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Biomechanical characterization of bioceramic composite based on hydroxyapatite and titania nanofibersfor biomedical applications

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Abstract—Hydroxyapatite (HAp) exhibits excellent biocompatibility with soft tissues, making it an ideal candidate for orthopedic applications. However, the low mechanical strength of hydroxyapatite ceramics restricts its use mainly to low load-bearing applications. So, in this study, composite material comprised of titanium dioxide (TiO2) nanofibers incorporated with bone derived hydroxyapatite was prepared to investigate the possibility of using it as bone substitute in high load bearing sites. Characteristics of produced materials were explored through physical, mechanical and in vitro studies. It was found that characteristics of the produced glass-ceramic materials clearly showed a marked dependence upon sintering temperature. Composite have superior mechanical features and at the same time, indicates a potential ability to form a new biologically interface with hard tissue and suitability for further investigations intended for medical applications.

Index Terms—Bioceramics, biomaterial, Bovine hydroxyapatite, Heat treatment, X-ray techniques, Mechanical properties, Microstructure, In vitro study, simulated body fluid (SBF), titania nanofibers

1. Introduction

One of the latest lines in materials science concerns materials for replacing defective bone parts. They may be subdivided into bioinert ones and bioactive ones inaccordance with their behavior in the body. Bioactive materials participate in metabolism and are resorbed and replaced by newly formed bone tissue. They include calcium phosphate glasses and glass ceramics, synthetic hydroxyapatite (SHAp), and biological hydroxyapatite (BHAp) [1].

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Hydroxyapatite (HA) [Ca_{10} $(PO_4)_6$ $(OH)_2$], is perhaps one of the most utilized biomaterial in this regard, because of their mineral component being similar to bone and teeth of the human body.Thestoichiometric HA has the chemical composition of Ca_{10} $(PO_4)_6(OH)_2$ with Ca/P molar ratio of 1.67. It is reported that HA is an excellent biocompatible material with hard tissues and exhibits osteoconductive properties, non-toxic, non-immunogenic behaviour. The HA can form a strong chemical bond with host bone tissue. This makesit recognized as a good bone substitute material. [2-6].

Unfortunately, the use of bioactive ceramics as bulk implants is usually limited to non-load bearing sites or less loaded portions because their fragility and also due to their inappropriate mechanical performances. Their practical applications as implants are still greatly restricted. Nevertheless, their field of application can be expanded[7-9]. Therefore, the development of bioactive materials that have improved and ultimately bone-like mechanical properties is desirable[10-12].

In recent years the use of nanotechnology has shown to be one of the most promising fields to enhance material properties for instance for biomedical applications[13]. The nanometer size of the inorganic component (mainly bonelike apatite) in natural bone is

considered to be important for its mechanical properties. Recent research in this field has also suggested that better osteoconductivity would be achieved if synthetic HAP resembled bone minerals more in composition, size, and morphology. Webster et al have shown significant increases in protein adsorption and osteoblast adhesion on the nanosized ceramic materials compared to traditional micronsized ones [14]. An increase in osteoblast function like proliferation, alkaline phosphate activity, and calcium deposition on nanoceramics was further evidenced where nanophase titania, alumina, and HAP show higher cell densities as a result of osteoblast proliferation in comparison to conventional micron-sized ceramics of these materials. The increased osteoblast function on nanophase ceramics is due to the increased adsorption of vitronectin over the surface of nanophase ceramics. The increased wettability of nanostructured ceramics enhances the adsorption of vitronectin in comparison to their conventional micro implant. Increased adsorption of vitronectin on nanophase ceramics induces increased oseteoblast adhesion and proliferation [15]. The benefits accrued by having nanoscale particles interact with a second phase also can be seen in another huge category of materials known as nanocomposites [14].Nanocomposite is one of the approaches to overcome the problem of low fracture toughness and poor wear resistance of HAp[16, 17]. The main idea of this study is to produce nanocrystallites of hydroxyapatite and to investigate the possibilities of reinforced glass-ceramics by titania nanofiber. The influence of the TiO_2 content (10 to 30 wt. %) on the physical and mechanical characteristics of the final materials was studied. In addition, the in vitro bioactivity of these materials was evaluated.

2. Experimental procedures2.1. Preparation of composite of hydroxyapatite/ titania nanofibers

Hydroxyapatite was mixed with 30 wt. % titania nanofibers in a ball millanduniaxially pressed at 600 MPa into green bodies using a 10 mm cylindrical dies. To obtain the best condition for sintering, many sintering conditions were investigated where, the compacted green body was sintered at various temperatures of 900, 1000 and 1150 °C, at a rate of 10 °C/min and dwell time for 2 h (Electrical heating furnace used is of type Nabertherm, model L08/14 made in Germany). The hydroxyapatite material used in this study was derived from natural bovine bones. Briefly, the procured bone samples were cleaned from substances, which include the ligaments and tissues stuck on the bone. 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. While,TiO_2 nanofibers were prepared by electrospinning a mixture of TiO_2 sol and poly vinyl-pyrrolidone (PVP,*M*w = 1 300 000, Sigma-Aldrich). In a distinctive procedure, 1.8 g of titanium tetraisopropoxide was mixed with 3 mL of acetic acid and 3 mL of ethanol in a glove box. After 10 min, this solution added to 7.5 mL of ethanol that contained 1 g of PVP, followed by stirring for ~1 h. The resulting light yellow solution was transparent with no precipitation after hours of stirring. electrospinning unit was used to produce the nanofibers. The electrospinning apparatus comprised of a plastic syringe attached to a capillary tip putted in carrier, a grounded collector, and a high-voltage supply (CPS-60 K02V1, Chungpa EMT Co, South Korea). The mixture was immediately loaded into a plastic syringe equipped with a tip made of plastics. The distance between the tip and the rotating cylinder was 16 cm. The applied voltage was set at16kV. The as-spun nanofibers were initially dried for 24 h at 80 ˚C. Finally, these nanofibers calcined at 500 °C for 3 h, where calcinattion was performed at this temperature to removethe PVP, decomposition of the inorganic precursor and achieve the crystallization of titania.

2.4. Analysis and testingmethods

Crystalline phases were examined by using X-ray diffractometer model no 202964-panalyticel empryan company. The set of characterization also comprised observation of the microstructure (SEM, quanta feg 250 and JEOL, JSM-T200).The bulk density and apparent porosity of sintered samples was determined using Archimedes̊ principle. The measurements of the mechanical properties comprised compression-strength tests, carried out with a universal test machine (UH-A, No 600283-03, shimazu corporation, made in japan), and Vickers microhardness (Veb Carl Zeiss Jena microhardness tester, made in GDR).**2.5 In vitro bioactivity assay in simulated body fluid**

Compact disks of the hydroxyapatite reinforced with TiO_2 nanofibers were put into SBF, proposed by Kokubo et al [18], 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[19- 21]. 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 phase purity and the crystallographic structural properties of the bovine bone hydroxyapatite (BHA)/TiO_2 nanofibers after sintering at 1150 ˚C were evaluated by X-ray diffraction. Spectra from the BHA/TiO_2 sample are depicted in Fig. 1. Diffraction peaks in these patterns can be well indexed tomixed phasesof calcium phosphate hydroxide (synonym of hydroxyapatite) compared with the standard diffraction patterns (JCPDS Card 04-008-4761 of pure HA), titania (card no. 01-082-0514, calcium titanium oxide (Card no 01-080-5717 and calcium phosphate Ca3 $(PO_4)_2$(Card 04-014-2292)

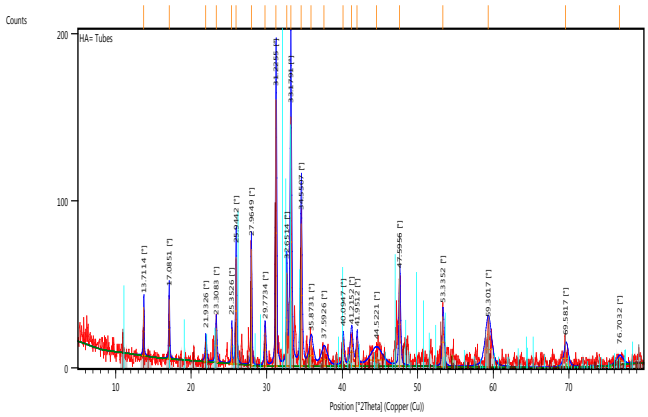

Fig. 1 XRD patterns of heat treated bovine bone hydroxyapatite/30% TiO_2 nanofibers prepared at 1150˚C

3.3 Morphological characterization

The crystallization of hydroxyapatite glass ceramic doped with 30wt% TiO_2 nanofibers has been examined in terms of microstructure inFig2.As shown in the figure, the inorganic nanofibers were well merged with the hydroxyapatite matrix and cause an elongation in hydroxyapatite particles. The microstructure of the sintered sample reveal that very clear grains with density of 3 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.We can point out that the evolution of microstructure and crystalline phases observed at sintering temperature of 1150˚C. The sintering temperature of 1150 ˚C was selected because this condition produced the optimum ceramics properties, as will defined in the next sections.

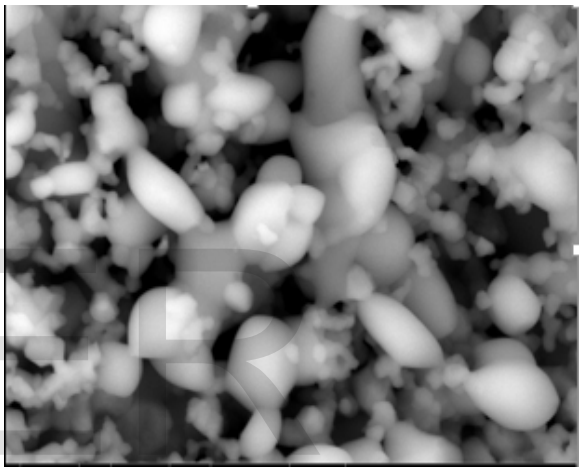

Fig. 2 microstructure of hydroxyapatite/ TiO_2 nanofibers at 1150 ˚C. The magnification in all panels is 13000X.

3.5. Physical Properties Measurement

Table 1 presents the densification parametersof BHA-TiO_2 compositein term of bulk density, percentage of porosity and water absorption after sintering at different temperatures. From the table, it is clear to notice that the porosity has an inverse proportion with increasing of the sintering temperature. Pores number decreased as the sintering temperature increased owing to enhance the densification of specimens. On other side, the results that obtained from the bulk density were illustred in the table, as temperature increased the density of specimens increased. The maximum density of 3.1 g/cm^3 achieved for the sample sintered at temperature 1150˚C which most of the pores were shrunk. 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 thetemperature usually enhances the bulk diffusion mechanisms which lead to densification [23]. Table. (1) alsoshows, the water absorption of the fired compositions as a function sintering temperature. One should observe that, sintered samples have reducing water absorption with increasing sintering temperature as the surface connected porosity decreases

Table 1. Experimental results of densification behaviour of the bovine bone hydroxyapatite/ 30%TiO_2 nanofibers sintered at different temperatures

Temperature	Porosity%	Density g/cm^3	Water absorption
900℃	**6.8**	**2.9**	**5.8**
1000℃	**5.5**	**3**	**4.4**
1150℃	**4**	**3.1**	**3**

3.4 Mechanical Properties Measurement

To determine the load carrying capacity of the produced ceramics, compression tests were performed on bovine bone hydroxyapatite/TiO_2 composite specimens at different conditions of sintering temperatures.The maximum strength was obtained at higher sintering temperature. This is referred to the high densification and bonding obtained at high sintering temperature. The highest strength for specimens sintered at 1150 ˚C was 115 Mpa.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 have demonstrated similar findings. Oktar et al. have added reduced quantities of lanthanum oxide to BHA (0.25, 0.50, 1 and 2wt %) to prepare composite structures having better compressive strength [24]. S. Salman et al [3] have used Ti, which is very popular in dental implant applications to reinforce bovine bone hydroxyapatite. The corresponding values of compression is 53.29 of BHA–Ti composites as they have been reported.Zhang et al. have used silver particles for toughening HA, but the biocompatibility of silver in human body may be a question mark. Oguzhan Gunduz et al [25] prepared composites of calcinated bovine bone-derived hydroxyapatite (BHA), doped with 2.5, 5, and 10 wt. % ZnO. The highest values of compressive strength were observed with 5% ZnO which equal 71.9Mpa.

Table 2 also shows the Vickers's hardness number of the produced ceramics. The high hardness resistance was achieved by applying 1150 □C as a sintering temperature.the same trend of compressive strength was obtained by the vichers hardness test too. The best results for microhardness were obtained at 1150 ˚C (800HV). By comparison this is also relative to the density values at the respective temperatures (3.1g/cm^3), indicating that the microhardness is also affected by density. The microhardness values obtained in this study are superior compared to those demonstrated in a study utilizing inert glass (CIG) addition to HA content. The best microhardness value obtained for CIG–BHA composites in the study was 507HV, which is appreciably lower than the highest values reported in this study [26].

Table 2. Experimental results of compression strength (in MPa) and Vickers microhardness (in HV) of the bovine bone hydroxyapatite/TiO_2 sintered at different temperatures.

Temperature	Compressive strength, MPa	Micro hardness, Hv
900℃	**65**	**550**
1000℃	**90**	**680**
1150℃	**115**	**800**

3.5 Assessment of in vitro bioactivity

Bioactivity of bovine bone hydroxyapatite/TiO_2 nanofibers 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 and SEM with EDAX. 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 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 span was based on the results obtained from previous in-vitro experiments performed in the same medium [27- 30]. The bioactivity has been investigated by checking the surface

morphology and the crystal structure of the deposited layer.

3.5.1 XRD analysis

The X-ray diffraction analyses results of the hydroxyapatite containing 30wt% TiO_2 nanofibers glass ceramic after soaking in SBF up to 30 d are shown in Fig. 3. Compared to Fig. 1which demonstrates the XRD pattern of hydroxyapatite containing 30wt% TiO_2 before soaking, upon being soaked in the SBF solution for 30 days, significant changes were observed in the XRDpatterns after immersion in SBF.As shown, the characteristic peaks of BHA/TiO_2 (30%) disappeared after soaking, new peaks were apparent after soaking. Thesenew peaks were assigned to be apatite according to the standard JCPDS (# 09-0432). This demonstrates that the soaking resulted in the formation of HA crystalline phase on the surfaces of the bioactive glasses ceramic. It is considered that a bonelike HAP layer, which precipitates on the surface of a bioactive material in human body, plays an essential role in forming the chemical bond of the bioactive material to the living bone.

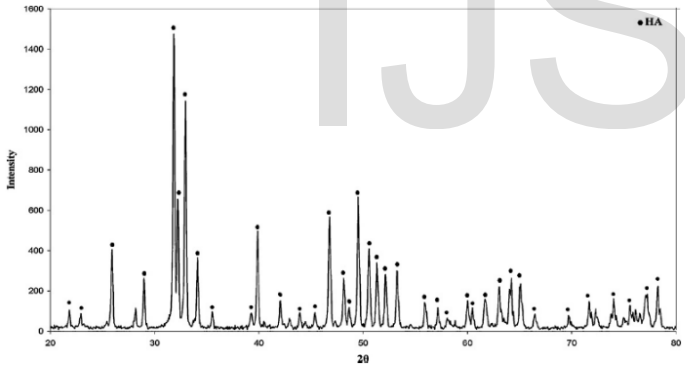

Fig (3). XRD patterns of BHA/TiO_2˚C after soaking

In SBF for 30 days

3.5.2 Microstructure of the new formed layer

Fig. 4shows the SEM micrographs with EDAX of the BHA/TiO_2 surfaces for 30 days soaking times in SBF.As shown,the surface morphology of the specimens changed, where the samples before and after incubation in SBF show completely different surface morphology.A new layer consisting of spherical particles was observed on the specimens. This layer should be apatite, as indicated from the XRD and EDAX result as for BHA/TiO_2 and from description of this layer by other studies, where morphology of this layer is quite similar to that of the apatite formed on the surfaces of bioactive glasses and glass-ceramics in SBF which described by other literature[28- 31].

The EDAX detected the elements of (Ca, P, O) with traces amount of Na, Mg, F and Cl. The Ca/P atomic ratio of the specimen HA was exactly 1.67, as was expected by composition of HA. There is no detection of any traces amount of titanium after soaking.

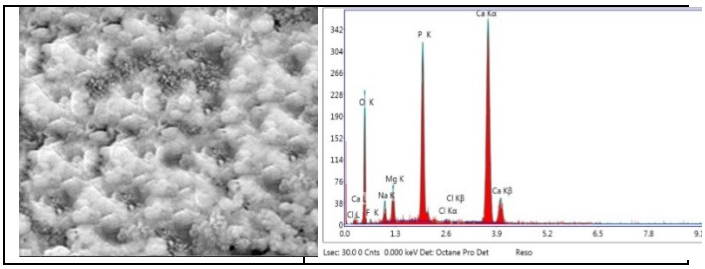

Fig. 4SEM photographs of the surface of BHA/TiO_2, with EDAX after soaking in SBF for 30 days

The mechanism of apatite formation on synthetic HAp has been documented, even if it is less frequently described than for bioactive glasses. It is a four-stage process, with accumulation of Ca^{2+} ions close to the negatively charge HAp surface, then the deposition of a calcium-rich amorphous CaP layer. As the calcium ions accumulate, the surface becomes positively charged and reacts with phosphate ions of the solution, forming a calcium-deficient CaP layer. This amorphous layer can then be transformed into a more stable bone-like apatite that will help cellular proliferation and differentiation to form native bone tissue. The time to crystallize an apatite layer at the surface of HAp is, however, relatively important, typically after 28 days of immersion in SBF [32]. While, in this study composite material of hydroxyapatite/TiO_2 nanofibers was used. Concerning apatite-forming tendency on TiO_2, It has been established that HA formation is closely related to the hydroxyl groups (–OH). The surface hydroxyl groups such as Ti–OH, Si–OH, Zr–OH, Ta–OH and Nb–OH are known to be efficient inducers for hydroxyapatite nucleation. It was mentioned that Ti–OH groups on titania, which have been proposed to be responsible for the apatite formation, are effective for apatite nucleation when they are arranged in a specific

structural unit [33] Accordingly, the titania nanofibers containing a significantly higher density of Ti–OH, would be expected to be the most efficient for hydroxyapatite formation.

Narges F. Fahim et al[33]studied the apatite-forming tendency on Silica particle-doped high aspect-ratio TiO_2 nanotube layers and reported that, the following factors are thought to be responsible for the induction and the formation of a dense, continuous apatite layer on TiO_2 nanotube surface: (1) the high aspect-ratio (higher surface area to volume ratio), which allows for the presence of plentiful OH groups on the surface before and/or after immersion in SBF that, in turn, affects the electrical charge of the host substrate; (2) the well-ordered pores with suitable sizes allow for the spontaneous growth of apatite nuclei, which act as novel pathways for transferring the calcium and phosphate ions from the surrounding body fluid, so that a dense and continuous layer can be formed; (3) a higher degree of roughness that is associated with high aspect-ratio nanotubular structures. Finally, formation of a thick layer of HA on TiO_2 nanotubes occurs by spontaneously taking up the calcium and phosphate ions in the SBF and incorporating minor ions, such as CO_3^{2-}, Na^+ and Mg^{2+} to develop a bone-like apatite layer (CHA), which can be represented by the approximate formula $(Ca, Mg, Na)_{10}(PO_4,CO_3)_6(OH)_2$.

It is well established that the mechanism of apatite formation on a functional groups like Ti–OH, Si–OH, Zr–OH, Ta–OH, and Nb–OH corresponds to the electrostatic interaction between surface layers and the ions in the SBF. All these functional groups have an isoelectric point zero charge (PZC) at pH values much lower than 7 and thus should thus is negatively charged in the body environment. The pH_{PZC} is the pH at which the material has a net surface charge of zero, where this is a characteristic property of the oxide that depends on the oxide crystal structure and on dielectric constant that controls the interfacial solvation behaviorfor pure silica and titania phases, the pH_{PZC} is ~2.5 and ~6, respectively, and the titania and silica–titania surfaces are thus negatively charged at the physiological pH of ~7.4 due to the deprotonated acidic hydroxide $[Ti–OH]^-$and/or $[Si–OH]^-$. Such a negatively charged surface can combine selectively with the positively charged Ca^{2+} ions in the fluid by being covalently and/or covalently coordinated bound to titania and/or silica surface to form $Ti–O–Ca^+$ and/or $Si–O–Ca^+$. As the calcium ions accumulate, the titanium surface gains an overall positive charge that, in turn, reacts with negatively charged phosphate ions (HPO_4^{2-}, PO_4^{3-}) to form an amorphous calcium phosphate, which transforms into apatite in SBF. Once apatite nuclei are formed, which can spontaneously grow into a dense and uniform layer by consuming the calcium and phosphate ions from the SBF and incorporating minor ions, such as carbonate, sodium, and magnesium, to develop a bone-like apatite layer since the SBF is already highly supersaturated with respect to apatite. We can speculate that some chemical bonding such as Ti–O–Ca–O–Ti and/or Si–O–Ca–O–Si, would be the initial steps for calcium-phosphate build up onto the surface of such materials[33].

Therefore it is reasonable to speculate that the mechanisms by which titania nanotubes induce apatite formation was similar to mechanisms in the present study by which titania nanofibers induce apatite formation, where titania nanofibers havehigh aspect-ratio (higher surface area to volume ratio), which allows for the presence of plentiful OH groups on the surface before and/or after immersion in SBF that, in turn, affects the electrical charge of the host substrate and a higher degree of roughness that is associated with high aspect-ratio nanotubular structures.Our results convincingly indicated the ability of these titania additives to promote apatite formation andstrengthen the grain boundary and grain densification.

Conclusion

This study presents the preparation and investigation of characteristics of composite materials of hydroxyapatite and TiO_2 nanofibers. Hydroxyapatite was derived from natural bovine bone, while, TiO_2 nanofibers were prepared by electrospinning a mixture of TiO_2 sol and poly vinyl-pyrrolidone. Results reveal thatcomposite of hydroxyapatite/TiO_2nanofibers have goodbio mechanical properties. Moreover, to have a compact implants, the suggested sintering temperature is 1150 ^oC as the best densification, porosity, strength and microhardness can be achieved at this temperature. Overall, this study introduces hydroxyapatite/TiO_2 nanofibers as promised candidate as bone substitute for the biomedical application.

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