemical results, of SBF containing the scaffolds. Decreasing of the percentage of Si ion in solutions containing more Hr powder samples indicates the presence of higher amount of Si element and it works as actively for formation Ca/P compounds precipitations. In fact, this element, Si ions, role very important at formation Ca/P compounds precipitations on the surface scaffolds. As an interesting biomaterial with potential orthopaedic and dental applications HA as good mechanical properties, osteoconductivity, bioactivity, and bone bonding ability. HA is not biodegradable and can be removed and remodelled in the host. If implanted directly, it may dislocate within the tissue. In fact, the nucleation was grown on Hr particles.

When the amount of Hr reaches to 15 wt%, the density starts to decrease. This is attributed to the overlapping of glass bonds and their subsequent failure. In all percentages of Hr (0, 5, 10, 15 and 20 wt.%), the pH value of the samples has increased during the first week of experiments. Higher concentration of Ca ions in the natural hydroxyapatite in comparison with non-natural one led to the movement of the calcium ions into the solution and the increase of pH value. Fig 10 indicated the ICP analysis of the NHA-Hr scaffold nanocomposite calcium ions concentration in the SBF solution for the sample containing 20 wt.% Hr has been recorded about 142 ppm. The reason for this reduction can be absorption of the calcium ions to the scaffolds surface which

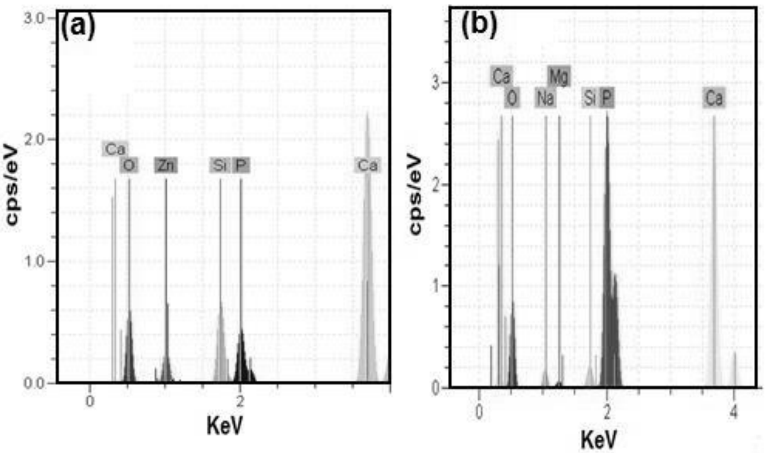

Fig. 8. EDX micrographs of HA-Hr scaffolds after soaking in SBF for 28 days with different concentration of (a) 0 wt. % and (b) 10 wt. % Hr

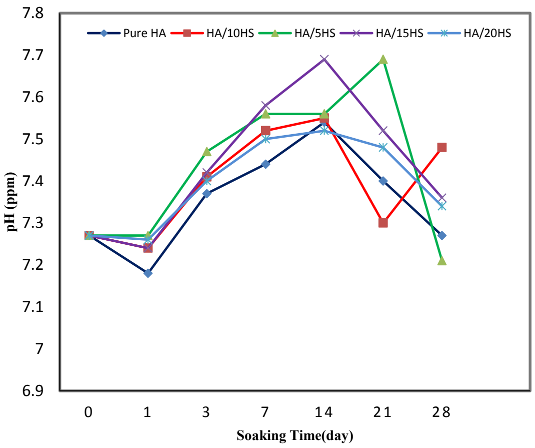

Fig.9. Change of pH value in SBF solutions of the samples in the during 28 days

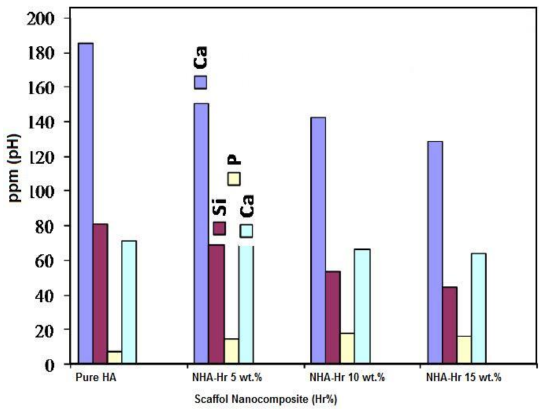

Fig.10. Ions changes in SBF solutions of the samples in the during 28 days

leads to apatite formation. In sample containing 10 wt.% Hr the calcium ions was about 142 ppm. Also, the results showed that decrease in Zn ion regarding to the increasing of Hr can be define as precipitation of Zn ion on the scaffold surface for boneOlike apatite preparation. Zn ion have an important role as a germinator on the apatite surface. In addition Fig 10 showed that decreasing in Si ions in the sample with 20 wt.% Hr can be because of high degradation rate of Si ions.

CONCLUSION

Pure Hr ($Ca_2ZnSi_2O_7$) powders were synthesized by the sol–gel method and Hr ceramics were prepared by sintering powder compacts. Best results were obtained at 1350°C for 5 h, where the compressive strength 1.21 MPa. The nanocomposite samples with 10wt. %HT have the maximum value of the density, which is attributed to the formation of Hr silicate phases between the matrix particles and subsequently the formation of glass bonds. A maximum value of 1.21 MPa was obtained for the compressive strength of HA-10 wt% HT nanocomposite sample.

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CONFLICT OF INTEREST

The authors confirm that this article content has not any conflicts of interest.

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