Preparation and evaluation of Bioceramic composite based on hydroxyapatite and titania nanoparticles for biomedical applications

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Abstract—in this study, composite material comprised of titanium dioxide (TiO2) nanoparticles incorporated with bone derived hydroxyapatite was prepared to investigate the possibility of using it as bone substitute in high load bearing sites. The structure and phase composition of the produced sintered bodies were characterized by X-ray diffraction (XRD) and scanning electron microscopy (SEM). Densification rate was evaluated by measuring apparent porosity. A mechanical characterization of the sintered samples was carried out by means of Vickers indentations and compressive strength determinations. The bioactivity was assessed by investigating the formation of apatite on the film surface after soaking in simulated body fluid. The results showed that the proposed composite present higher densification, better mechanical characteristics compared to pure hydroxyapatite. Moreover, the results also showed bone like apatite was formed on the surface of hydroxyapatite/TiO2 nanoparticles soaked in simulated body fluid. The results obtained demonstrate that hydroxyapatite can be considered suitable as filler in biomedical applications while glass-ceramic matrix composite reinforced with titania nanostructure can be used in high load bearing applications from the point of view of the biomechnical properties. This behavior suggests a possibility of a further favorable in vivo response.

Index Terms— Bioceramics, biomaterial, Bovine hydroxyapatite, Mechanical properties, In vitro study, simulated body fluid (SBF), titania nanoparticles

1. Introduction

Bone is one of few human tissues that can regenerate themselves. For instance, the fracture of bone activates osteogenesis and ultimately the fracture heals without any scar formation. Although bone has its own ability to repair, the ability decreases with age, is affected by diseases and other factors, and is limited to small bone defects. Thus far, grafts are necessary to assist bone repair when bone loss is too big, for example in cases of excision of bone tumour, bone sarcoma or other bone diseases, in cases of bone loss in accidents, and in complicated fractured bones that cannot repair themselves. Several grafts can be the choice in clinics for bone repair ex. (autograft, allograft). The shortcomings of autograft and allograft justified the development of artificial bone grafts - biomaterials. In the last century, different biomaterials, including metals, polymers, calcium phosphate biomaterials, bioglasses have been studied for bone repair [1-6].

Calcium phosphates based materials are preferred as bone grafts in hard tissue engineering because of their superior biocompatibility and bioactivity [7].

However, the low mechanical strength of normal HAp ceramics restricts its use mainly to low load-bearing applications. Recent advances in nanoscience and nanotechnology have reignited interest in the formation of nanosized HAp and the study of its properties on the nanoscale. Nanocrystalline HAp powders exhibit improved sinterability and enhanced densification due to greater surface area, which may improve fracture toughness, as well as other mechanical properties [8].

Nanomaterial–tissue interactions strongly influence properties and thus extend or limit new application areas. Nanomaterials, due to high surface areas and roughness, have large surface energy and unique surface properties arising from surface defects, nonregular grain boundaries, etc. All of these unusual properties are inherent for nanomaterials and will affect interactions with proteins, which are natural nanoscale entities. For example, the increased surface area and nanoscale surface features of nanomaterials can provide more available sites for protein adsorption and, thus, alter the capability for cellular interactions. One

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straightforward explanation lies in the fact that natural tissues and associated extracellular matrices are composed of nanostructured materials. It is not yet known exactly how nanoparticles interact with tissue. It is suggested that nanoparticles, because of their small size, may act like haptens to modify protein structures, either by altering their function or rendering them antigenic, thus raising their potential for autoimmune effects [9].

The recent trend in bioceramic research is mainly concentrated on developing biocomposites [10], where there is another an approach to solve the mechanical limitations of bioactive glasses and ceramics for load-bearing applications [6]. Here, we report on titanium oxide (TiO_2) surface nanostructures utilized for reinforcing hydroxyapatite nanoparticles for orthopedic implant considerations. Specifically, the effects of TiO_2 nanoparticle on biomechanical properties of nano crystalline hydroxyapatite for bone regeneration will be discussed.

2. Experimental procedures

2.1. Bone-derived HA/TiO_2 nanoparticles processing

Hydroxyapatite was mixed with titania nanoparticles in a ball mill under variation of the titania nanoparticles contents. The percentage of TiO_2 nanoparticles was 10, 20 and 30 wt. % from total amount of hydroxyapatite. By this method three types of ceramic samples containing different percentages of TiO_2 nanoparticles were prepared. (BHA)/TiO_2 powder was uniaxially pressed at 600 MPa into green bodies using a 10 mm cylindrical dies and sintered at various sintering temperatures of 900, 1000 and 1150℃. The hydroxyapatite material used in this study was derived from the shaft portion of the bovine femurs, of the natural bovine bones. The procured bone samples were cleaned from substances, which include the ligaments and tissues stuck on the bone. The samples were washed with distilled water. The cleaned bone samples were degreased by immersing in acetone. After washing, the treated bone samples were preheated at 160 ◦C for 48 h. The cleaned bones were grinded to particle size less than 200μm then calcined to 800 ˚C at heating rate of 10 ◦C/min with soaking time of 1 h to completely remove organics and to sterilize the samples. The obtained calcined bone powder was further grinded by ball milling to decrease the particle size to 63 μm which were used for processing. The size of the hydroxyapatite crystals as assessed from the SEM images ranged from 100 to 800 nm. While, Synthesis of TiO_2 nanoparticles was adopted from reference [11], with some variations. ‘‘sol–gel method’’ was used in this study for TiO_2 nanoparticle preparation. Titanium tetraisopropoxide (TTIP, Ti $(OCH(CH_3)_2)_4$, 97%, sigma-Aldrich) was used as a precursor to prepare TiO_2 sol using a mixture of ethanol and nitric acid. The precursor solution was a mixture of 20 ml TTIP and 100 ml ethanol. The mixture was stirred for 1 h. The mixture of 20 ml of water, 20 ml ethanol and 10 ml HNO_3 solution was then added drop wise into the first mixture. The reaction was performed at room temperature while stirring constantly for 2 h. After aging for 24 h, the sol was transformed into gel. In order to obtain nanoparticles, the gel was dried at 100 °C overnight in an oven to evaporate water and organic material to the maximum extent. Then the powders were annealed at 500 °C for 3 h to obtain the desired TiO_2 nanocrystalline. The SEM images of TiO_2 nanoparticles reveal aggregates of near-spherical particles of size within the range of 50–250 nm

2.4. Analysis and testing methods of materials characterization

Phase identification of the sintered compact disks were determined by X-ray diffraction model no 202964-panalyticel empryan company. The specimen surface microstructure was studied by scanning electron microscope (quanta feg 250 and JEOL, JSM-T200). Densification in term of apparent porosity was measured by using Archimedes’ method. Compression-strength tests was carried out with a universal test machine (UH-A, No 600283-03, shimazu corporation, made in japan), while, Vickers microhardness was measured using Veb Carl Zeiss Jena microhardness tester, made in GDR).

2.5 In vitro bioactivity assay

Compact disks of the hydroxyapatite reinforced with TiO_2 nanoparticles were put into SBF, proposed by Kokubo et al [12], at 36.5 ˚C in polyethylene containers. The SBF solution has a composition and concentration similar to those of the inorganic part of human blood plasma. [13- 15]. The solution was buffered to pH 7.4 with tris-(hydroxyl methyl)-amino methane and hydrochloric acid. The soaking times were 30 days. After being soaked, the disks were rinsed with double distilled water and dried at room temperature. The in vitro bioactivities of the

hydroxyapatite composite were evaluated by studying the changes in the crystalline phases and microstructure formed on the surfaces of the disks by XRD and SEM.

3. Results and discussion

3.1 Crystallographic Investigation

The results of the crystallographic analysis of the produced samples, carried out with X-ray diffraction, are presented in Fig. 1The X-ray diffraction patterns of BHA/TiO_2 composite samples reveal peaks associated with HA (Card no. 01-074-977). Also, calcium phosphate Ca_3 $(PO_4)_2$ peak can be observed (Card no. 01-073-4869). The calcium titanium oxide with a high intensity is observed (Card no. 04-007-8803). Also TiO_2 peak can be detected Card no. 01-080-2547). These patterns confirm that the titania was well incorporated in the hydroxyapatite matrix.

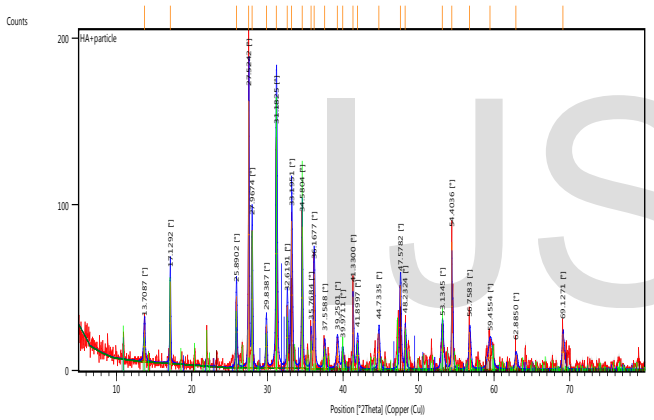

Fig. 1 XRD patterns of heat treated hydroxyapatite/ TiO_2 nanoparticles prepared at 500˚C

3.3 Morphological characterization

Representative SEM pictures of bovine bone hydroxyapatite/TiO_2 samples sintered at 1150℃ are respectively depicted in Figs. 2. As these images show that, very clear grains with density of 2.99 g/cm^3. A high densification regime is suggested after sintering at 1150 ˚C which showed that the sintering process almost complete and subsequently grain growth occurred at temperature 1150˚C. Furthermore TiO_2 nanoparticles 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 1150˚C and for hydroxyapatite glass ceramic doped with 30wt% TiO_2 nanoparticles, where these compact materials with 30wt% titania nanoparticles and at 1150℃ represent the best physical and mechanical characteristics so it was chosen as will defined in the next sections

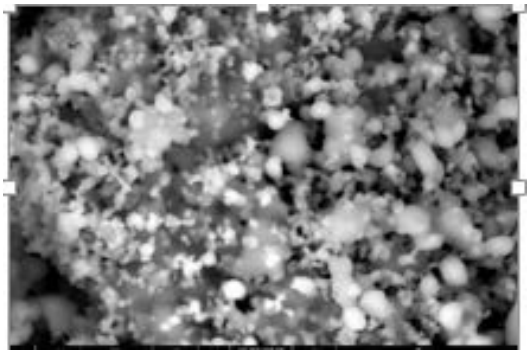

Fig.2 Microstructure of hydroxyapatite/TiO_2 nanoparticles at 1150 ˚C.

3.5. Physical Properties Measurement

Fig 3 presents the apparent porosities of sintered bovine bone (BHA) and BHA-TiO_2 composite at different sintering temperature. It can be observed that with increasing sintering temperature, porosity of obtained glass ceramics either pure hydroxyapatite or composite significantly decreased, where it is interesting to note that the hydroxyapatite samples for example fired at 900˚C result in materials with high porosity percentage (13.6) while at 1150 low porous materials was obtained (10.6). This behavior could be understood in term of the temperature where temperature is one of the most important factors that control sintering. Since diffusion is responsible for sintering, clearly increasing temperature will greatly enhance the sintering kinetics, because diffusion is thermally activated. Therefore, increasing the temperature usually enhances the bulk diffusion mechanisms which lead to densification [16]. As well, the influence of the titania nanoparticles content on the densification behavior in term of porosity is clearly to extract from the displayed figure. Progressive addition of titania nanoparticles produces a decrease of samples porosity due to the fact that the added materials develop a large amount of high viscosity phases causing reduction of the open porosity.

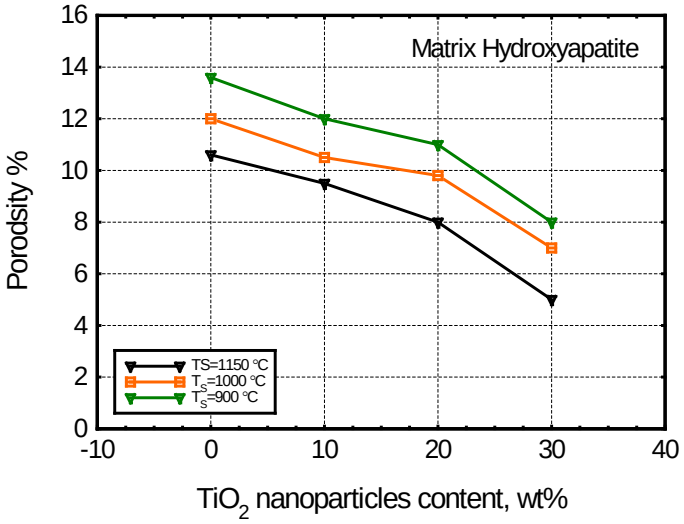

Fig.3 Porosity as % of hydroxyapatite /TiO_2 nanofibers at (900, 1000, 1150˚C

3.4 Mechanical Properties Measurement

Fig. 4 shows the compressive strength of bovine bone hydroxyapatite (BHA) and various BHA composites (10, 20, and 30wt% TiO_2) prepared at different temperatures (900, 1000, and 1150 ˚C). The maximum value for compressive strength was obtained for samples prepared at 1150 ˚C in the order 30wt% (63MPa), 20wt (56MPa), 10wt% (46MPa) and finally 0 wt. % TiO_2 (40 MPa). The maximum strength of all samples obtained at temperature 1150℃. This was attributed to complete sintering process of specimen with highest density. At lower temperature the higher result of porosity percentage contributed to lower strength of specimens. This result due to incomplete sintering process with high pores distribution on the surface. Moreover it can be observed that with increasing titania content, compressive strength of dense bovine bone ceramics significantly increased. This was mainly because of the porosities decreased as the weight fraction of the titania increased. The strength is a property which is strongly dependent on the porosity and microstructural defects of the specimen. Previous studies focusing on the use of other materials to form BHA composites which have better compressive strength. Faik Nu¨zhet Oktar et al [17] have added 5 wt. % and 10 wt. % of SiO_2, MgO, Al_2O_3 and ZrO_2 (stabilized with 8% Y2O3) to prepare composite structures having better compressive strength. Viviane V. Silvaet al [18] used ZrO_2, which have good biocompatibility, to reinforce bovine bone hydroxyapatite.

On other side, the results that obtained from the vickers hardness test were illustred in Fig 5. the same trend of compressive strength was obtained by the vichers hardness test too. The best results for microhardness (Fig. 5) were obtained at 30wt% TiO_2 content at 1150 ˚C (765HV). By comparison this is also relative to the density values at the respective temperatures (2.99 g/cm^3), indicating that the microhardness is also affected by density. In general, when comparing microhardness and compression values of composite hydroxyapatite and hydroxypatite alone, (Fig. 6 and Fig. 5) the reinforcement role of TiO_2 becomes extremely evident and valuable.

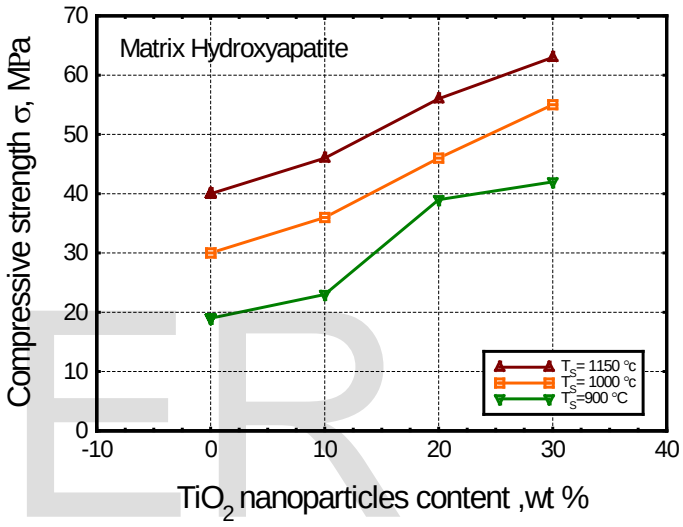

Fig.4 compressive strength of hydroxyapatite /TiO_2 nanoparticles at (900, 1000, 1150˚C)

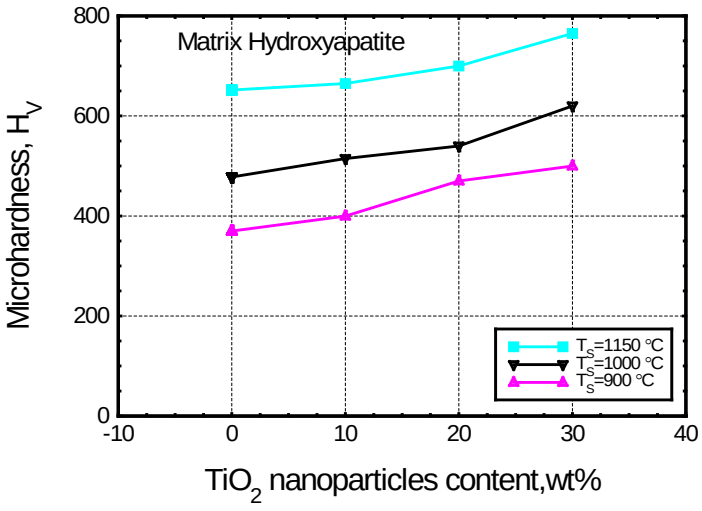

Fig.5 micro hardness of hydroxyapatite /TiO_2 nanoparticles at (900, 1000, 1150˚C)

3.5 Assessment of in vitro bioactivity

The effect of titania nanoparticles additions on bioactivity of bovine bone hydroxyapatite have been analyzed by soaking the produced compact disks in SBF solution for 30 days. The characterization of the glasses ceramic, after the treatment in SBF, was performed through XRD analysis. It is necessary to point out that the results of the physical and mechanical properties were taken into account to select the samples for the in vitro study. Samples sintered at 1150 ˚C and 30 wt. % titania content shows higher densification behavior and superior mechanical properties. Therefore, the bioactivity was tested on these samples. Finally it is worth to note that the immersion period of the glass-ceramic in SBF was up to 4 weeks. The choice of this time was based on the results obtained from previous in-vitro experiments [19-21].

The results of XRD analysis of HA-30 wt% TiO_2 composite, immersed in simulated body fluid for 30 days, are shown in Figs. 6. Observation of the peaks, as shown in Fig. 6 compared to Fig 1, reveals that all peaks which indexed as $Ca_3(PO_4)_2$, $CaTiO_3$ and TiO_2 completely disappeared. The diffractograms of the sintered samples after immersion in SBF (Fig 6) show only the characteristic pattern of hydroxyapatite (as deduced by comparing to the standard JCPDS File No. 04-008-4761 of pure HA).

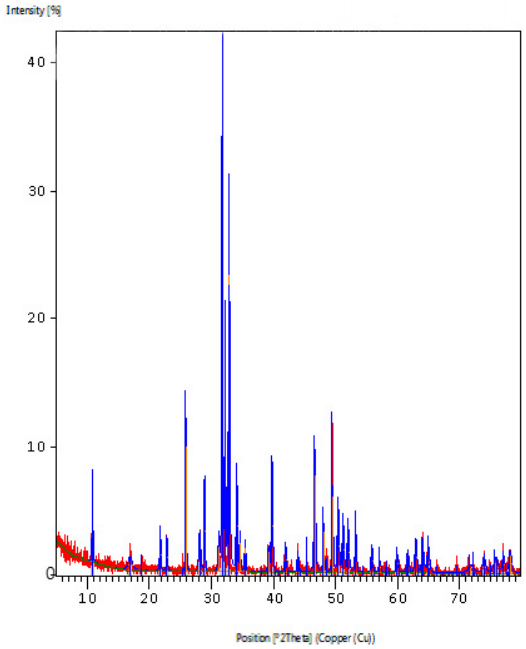

Fig (6). XRD patterns of BHA/TiO_2˚C after soaking

It could be expected that, the combination of bovine bone hydroxyapatite and TiO_2 nanoparticles allows two possible reactions leading to surface nucleation and subsequent mineral (apatite) growth. HA formation on the composite surfaces can be triggered by the surface condition of both the bovine bone hydroxyapatite and the titanium dioxide nanoparticles. Therefore two different routes or mechanisms of HA formation can be suggested, as discussed next.

Firstly when a material is incubated in SBF solution, the formation of apatite layer on the surface of pellet goes through a sequence of chemical reactions like spontaneous precipitation, nucleation and growth of calcium phosphate. It has been suggested that surface chemistry plays an important role in this process and even the functional groups of materials have a large effect on the bone bonding property. It is well known that HAp structure consists of Ca, PO_4, and OH groups closely packed together. The OH^- and PO_4^{3-} groups are responsible for negativity of HAp surface and Ca^{2+} ions form the positive group. The process of apatite formation mainly depends on negative group, which in turn depends on the large number of negative ions (i.e. OH^- and PO_4^{3-}) on the surface. During incubation period, the positive Ca^{2+} ions from SBF are attracted by the OH^- and PO_4^{3-} ions present on HAp surface. Therefore, the surface gains positive charge with respective to the surrounding SBF and further attracts the negatively charged OH^- and PO_4^{3-} ions from the SBF. This leads to formation of the apatite layer [22].

Secondly a similar reaction path is conceivable due to the presence of titanium dioxide nanoparticles within the hydroxyapatite matrix. TiO_2 is able to absorb water at the surface, resulting in titanium hydroxide (Ti–OH) groups. In the buffered and neutral supersaturated SBF solution (pH 7.25), the Ti–OH groups dissociate leading to a negatively charged TiO_2 surface that provides sites for calcium phosphate nucleation. This mechanism has been proposed for sol-gel derived TiO_2 gels and films. Furthermore, it has been reported that Ti–OH groups in TiO_2 gels are particularly effective in inducing apatite formation if TiO_2 is present in its anatase modification [23].

Mei Weia et al reported that, in the case of calcium-containing titania gels heat treated, the calcium ion is released from the gels into SBF and the amounts of the released calcium ion is increased with

increasing CaO content of the gels. This means that when these gels are soaked in SBF, larger amounts of Ti-OH groups are formed on the surface of the gels via exchange of the Ca^{2+} ion in the gels with H_3O^+ ion in SBF and the ionic activity product of the apatite in SBF is increased with increasing CaO content of the gels. It is expected that thus formed Ti-OH groups induce the apatite nucleation and the increased ionic activity product accelerate the apatite nucleation [24].

Weeraphat Pon-On et al indicated that formation of silinol-like groups of Ti/OH and of the perovskite structure $CaTiO_3$ nodules during the synthesis process would promote the formation of apatite when they interact with the SBF. The net ionic charge on the composite surface will trigger the precipitation of calcium phosphate from the SBF. Webster et al. reported that $CaTiO_3$ promotes osteoblast adhesion [25]. Furthermore, It has been reported that bulk TiO_2 substrates processed with nanoparticles have approximately 35% more surface area compared to the respective conventional materials, leading to a 30% greater osteoblast adhesion [23].

Briefly, it has been shown that hydroxyl groups and the negatively charged surface at pH 7.4 are properties which enhance the chemical bonding of TiO_2 coatings to bone. In addition, there is also evidence of strong surface structural dependence to the calcium phosphate formation properties on these coatings. The surface topography at the nanometer level has been shown to influence the in vitro bioactivity. This phenomenon was related to the charge density and the topographical matching of the titania surface and calcium phosphate crystal size found in bone (i.e. not with the matching of the atomic distances in single crystals). [26].

Therefore it is reasonable to speculate that above mentioned reasons lead to induce apatite formation by titania nanoparticles

Conclusion

The present study showed the preparation of composite material of hydroxyapatite and titania nanoparticles to investigate the possibility of using it as implant in high load bearing sites. The characterization of hydroxyapatite/TiO_2 nanoparticles composites have demonstrated the potential of a novel biomaterial composite. densfication rate, microhardness and compressive strength could be enhanced by controlling titania nanoparticles content and sintering temperature. Overall, this study introduces hydroxyapatite/TiO_2 nanoparticles as promised candidate for medical application.

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