Effect of Adding Titania and Alumina on the Bioactivity Properties of Porous Hydroxyapatite via Replication Method for Bone Reconstruction

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Abstract. In this investigation, Hydroxyapatite/Titania and hydroxyapatite/Alumina porous composites at five various proportions were manufactured by replication method A.K.A the polymeric sponge method. HAp powder was fabricated by a "solid-state reaction" in molar proportion between ( TCP)Tri calcium phosphate and Ca(OH) 2 Calcium hydroxide. The microstructures were examined by utilizing (XRD), (SEM) and EDX". Incubated for thirty days in SBF (Simulation Body Fluid), a thin layer like apatite formed completely on a bone specially on the surface of the samples was. There's a very low degeneracy degree when amount of the reinforcements materials increased.

Introduction

Generally Hydroxyapatite HAp, "Ca10 (PO4)6(OH) 2 is an inorganic minerals” that found in human bone. It has a ton of utilization in orthopedic and dental health due to its incredible bioactivity [1]. Biomaterials usually consist of two materials in a molar proportion they are Calcium (Ca) and Phosphorus (P)[2, 3]. However, available commercial hydroxyapatite show an extremely low porosity and it can't be used "viably for adsorption for biomedical applications", yet for the most part of its use in drug delivery, protein and etc from biomedical application [4]. On the last decay, replication strategy has been shown to be a positive and useful way because of its potential in assembling HAp/TiO 2 composites with high porosity, "brilliant interconnections between the macropores, and pore shape similarity” with cancellous bone [5]. It has been known that HAp/TiO 2 can be an awesome blend of mechanical properties for bioactivity application [6]. Due to its "high specific strength and prevalent corrosion resistance” [7]. Titania are the best oxides for biomaterials, dental and orthopedics health applications. TiO2 additionally shows a magnificent penetrability and high biocompatibility for boost the cell suitability [8]. Executing of the HAp/Titania material in a living bodies depend on these elements, strength of the HAp structure, which is affected by the manufacturing method of the embed materials, sintering measure which are the main manufacturing steps that influenced for the most their final properties in application [9]. Hydroxyapatite is the base material of apatite, which is the fundamental component in "bone and teeth” [10].

Alumina is a solid ceramic material that utilized additionally as a "reinforcing material” in biocomposites. Polycrystalline alpha alumina properties is: strength, excellent resistance to impact, Good flexural strength, resistance to micro crack and very good compressive properties which are
gained with average grain sizes almost up to of < 4 microns and purity almost up to >99.7%". Mechanical properties of alumina used in Clinical utilizations of Al₂O₃ specially in knee prostheses, bone and dental screws, alveolar edge and maxilofacial remaking, bone substitutes, corneal substitutions and segmental bone substitutions". [11]

Hydroxyapatite can be made by procedures like: "wet substance, precipitation and it tends to be separated from bovine bones (bio-squander) or by solid- state procedure. Calcination of HAp powder is normally done at temperatures between (1000-1250 °C). At elevated temperature, HAp dehydroxylation will occur and it decomposed to (TCP) which has higher number disintegration rate [12]. Various Fabrication techniques are used for cancellous ceramics structure [13, 14], these methods are: - "replication method, sacrificial method and reactions method". The polymeric sponge procedure is one of the interesting and easy to work with method to fabricated the macro and micro porous ceramic represents by these two components: hydroxyapatite-Titania. [15].

The objective of this work is to enhance and develop new technique to make HAp biocomposites from these raw materials (tri calcium phosphate and calcium hydroxide) by solid- state reaction and reinforcing it with these two materials (Titania and Alumina particles) to make a bioceramic composite and study their impact on bioactivity properties of samples for bone recovery.

Experimental Procedure

Preparation of the Samples

In this study a new method for fabrication of highly porous HAp/ TiO₂ and HAp/Al₂O₃ very biocompatibility ceramic scaffolds was synthesis. First hydroxyapatite powders was produced by (solid state synthesis). The initial materials that used were commercially available and they are: calcium hydroxide Ca(OH)₂ (Himedia, India) and tri calcium phosphate (TCP) (Himedia, India). The powders were mixed in molar ratio (3:2) and were stirrer and diffused in water which is de-ionized water at (solid loading 55%). Then added 5, 10,15,20 and 25 wt% of both TiO₂ and Al₂O₃ from the mixer were filled with mixer and 4% PVA (poly vinyl alcohol) was added as a binder and were magnetic stirred for almost 2 hrs until the PVA completely melt and the pH of the slurry is 12 then all set for the dipping process .

Replication sponge method that have used in this article were each cut into a dimension of 1 cm³. The sponges were manually squeezed and put in a (bell jar) and then evacuated up to 10⁻¹ Torr to eliminate the trapped air and make certain that the sponge is totally filled with the slurry. Any surplus slurry was extracted by firmly press the specimens through a “roller”. The filled specimens were dried in domestic microwave almost for (10 to 25) min in accord with viscosity of solid loading. The specimens were fired at “(1250 °C) with rate of heating (5 °C/min) and 3 hrs for soaking in the same degree. The Crystallographic & morphological studies were done by utilizing optical microscopes, XRD, Scanning electron microscope and EDX will be discussed.

Immersion in SBF Solutions

Selected the best ratio for immersion, Sample of (25 TiO₂+ 75 HAp wt. % and 25 Al₂O₃ + 75 HAp wt. % scaffolds) 1 cm³ was placed in plastic jar with 20 ml of (5xSBF). The jar was covered with an a sealed and the constant temperature at 37°C. After thirty days in contact with simulation body fluids in order to perform various properties wash them with distilled water and drying it with domestic microwave to make it ready for SEM and XRD analysis. Table (1) shows SBF composition.
Table 1. shows SBF composition. [16]

| ITEM      | SBF×5 (g/l) |
|-----------|-------------|
| NaCl      | 40.18       |
| NaHCO₃    | 1.76        |
| KCl       | 1.125       |
| K₂HPO₄    | 1.15        |
| MgCl₂.6H₂O| 1.555       |
| CaCl₂     | 1.465       |
| NaSO₄     | 0.36        |

Results and Discussion

Morphological Characterization

The figures of an optical and SEM pictures of HAp/TiO₂ samples sintered at 1250 °C are respectively showed in Figs. 1 & 2. A high densification area is suggested after sintering at 1250 °C which showed that the firing process almost done and then grain growth happen at temperature 1250°C with no dissolved of hydroxyapatite to TCP Furthermore TiO₂ particles were well merged with the hydroxyapatite matrix and causes formation of glassy phase. We can point out that the evolution of microstructure and crystalline phases observed at sintering temperature of 1250°C and for hydroxyapatite glass ceramic doped with 25 wt% TiO₂ particles.

While in HAp/Al₂O₃ the optimum sintering temperature for composite was detected, according to the data of the experiment, to be 1250 °C. The unification of the packed closely to the particles showed to be finished according to the firing as shown in SEM image in Fig. 3. In the situation of the HAp/Al₂O₃ composite specimens, the creation of TCP phase was seen. It was reported that α- and β-tri calcium phosphates are very biodegradable apatite forms compared with the HAp structure, which is very connected with the exact temperature of the firing. The recently created composite structure of the samples was identified.

Figure 1. Optical micrograph of polymeric foam.
Figure 2. SEM image of HAp/TiO$_2$ porous ceramic fired at 1250 °C.

Figure 3. SEM image of HAp/Al$_2$O$_3$ porous ceramic fired at 1250 °C.

Analysis of XRD Pattern for HAp/TiO$_2$

Fig. 4 show the XRD analysis, the “phase components” of the 25wt.% TiO$_2$ composites appeared the existence of “HAp, α-TCP, β-TCP, rutile (titanium oxides) and perovskite (CaTiO$_3$)” as determined by XRD at sintering temperature 1250 °C. The next equation has been suggested to calculate the degeneration of HAp when it is sintered at high elevated temperature [17]:

$$\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 \rightarrow 3\text{Ca}_3(\text{PO}_4)_2 + \text{CaO} + \text{H}_2\text{O} \uparrow$$  \hspace{1cm} (1)

Furthermore, Titanium oxide is a chemically stabilized material that have a transformation from first phase anatase to second phase rutile at temperatures ranging between (450 and 950 °C) [18]. Yet, the creation of “CaTiO$_3$” suggest that the processing between (HAp and Titania) has done through thermal processing. The next chemical reaction is proposed:

$$\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 + \text{TiO}_2 \rightarrow 3\text{Ca}_3(\text{PO}_4)_2 + \text{CaTiO}_3 + \text{H}_2\text{O} \uparrow$$ \hspace{1cm} (1)

In accord with stage compositions it is clear that “CaTiO$_3$” is a result of the processing “firing process” that done between “TiO$_2$ and CaO”, which is one of the degeneration materials of HAp (Eq. (1)). Increasing the amount of Titania leads to a greater concentration of “CaTiO$_3$” in the composite and the existence of β-TCP is obvious, which leads to that portion of α-TCP has transformed to β-TCP. According to the results, the reactivity of Titania with HAp is very high, leading to the disassociation of the majority of the HAp phase, forming $\text{Ca}_3(\text{PO}_4)_2$ during the reaction.
Figure 4. XRD patterns of HAp/ TiO\textsubscript{2} powder.

Analysis of XRD Patterns for HAp/ Al\textsubscript{2}O\textsubscript{3}

Fig. 5 reveal XRD analysis, the addition of Al\textsubscript{2}O\textsubscript{3} resulted in decreased HAp/ β– TCP ratio in the samples, with much larger reduction at higher levels of Al\textsubscript{2}O\textsubscript{3} addition. While only Al\textsubscript{2}O\textsubscript{3} was the secondary phase found at the maximum ratio (25 wt. %). The formation of CaAl\textsubscript{2}O\textsubscript{4} by “solid state reaction between Al\textsubscript{2}O\textsubscript{3} and CaO” lowers the existence of calcium demanded for the “formation of stoichiometric HAp” that way directing to the formation of an increased value of β– TCP. The next chemical reaction is proposed: [19]

\[
\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 + \text{Al}_2\text{O}_3 \rightarrow 3\text{Ca}_3(\text{PO}_4)_2 + \text{CaAl}_2\text{O}_4 + \text{H}_2\text{O} \uparrow
\]

Figure 5. XRD patterns of HAp/ Al\textsubscript{2}O\textsubscript{3} powder.

Bioactivity Test

To study the bioactivity behavior of the samples in vitro study and test was done. In (vitro test) was done in “(SBF) simulated body fluid” made in accord with the “Kokubo formula” [20, 21] under a exact temperature which is (37 °C) and pH (7.2). Templates were removed from the SBF mixer after
Bioactivity tests were accomplished on the scaffolds which presented the highest mechanical and physical characterization.

**Assessment of HAp/TiO2 in Vitro Bioactivity**

Fig. 6 SEM images of specimens HAp/ TiO2 soaked in simulation body fluids solution for “30 days” shows the apatite layer after the immersion, the SEM image looks like typical “cauliflower” morphology, the porous sample exhibits the expected behavior; the gradual degradation of the both materials agreed to the formation of new HAp on the porous sample surface. This result is agree well with Fidanchevsk et al [22] as they reported in their article the apatite nucleation was also spotted on TiO2 particles. After four weeks, the matrix was completely covered with thin layer of new appetite.

Fig.7 of XRD analysis and if compared between them it notice that all the peaks indicated as Ca₃(PO₄)₂, CaTiO₂ are totally vanish and HAp phase had also replaced and become less in height in particular areas. When the material soaked with SBF solution, creation of apatite layer on the tops of porous samples carry out a serious of chemical reaction like a casual precipitation and development of calcium phosphate. It’s recognized that “HAp structure made of Ca, PO₄, and OH groups” carefully loaded together. The OH⁻ and PO₄³⁻ groups are in charge for “negativity of HAp surface and Ca²⁺ ions of the +ve group”.

The procedure of hydroxyapatite forming generally rely on “negative group, which in rely on the many numbers of -ve ions (i.e. OH⁻ and PO₄³⁻) on the surface”. Through incubating time, “the +ve Ca²⁺ ions from SBF are pulled by the OH⁻ and PO₄³⁻ ions appear on HAp exterior”. Thus, the exterior gets the +ve charge with particular to the enclosure “SBF and attracts the -ve charged OH⁻ and PO₄³⁻ ions from the SBF. This leading to creation of the apatite layer” [23]. Titania is fit to suck in water at the exterior of HAp, manufacture in “titanium hydroxide (Ti–OH)” groups. In the neutral and buffered fully saturated SBF solution (pH 7.2), the Ti–OH groups detach directing to a -ve charged Titania surface that supplies location for calcium phosphate nucleation. Moreover, it notified that Ti–OH groups in Titania particles they are efficient in promote apatite formalization of Titania [24].

EDX analysis of HAp/TiO2 showed that presence of these elements “Ca, P, O and Ti” in HAp/TiO2 porous composites. Usually, the existence of Ca and P ions have an important factors in bone growth [25]. In other studies manufactured HAp with various Ca/P molar ratios utilizing “fish and bovine bones” [26, 27], which were so close to theoretical value of Ca/P molar ratio level. Moreover, this method HAp/TiO2 the Ca/P molar ratio value was closely to (1.70) as shown in table (2), which is very close to the theoretically molar ratio of Ca/P (1.67) value of Hydroxyapatite this ratio is before immersion while after “immersion in (SBF) for 30 days” this ratio lowered to (1.32) for the chosen sample. This decrease refereeing to “deposition of a non-stoichiometric hydroxyapatite from simulation body fluids”.

![Figure 6. SEM pictures of HAp/ TiO2 after dipping in SBF solution for 30 days.](image)
Assessment of HAp/Al\textsubscript{2}O\textsubscript{3} in Vitro Bioactivity

Where in HAp/Al\textsubscript{2}O\textsubscript{3} the SEM images in Fig.8 reveals resulte of the manufactured scaffolds. The simulation body fluid, prepared by T. Kokubo\textsuperscript{23}, simulated the environment of the bloody-plasma in the body. The intensity of the significant ion types, Ca\textsuperscript{2+}, Al\textsuperscript{3+} and PO\textsubscript{4}\textsuperscript{3-}, are given. HAp scaffolds in pure form exhibited the predictable attitude; the progressive consuming the ions as together responsible for the creation of the new Hydroxyapatite on the porous surface. The capability of a Ca-apatite creation on the surface was extremely reported in the past \cite{28, 29} as shown Fig. 9. Otherwise the reactivity of the HAp/Al\textsubscript{2}O\textsubscript{3} composite in the SBF environment was little different. As could the PO\textsubscript{4}\textsuperscript{3-} ions were totally exhausted within \cite{30}; furthermore, the Ca\textsuperscript{2+} and Al\textsuperscript{3+} cation condensation had an increasing tendency.

The newly created phase in practice covered the composite samples surfaces is in good agreement with Basar et al. \cite{31}. The performed EDx analysis could not completely recognize between the creations of the Ca\textsuperscript{2+} or Al\textsuperscript{3+} bonded hydroxyapatite structures. The chance of the creation of both phases is nearly confirmed. X. This agree with Chatzistavrou et al. \cite{32}which explained the mechanism of apatite creation on a HAp/Al\textsubscript{2}O\textsubscript{3} composite surface.
Figure 9. XRD analysis after immersion in SBF solution for 30 days.

The Ca/P ratio before the 1.64 before the immersion, this ratio decreases to 1.37 after the immersion as shown in table (2), due to the same reasons above and in the end of the immersion a new apatite layer was covering the foam.

| Materials      | Ca/P Ratio Before | Ca/P Ratio After |
|----------------|-------------------|------------------|
| HAp/TiO₂       | 1.7               | 1.32             |
| HAp/Al₂O₃      | 1.64              | 1.37             |

To compare between the two additives it’s found out there is a minimum impact on the dissolution rate of the hydroxyapatite of the sample in SBF solution as shown in SEM images but in ratio acceptable to use as an implant in the bone reconstruction.

Conclusion

The present study successfully prepared a composite material of hydroxyapatite and titania by solid state reaction via polymeric sponge method to create porous ceramic and investigate the possibility of using it as implant as cancellous bone for bone regeneration. The characterization of HAp/TiO₂ and HAp/Al₂O₃ porous ceramics composites have demonstrated the potential of a novel biomaterial composite. Microstructure and in-vitro degradation could be enhanced by controlling titania and alumina ratio by controlling the rheological properties. The adding of the two reinforcement materials have a minimum effect on degradation rate of the hydroxyapatite. First of all, this study presents a new procedure to prepare HAp powder and can be reinforced with TiO₂ and Al₂O₃ particles to fabricate porous ceramic composites as perfect candidate for bone reconstruction and regeneration.

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