Introducing the fluorine doped natural hydroxyapatite-titania nanobiocomposite ceramic

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ABSTRACT

In the present research, natural hydroxyapatite (NHA) was synthesized from bovine bones and then fluorine was doped into the NHA matrix to produce fluorine doped NHA (FNHA; natural fluor-hydroxyapatite) in optimum conditions. At the end an FNHA-TiO2 nanobiocomposite ceramic with excellent biocompatibility and good chemical stability was synthesized through a mechanochemical route and a subsequent two step sintering (TSS) process. Thermal gravimetric analysis (TGA), Differential scanning calorimetry (DSC), X-ray fluorescence (XRF), X-ray diffraction (XRD), scanning electron microscopy (SEM), inductive coupled plasma (ICP), and energy-dispersive X-ray spectroscopy (EDX) were used as the means for gathering and analysis of the results. According to the obtained results, TiO2 can prevent early decomposition of FNHA by the formation of the CaTiO3 phases and hence strengthen the interactions between the apatite particles which results in the increase of the mechanical properties. Besides, TiO2 provides more Si–OH nucleation sites for the formation of the apatite layers and hence more bioactivity.

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1. Introduction

Hydroxyapatite (HA) is a naturally occurring mineral form of calcium apatite with the formula Ca5 (PO4)3(OH). It is usually written as Ca10 (PO4)6(OH)2 with the Ca/P ratio being 1.67. HA is the hydroxyl end member of the complex apatite group [1]. Among different calcium phosphate ceramics, HA has been extensively used as a substitute material for bone or damaged teeth because of its crystallographic similarity to various calcified tissues of vertebrates [2]. However, HA is faced with an intrinsically high dissolution rate in a biological environment and poor corrosion resistance in acid solutions [2]. Moreover, HA has a poor thermal stability which results in its decomposition into other phases and hence undesirable fast dissolution rate in vivo [3].

Fluorine doped hydroxyapatite (FA; Ca10 (PO4)6F2) has been revealed to be a viable alternative to bone because of its good biocompatibility, low solubility, and also high thermal and chemical stability [4]. Pure fluorapatite (FA; Ca10 (PO4)6F2) is known to have a much lower solubility in biological fluids than HA, because FA has a greater stability compared to HA, both chemically and structurally. According to a study conducted by Eslami et al. [5], with increasing the incorporation of F into the HA structure thermal stability is considerably increased. By varying the amount of Fluoride substitutions the solubility as well as biological lifetime can be fine-tuned. The results of cell culture demonstrated that the F content affects the cultured cells behaviours in different ways: a high F content leads to a low surface potential, which favours cell attachment. However, a decrease of the Ca2P release could inhibit cell proliferation. Fluorine can also act as a superior substitute to fluoride ions released from FHA in order to optimize the bioactivity of the implants to control the amount of fluoride ions released from FHA [4]. Besides, the mechanical properties of fluorapatite and all other calcium phosphates are generally inadequate for many load-carrying applications [7]. These bioceramics also have a low density and hence poor mechanical properties [7]. Research works
carried out in this relation show that the combination of calcium phosphates and other compounds can improve the poor mechanical properties of calcium phosphates [7].

Some attempts have been made to develop the HA-based composites such as the HA-Al2O3 [8], HA-ZrO2 [9], and HA-TiO2 [10] composites. These studies point to the occurrence of two major phenomena including the dissociation of HA to tricalcium phosphate (TCP) and interfacial reactions between HA and reinforcement ceramic phase which can lead to the formation of CaZrO3 and CaTiO3 in HA-ZrO2 and HA-TiO2 systems, respectively. However, only a few studies have concentrated on the use of metallic oxides such as TiO2 for the preparation of the FA-based biocomposites [11]. Therefore, the main objective of the present research is the fabrication of FNHA-TiO2 biocomposite which can present the advantages of both TiO2 and FNHA.

HA–TiO2 nanobiocomposite is one of the most important achievements of the mentioned researches. It is produced by different methods such as chemical co-precipitation [12], in situ precipitation [13], photo-induced formation [14], sol-gel dip coating [15], and gas tunnel type plasma spraying [16]. In addition to the above-mentioned methods, the high energy ball milling process is a simple dry method to obtain any quantity of powder with controlled microstructure [17]. Usually, the powder obtained by the mechanochemical route has a suitable structure because of the disorderliness of surface-bonded species resulted by pressure [18]. The benefits of ease, reprocessing capability, and low processing cost are the most significant advantages of this method [19]. Moreover, it is not necessary to precisely control the melting conditions and the obtained powders have nanostructural characteristics [20].

Despite the poor mechanical properties of hydroxyapatite and its compounds, their unique biological properties lead us to think about working on improving their properties instead of completely replacing them by other materials. In the present study, at first the HA powders were produced in natural form through a very simple method. To obtain better chemical stability, a controlled amount of fluorine was injected into the hydroxyapatite matrix. Finally, an FNHA-TiO2 bioceramic composite with an optimum amount of TiO2 was fabricated and its chemical stability and biological property were assessed. The obtained results promised a nanobiocomposite with good chemical stability and biological properties.

2. Materials and methods

2.1. Preparation of the powder samples

As a brief reiteration of the methods in our previous work [21].

Table 1
XRF analysis of the hydroxyapatite powders.

<table>
<thead>
<tr>
<th>Element</th>
<th>Ca</th>
<th>P</th>
<th>Na</th>
<th>F</th>
<th>Mg</th>
<th>Sr</th>
<th>Cl</th>
<th>Si</th>
<th>S</th>
<th>Al</th>
<th>Cu</th>
<th>Zn</th>
<th>Fe</th>
<th>K</th>
<th>Zr</th>
<th>Ca/P Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>wt%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>70.2</td>
<td>21.09</td>
<td>1.14</td>
<td>1.09</td>
<td>0.64</td>
<td>0.40</td>
<td>0.18</td>
<td>0.12</td>
<td>0.06</td>
<td>0.05</td>
<td>0.05</td>
<td>0.04</td>
<td>0.04</td>
<td>0.02</td>
<td>0.007</td>
<td>3.33</td>
<td>100.04</td>
</tr>
</tbody>
</table>

Table 2
Ion concentrations (mmol/dm3) of SBF and Human Blood Plasma.

<table>
<thead>
<tr>
<th>Ionic concentration (mmol/dm3)</th>
<th>Simulated body fluid</th>
<th>Blood plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺</td>
<td>142.0</td>
<td>142.0</td>
</tr>
<tr>
<td>K⁺</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>147.8</td>
<td>103.0</td>
</tr>
<tr>
<td>HCO₃⁻</td>
<td>4.2</td>
<td>27.0</td>
</tr>
<tr>
<td>HPO₄²⁻</td>
<td>1</td>
<td>1.0</td>
</tr>
<tr>
<td>SO₄²⁻</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>pH</td>
<td>7.4</td>
<td>7.2–7.4</td>
</tr>
</tbody>
</table>

Bovine bones were boiled for 12 h to remove their fat. In order to remove moisture, the wet bones were heated to 110°C and kept at this temperature for 2 h. To prevent the blackening with soot during heating, the bones were cut into approximately 10-mm thick pieces and heated to 550°C for 2 h in air to allow evaporation of organic substances. The blackened bone pieces were heat-treated for 3 h at 850°C. The powdered mixture was then loaded into a hardened steel bowl along with a ceramic ball with 10 mm diameter and 110 gr mass. The ball-to-powder weight ratio (BPR) was 15, and the rotational speed was 600 rpm. The sample was subsequently heated in a furnace to 850°C for 8 and 10 h. The time of milling by using the planetary ball mill was set on 10 h. The XRF analysis which confirms a successful synthesis of HA is presented in Table 1. It is worth mentioning that the amount of Ca/P in the produced HA (natural hydroxyapatite) is more than that in the non-natural one (Ca/P(HA) = 1.67 and Ca/P(NHA) = 3.33).

2.2. Synthesis of FNHA

The natural hydroxyapatite (NHA) powders obtained from the abovementioned process were again ball milled for 0.5 h to homogenize the product. The FNHA particles were produced by blending NHA and CaF₂ by a high energy planetary mill. The BPR of 15 and rotational speed of 600 rpm were used for the milling process in which the overall mass was 135 g and milling durations were 4, 6, and 8 h.

2.3. The composite formation

An FNHA-TiO₂ nanobiocomposite powder with different percentages of TiO₂ (5 wt%, 10 wt%, and 15 wt%) was mechanically activated through high energy ball milling. The BPR was 10 and the speed of vial was 600 rpm. The powders were weighed in amounts of 0.3 g and pressed in a 6 mm diameter steel disc mould for 2 min under a force of 9000 N. Thereafter, the mixed powders were cold pressed under 600 MPa, and the prepared compact samples were sintered through a two-step sintering (TSS) process (T1 = 1150°C, T2 = 950°C). The sintered specimens were cooled slowly in a furnace at the cooling rate of 10°C/min (The TSS diagram was sent to the Journal office as a supplementary file).

2.4. The characterization procedure

Phase and structural analyses were carried out by using XRD (Philips, X’Pert MPD diffracto-meter) using CuKα radiation (λ = 0.15418 nm) over a 20 range of 20–70°. The achieved experimental patterns were compared with the standard patterns compiled by the Joint Committee on Powder Diffraction and Standards (JCDPS), represented by card no. 09-432 for NHA. Scanning electron microscopy (SEM) (Philips XL30, Eindhoven, The Netherlands) was used to observe the morphology of the surface. The Ca/P ratio was determined by using energy-dispersive X-ray (EDX) spectroscopic microanalysis. The samples were coated with Au for 2 min by spraying in high vacuum at the accelerating voltage of 35 kV to be suitable for SEM investigations. The concentrations of Ca, Si, ... ions in Simulated Body Fluid (SBF) after soaking were tested using inductive coupled plasma atomic emission spectroscopy (ICP-AES; Zais 110394c). The elemental analysis of hydroxyapatite powder (raw material) was performed by X-ray
fluorescence spectrometry (XRF, Bruker-S4 Pioneer, Germany). The DSC experiments were performed on a differential scanning calorimeter (DSC 60, Shimadzu Co., Japan). The samples were accurately weighed in aluminium pans and heated at 10 °C/min under a nitrogen flow. Thermal gravimetric analysis (TGA) of the as-prepared powders was carried out on a Mettler-Toledo TGA 851e.

2.5. In vitro bioactivity evaluation

The SBF (Simulated Body Fluid) test is a technique that is used to evaluate the in vitro bioactivity of materials [22]. In this method, the selected materials are immersed in an aqueous SBF solution in which the characteristics of human blood plasma have been simulated. After immersion for a certain period, the bioactivity is evaluated by the amount of the HA layer formed on the surface of the samples [23]. The SBF solution is produced in laboratory with the ionic concentration nearly similar to human blood plasma, according to the procedure proposed by Kokubo (known as Kokubo method) [24]. The appropriate quantities of reagents comprised of NaCl, NaHCO3, KCl, K2HPO4·3H2O, MgCl2·6H2O, CaCl2, Na2SO4, and tris buffer are dissolved in 1 l of double distilled water so as to have ionic concentration of various inorganic ions similar to those of the human blood plasma [24]. Table 2 provides information on ions concentration of SBF obtained by Kokubo method and its comparison with human blood plasma (see Fig. 1).

3. Results and discussion

The DSC/TGA data for the as-prepared NHA powder are shown in Fig. 2 in which the first endothermic peaks at 100 °C and 250 °C are related to the absorbed water. As can be seen, two exothermic peaks were obtained between 400 °C and 600 °C without any significant weight loss. So, it can be claimed that they are corresponding to some kind of phase transformation (α-TCP (Ca3(PO4)2) to α’ and β-TCP). This is verified by Fig. 3 in which the X-ray patterns of the as-synthesized NHA powder and that of calcined at 560 °C are presented. It is observable from this figure that both patterns contain HA peaks; however, with increasing the temperature to 560 °C, distinctions become visible in the appearance of the new β-TCP phases. Another exothermic peak, which can be seen at 750 °C, probably represents DCP (Ca2P2O7). A weight loss close to 21% is observed in the TGA graph. This leads to the conclusion that NHA does not have a suitable stability in high temperatures and chemical solutions.

As it was mentioned before, NHA is able to form FNHA with the crystallographic substitution of OH⁻ by F⁻ which has a much lower solubility than NHA, making it an alternative potential candidate for bone repair. Fig. 4 shows the TGA graph for NHA at different percentages of fluorine. Several important points can be inferred from this figure. First, the weight loss of NHA has been markedly reduced by adding fluorine which means an increase in chemical stability. Second, in low percentage of fluorine, there will be a thermal instability at 850 °C like pure NHA which means that there is an optimum content of the fluorine doped in NHA. As can be seen in this figure, by increasing the fluorine content, the OH groups are completely substituted by fluorine during which FA with good chemical and thermal stability is formed. These observations suggest that when the fluorine content in the NHA matrix is high enough, the thermal stability of FNHA improves and in the corresponding expression the decomposition of FNHA would be effectively postponed.

Fig. 5 indicates the XRD patterns of the NHA doped with CaF2 at various milling times. The broadening of the peaks can be seen with increasing the milling time which results in crystallite size refinement [25]. It is also worth considering that FNHA is completely formed at 8 h, which implies that the doping process has been completed at or before this milling time. To further explore the
Fig. 4. TGA graph for NHA at different percentage of fluorine.

Fig. 5. XRD patterns of the NHA doped with CaF$_2$ at various milling times.

Fig. 6. The FHNA- TiO$_2$ nanobiocomposite samples after TSS process; a) 5%wt TiO$_2$, b) 10%wt TiO$_2$, and c) 15%wt TiO$_2$.
Fig. 7. X-ray diffraction pattern of sintered FNHA/TiO₂ nanobiocomposite.

Fig. 8. TEM images of the sintered samples; a) 10%wt TiO₂ and b) 15%wt TiO₂.

Fig. 9. The changes in a) calcium ions concentration and b) pH values versus immersion time in the SBF solution.
phenomenon of being doped, the magnified patterns of the mentioned samples are also given in Fig. 5. It can be inferred from this figure that in addition to widening, the FNHA characteristic peaks (reflections) gradually shifted to the right hand side. This stems from the reduction of the $a$-axis length of the hexagonal HA crystals lattice due to lower ionic radii of $F^{-}$ (0.065 nm) compared to $OH^{-}$ (1.32 nm) which introduces distortion into the lattice with incorporation of fluorine ions instead of hydroxyl groups in the apatite structure. This observation is in good agreement with the fact that fluorine addition tends to decrease the lattice parameter $a$, but does not obviously affect the lattice parameter $c$.

Also, FHA has the release of fluoride at a controlled rate which can lead to the provision of a strong bone with good mechanical and functional properties [26]. Accordingly, different percentages of TiO$_2$ (5, 10, and 15) was added to FNHA and the produced composite was sintered by the TSS process. The FNHA-TiO$_2$ nanobiocomposite samples after the TSS process are shown in Fig. 6. As can be seen in Fig. 6a, b and c, with increasing the TiO$_2$ content reinforced in the FNHA matrix, the compaction increases. Before explaining the observed event, it is necessary to note that for pure FNHA the interconnection of the apatite particles during the TSS process yields high density of the sintered specimen. However, it was reported that [27] with excess temperature, the decrease in densification is observed for pure FNHA. The reduction of the FNHA density can be attributed to the decomposition that results in the formation of calcium phosphates as mentioned earlier. The above interpretation will vary with the addition of TiO$_2$ to FNHA. In fact, TiO$_2$ can prevent early decomposition of FNHA and consequently strengthen the interactions between the apatite particles, which leads to the increase of compaction as shown in Fig. 6. X-ray diffraction pattern of the sintered FNHA-TiO$_2$ nanobiocomposite is illustrated in Fig. 7. The peaks relevant to FNHA and TiO$_2$ as major phases can be seen. One can also observe the TCP, CaF$_2$ and CaTiO$_3$ phases. It is notable that when FNHA completely decomposes to calcium phosphate phases, the density of composite begins to decrease. The formation of the CaTiO$_3$ phases indicates that the calcium phosphate phases have not been able to completely form.

TEM photographs of the sintered samples at various content of TiO$_2$ shown in Fig. 8a and b. As can be seen, there is almost no change in the size of the grains by increasing the TiO$_2$ content. From the previous interpretations (explanations about Figs. 6 and 7) and also TEM images, it can be concluded that TiO$_2$ can only prevent early decomposition of FNHA but not change the FNHA grain size. This means that the compaction observed in Fig. 6 is not because of the decrease of the mean grain size but is because of the undecomposed FNHA resulted by the increase of TiO$_2$. The formation of the CaTiO$_3$ phases (see Fig. 7) indicates that TiO$_2$ can successfully prevent decomposition of FNHA to calcium phosphate phases.

The bioactivity of ceramics has been defined as “the bond ability with host bone tissue” [28]. The in vitro bioactivity is assessed by
Fig. 11. EDX analysis of samples with 10% and 15% TiO₂ (points A, B in Fig. 10c, d): a) FNHA-10%TiO₂- point A; b) FNHA-10%TiO₂- point B; c) FNHA-15%TiO₂- point A.
examination of the growth of bone like apatite on the surface of the samples after soaking in Kokubo's SBF solution. The dissolution curves can be very helpful as they indicate the changes in calcium ions concentration and the pH values versus the immersion time in SBF (Fig. 9). It is understood from this figure that the pH value as well as the Ca ion concentration increased during the first two weeks of experiments (Fig. 9a, b). Here, it can be claimed that the Ca ions concentration in fluorine natural hydroxyapatite (FNHA) has a higher amount than that in the non-natural one (see XRF analysis for NHA in Table 1), leading to the instability of the FNHA-TiO2 nanobiocomposite and hence the entry of the calcium ions into the SBF solution. The event leads to the pH increase in the first two weeks of experiments.

As can be observed in Fig. 9a, the Ca ions concentration in pure FNHA is higher than that in the FNHA-TiO2 nanobiocomposite samples. This event may be originated from the more density as well as chemical stability of the FNHA-TiO2 nanobiocomposite and hence less reacting with the SBF solution in the first two weeks. In other words, the release of the Ca ions from pure FNHA is higher than that from the FNHA-TiO2 nanobiocomposite because of its lower chemical stability (see Fig. 6).

The release of the calcium ions from the surface into the SBF solution leads to the formation of many silanol (Si–OH) groups on the surface. The silanol groups are heterogeneous nucleation sites for the apatite layers. As can be seen from Fig. 9a, the Ca ions concentration begins to decrease for pure FNHA and tends to be fixed in the FNHA-TiO2 nanobiocomposite samples after two weeks. This phenomenon could be explained by the notion that the SBF solution eventually becomes saturated from the Ca ions (after 2 weeks), so that the calcium ions tend to leave the solution. In other words, a balance between the Ca ions release and Ca ions absorption is achieved. It should be noted that there are two places for the absorption of calcium ions. The first place is the surface of the apatite nuclei and the second place is the surface on which the apatite has not been formed. Obviously, the Ca ions concentration in the surface of apatite nuclei is less than that in other surfaces and that is why the Ca ions (in the form of CaP ions) in the solution have a tendency to migrate to the apatite nuclei. This is a mechanism for the growth of the apatite layer in the SBF solution. It is necessary to note that the Si–OH nucleation sites also play a key role in the reduction of Ca ions. In other word, as these nucleation sites increase the absorption of calcium ions into these sites increases as well, so that the concentration of the Ca ions in the SBF solution and consequently the pH value are reduced (Fig. 9a, b). It is clear from Fig. 9b that the optimum amount of pH (close to the pH of human blood plasma) has been achieved after 21 days for the FNHA-TiO2 nanobiocomposite samples. The above results are confirmed by SEM images and the EDX analysis.

The SEM images of the FNHA–TiO2 nanobiocomposite samples after immersion in the SBF solution for 21 days are shown in Fig. 10. As can be seen, the pure FNHA has a lower ability in apatite formation compared to other samples composed of FNHA-TiO2 (Fig. 10a). Fig. 10b shows that with increasing the TiO2 content to 5 wt%, the apatite precipitations (light porous areas) have increased. According to Fig. 10c and d, the apatite formation on the surface of the samples continues with the increase of the TiO2 percentage in the FNHA-TiO2 nanobiocomposite, which is attributed to more availability of nucleation sites as mentioned in the previous section.

The EDX analysis of the samples with 10% of TiO2 (points A and B in Fig. 10c) and 15% of TiO2 (point A in Fig. 10d) is shown in Fig. 11. As can be seen in this figure, the surface on which there is no the apatite layer (point B in Fig. 10c) has a higher Ca ions concentration than the surface on which the apatite layer is precipitated (point A in Fig. 10c). This confirms what was stated in this study regarding the growth of the apatite layer. On the other hand, the samples with higher amount of TiO2 (point A in Fig. 10d) have more Si than the samples with a lower amount of TiO2 (see the EDX analysis of point B and point A in Fig. 10c and d, respectively). This also confirms that with increasing the percentage of TiO2 up to 15, the Si–OH nucleation sites also increase which leads to the ease of formation of the apatite layers and hence more bioactivity.

4. Conclusions

In the present study, an FNHA-TiO2 nanobiocomposite ceramic with excellent biocompatibility and good chemical stability was synthesized. The FNHA is completely formed at 8 h of milling, which implies that the doping process has been completed at or before this milling time. The SEM images of the sintered samples showed that with increasing the TiO2 content reinforced in the FNHA matrix, the compaction increases, although the grain size remains constant. TiO2 can prevent early decomposition of FNHA by the formation of the CaTiO3 phase and hence strengthen the interactions between the apatite particles which results in the increase of compaction. The pH values as well as the Ca ions concentration in the SBF solution have increased during the first two weeks of experiments due to more Ca concentration in natural-fluorhydroxyapatite than that in the non-natural one, leading to the instability of the FNHA-TiO2 nanobiocomposite and hence the entry of the calcium ions into the SBF solution. The more Ca ions concentration in the SBF solution for pure FNHA compared to FNHA–TiO2 nanobiocomposite samples is related to the more density as well as chemical stability of the FNHA–TiO2 nanobiocomposite and hence less reacting with the SBF solution in the first two weeks of experiments. As the Ca ions concentration in the surface of apatite nuclei is less than that in other surfaces, the Ca ions in the SBF solution migrate to the apatite nuclei. This is a mechanism for the growth of the apatite layer in the SBF solution. As the percentage of TiO2 increases, the Si–OH nucleation sites also increase which leads to the ease of formation of the apatite layers and hence more bioactivity.

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