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Research Article

STUDY OF IN VITRO BIOACTIVITY AND CHARACTERIZATION OF HA-TIO2 BASED 45S5 BIOCOMPOSITES

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ABSTRACT

Bioactive glass (45S5), its lean mechanical strength limits and bioactivity in load bearing positions was reinforced by introducing titania (TiO_2) and HA. HA-TiO_2 based 45S5 biocomposites having general composition 45S5 (100-2X)+XHA+XTiO_2 (where X=0 to 20 wt%) were prepared by uniaxial pressing followed by sintering at 1000˚C. In order to determine its in vitro bioactivity these samples were exposed to SBF solution for different time intervals. The samples were evaluated by XRD, FTIR, SEM before and after soaking in SBF. FTIR analysis has shown the presence of apatite formation on the surface of the samples. Further bioactivity of biocomposites increases with increasing concentration of HA.

INTRODUCTION

Since the 1970s, various types of ceramic, glass and glass–ceramic materials have been proposed and used to replace damaged bone in many clinical applications. Among them, hydroxyapatite (HA) has been successfully employed for its excellent biocompatibility. On the other hand, the bioactivity of HA and its reactivity with bone can be improved by the addition of appropriate amounts of TiO_2 and bioactive glasses, thus obtaining BG- HA-TiO_2 based biocomposites. The loss of an organ or tissue due to cancer, disease or trauma is a critical problem in human health care. A challenging promising approach to address such issue is to create biological or hybrid replacement for implantation into the body, make use of the self-healing potential of the body itself, as proposed in the framework of the emerging tissue engineering [1-4]. Hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$) has the ability to form strong chemical bonds with natural bone because of its similar chemical and crystallographic structure like the apatite of living bone. But one of its primary restrictions in clinical use is load-bearing implants has poor mechanical property [5–7]. Two creative approaches for these mechanical limitations are use of bioactive ceramics as coatings and bioactive phase in composites [8].

Bioactive ceramics, such as bioglass and dense hydroxyapatite (HA), have been developed over the last two decades. Their accomplishments in the field of biomedical applications, especially in prosthetic applications now a days attracting [9].Calcium phosphate ceramics, especially HA, are currently used as biomaterials for many applications in both dentistry and orthopedics, because they form a real bond with the surrounding bone tissue when implanted. Nevertheless, due to the poor mechanical properties of bulk HA ceramics, such materials cannot be used as implant devices for load-bearing applications [10]. Artificial implants, such as the total hip replacement, are successful for a limited time, but all orthopedic implants lack three of the most critical characteristics of living tissues: (a) the ability to self-repair; (b) the ability to maintain a blood supply; and (c) the ability to modify their structure and properties in response to environmental factors such as mechanical load. A recent study showed that 24% of charnley hip operations required revision surgery [11]. It is well known that the incorporation of a ceramic reinforcement e.g.(fibres, whiskers, platelets or particles) in a ceramic matrix improves the mechanical properties. In return, compared with the monolithic matrix behavior, the presence of a reinforcement opposes the sintering process [12].

Therefore, the present work is concerned with the preparation and characterization of novel biocomposites containing bioactive glass (BG), HA and Titania. Also, these biocomposites were followed in simulated body fluid (SBF), FTIR and SEM techniques is used to verify the formation of a bone-like apatite layer on their surfaces by doing in vitro test.

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Experimental

Preparation of Samples

Preparation of Bioglass

Bioglass (45S5) with the chemical compositions as given in Table 1, were prepared from reagent grade chemicals were weighout and properly mixed and melted in 100 ml platinum crucible at 1400± 5°C with air as furnace atmosphere for 4 hours, melted glasses were poured in water to prepare frit and it was milled to a powder form in a porcelain ball mill for 24 h.

Production of HA

In the present study, calcium nitrate tetrahydrate ($Ca(NO_3)_2$ $4H_2O$) (CNT) (G.S. Chemical Testing Lab. & allied industries, India), phosphoric acid (H_3PO_4)(Loba Chemie Pvt. Ltd, India) and ammonia (NH_3) (Loba Chemie Pvt. Ltd, India)) were used as initial precursors. The schematic presentation of the procedure [13] is given in Fig. 1.

Preparation of BG/HA/TiO_2 composites

The HA and TiO_2 powder were mixed each in weight ratios 5,10,15 and 20% with bioglass (45S5) powder, compacted at 1500 MPa pressure into cylindrical samples (1 cm,1 cm) and were sintered at 1150°C to prepare the composites as shown in Table 1.

Table 1 Composition of Bioactive glass and Bio-composite (BGHATi1, BGHATi2, BGHATi3, BGHATi4)

Bio-composite Samples	Composition (wt %)		
	BG (45S5)	HA	TiO_2
BGHATi1	90	05	05
BGHATi2	80	10	10
BGHATi3	70	15	15
BGHATi4	60	20	20

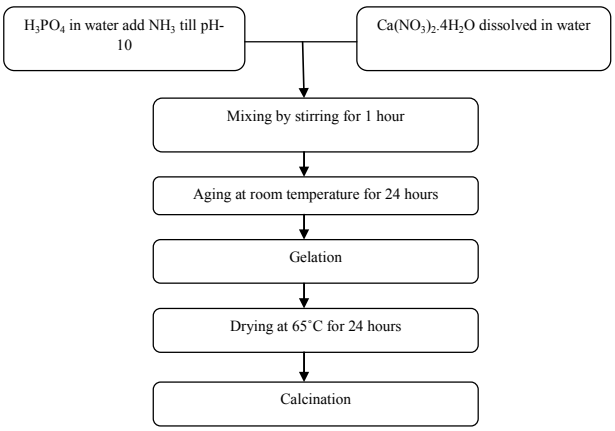


Fig. 1 Flow chart of hydroxyapatite preparation by the sol-gel route.

Preparation of SBF

Kokubo and his colleagues developed simulated body fluid that has an inorganic ion concentrations similar to those of human body fluid in order to reproduce in vitro formation of apatite on bioactive materials [14]. The SBF solution was prepared by dissolving reagent-grade NaCl, KCl, $NaHCO_3$, $MgCl_2.6H_2O$, $CaCl_2$ and KH_2PO_4 into double distilled water and it was buffered at pH=7.4 with TRIS (trishydroxymethyl amino methane) and 1N HCl at 37°C as compared to the human blood plasma (WBC). The ion concentrations of SBF are given in the Table 2[14].

Table 2 Ion concentration (mM/litre) of simulated body fluid and human blood plasma

	Na^+	K^+	Ca^{2+}	Mg^{2+}	HCO_3^-	Cl^-	HPO_4^{2-}	SO_4^{2-}
Simulated Body Fluid	142.0	5.0	2.5	1.5	4.2	148.0	1.0	0.5
Blood plasma	142.0	5.0	2.5	1.5	27.0	103.0	1.0	0.5

Powder X-ray diffraction (XRD) measurements

The biocomposite samples were ground to 75 microns and the fine powders were subjected to X-ray diffraction analysis (XRD) with RIGAKU-Miniflex II diffractometer adopted Cu-Kα radiation (λ = 1.5405A°) with a tube voltage of 40 kV and current of 35mA in a 2θ range between 20° and 80°. The step size and measuring speed was set to 0.02° and 1° per min respectively, in the present investigation. The JCPDS-International Centre for Diffraction Data Cards were used as a reference.

Structural analysis of bioactive glass by FTIR Reflectance spectrometry

The structures of biocomposite samples were measured at room temperature in the frequency range of 4000–400 cm^{-1} using a Fourier transform infrared spectrometer, (Bruker Tensor 27 FTIR,USA). The fine bioactive glass powder samples were mixed with KBr in the ratio of 1:100 and the mixtures were subjected to an evocable die at load of 10 bar pressure to produce clear homogeneous discs. The prepared discs were immediately subjected to IR spectrometer to measure the reflectance spectra in order to avoid moisture attack.

In vitro bioactivity study of bioactive glass composite

In order to evaluate the formation of (calcium phosphate) apatite layer on the surface of the samples after immersion in SBF solution, the samples (2 gm) were immersed in a 20ml of SBF solution in a small plastic container at 37°C having pH 7.40 in an incubator at static condition for a period of 1, 3, 7, 14 and 21 days. After soaking, the samples were filtered, rinsed by doubly distilled water, and dried in an oven at 100±3°C for 2 hours before analysis by FTIR.

pH measurement

The biocomposite powder (2 gm) was soaked in a 20 ml of SBF solution at 37°C for different time period and the pH was measured by using microprocessor based pH-EC meter (model-1611,ESICO-USA).

Density and Mechanical Properties Measurements

Archimedes principle was applied to obtain the density of biocomposite samples using distilled water as buoyant. All the weight measurements were taken using a digital balance (Sartorius, Model: BP221S, USA) having an accuracy of ± 0.0001 g. Density (ρ) of sample was obtained by employing the relation (1) as given below:

$$\rho = \frac{Wa}{Wa-Wb}\rho b \qquad \text{------- (1)}$$

where W_a is the weight of sample in air, W_b is the weight of sample in buoyant and ρb is the density of buoyant. Micro indentations were made on the polished surfaces of bioactive glass composite using a diamond Vickers indenter of a micro

hardness testing machine (Future - Tech Corp, Tokyo, Model FM - 7e, Japan).

The size of the specimen was 20 mm x 20 mm x 20 mm according to ASTM Standard: C730 - 98. The indentations have been made for loads ranging between 30 mN and 2000 mN, applied at a velocity of 1 mm/s and allowed to equilibrate for 15 seconds before measurement. Microhardness (H) (GPa) of specimen is calculated using the formula (2) as given below:

$$H_v = 1.854\frac{P}{D2} \quad \text{------ (2)}$$

Where H_v hardness value, P (N) applied load on specimen and D (m) is the diagonal of the impression.

RESULTS AND DISCUSSION

Characterization

Phase analysis

The prepared samples of bioglass/HA composites are BGHATi1, BGHATi2, BGHATi3 and BGHATi4 (all of the samples in Table 1) were characterized by XRD. X-ray powder diffraction data of the prepared biocomposites are shown in Fig. 1. The patterns of BGHATi1 composite having high % of BG content do not show any peaks for BG due to its amorphous nature and because the intensity of titania peaks is very weak denoting lower content of titania in the BGHATi1 composite.

These peaks are recorded at d (A°) = 3.24, 2.99 and 2.61 forming calcium titanium silicate [$CaTi(SiO_5)$] compound (Card No.: 73-2066) and proving interaction between TiO_2 and BG powders. Fig. 1 shows the increase of the intensity of calcium titanium silicate oxide peaks denoting more reaction found between the BG and TiO_2 [15] as well as the appearance of some peaks of titania (rutile form) at d (A°) = 2.49, 2.18, 2.06, 1.68 and 1.62 (Card No.: 04-0551) as a result of the conversion of anatase to rutile form that are not reacted with BG powder. In this domain, anatase transforms into rutile form at any temperature between 600 and 1000˚C [16]. For BGHATi3 composite, Fig. 1 shows the increase of intensity of $CaTi(SiO_5)/TiO_2$ peak at d (A°) = 3.25 with disappearance of the peaks at d (A°) = 2.99.

The peaks of rutile increases and became almost four times compared to those of BGHATi4 composite as a result of the highest titania content in the BG/titania composite proving the presence of part of titania powder which does not react with BG powder and converts completely to the rutile form.

This results show possible reactions during transformation at temperature from 1000 to 1200°C as follows [17]

$$Ca_{10}(PO_4)_6(OH)_2 + TiO_2 \longrightarrow 3Ca_3(PO_4)_2 + CaTiO_3 + H_2O \quad \text{---(3)}$$

$$Ca_{10}(PO_4)_6(OH)_2 + SiO_2 + TiO_2 \longrightarrow 3Ca_3(PO_4)_2 + CaTiSiO_3 + H_2O \quad \text{---(4)}$$

$$Ca_8(PO_4)_6(PO_2OH) + 3/2\ Na_2O \longrightarrow Ca_3(PO_4)_2 + Na_2Ca_6(PO_4)_5 + 1/2H_2O + O_2 \quad \text{---(5)}$$

$$TiO_2\ (anatase) \longrightarrow TiO_2\ (rutile) \quad \text{---(6)}$$

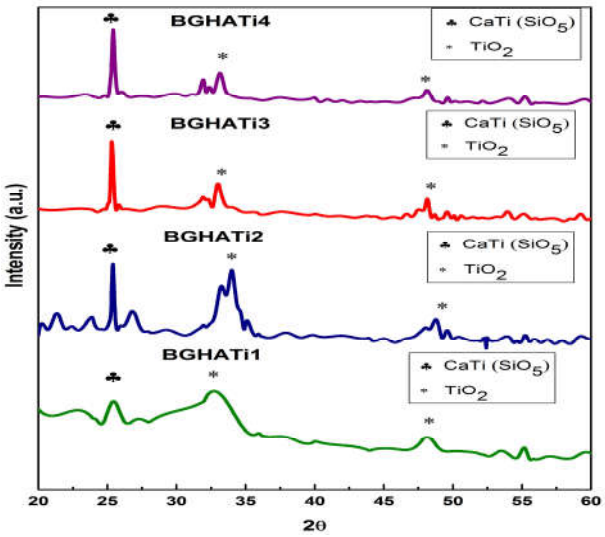
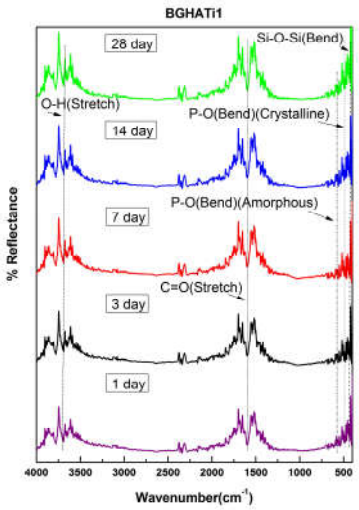


Fig. 2 X-ray diffraction of the prepared BG/HA/TiO_2 composites.

FTIR assessment

Fig. 3, 4 shows the FT-IR spectra of titania (TiO_2), bioactive glass (G), hydroxyapatite (HA), and their biocomposites (BGHATi1, BGHATi2, BGHATi3, and BGHATi4). The FTIR spectra of BGHATi1 composite having high % of BG showed the typical bands for BG structure with broadening in their bands. The band at 1090 cm^{-1} corresponds to the Si–O–Si asymmetric stretching mode, the band at 792 cm^{-1} is associated with a Si–O–Si symmetric stretching and the band at 476 cm^{-1} is assigned to the Si–O–Si symmetric bending mode and the shoulder at 950 cm^{-1} is related to the Si–O–Ca [18]. FTIR spectrum of BGHATi1 composite showed that the bands at 596 and 603 cm^{-1} are associated with the stretching vibration of phosphate groups [19]. The spectra of BGHATi2 and BGHATi3 composites have same behavior like BGHATi1 with broadening in the bands with some shift compared to original BG, HA, titania. The intensity of Ti–O–Ti/Si–O–Si and PO_4^{3-} bands increased gradually as a result of the increase of titania content proving interaction between BG, HA and titania forming $CaTi(SiO_5)$ compound as in BGHATi4 composite containing the highest content of titania. Fig. 3, 4 after soaking in SBF for different time periods is a part of hydroxyapatite in the composition of prepared composite, it was expected to find the hydroxyl group peaks on 3570 cm^{-1}.



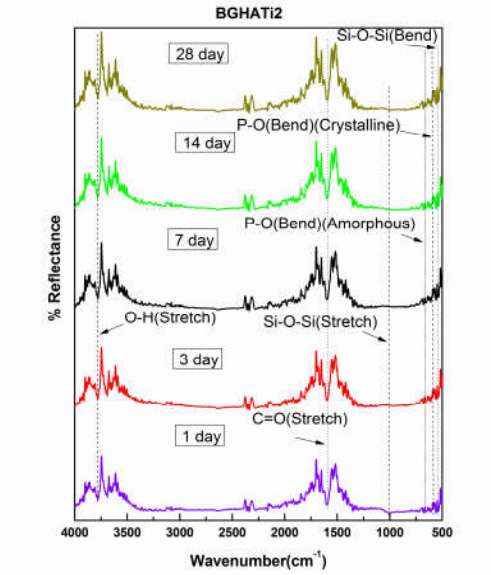


Fig. 3 The FTIR of BGHATi1, BGHATi2 composite post immersion in SBF after 1,3,7,14,28 days.

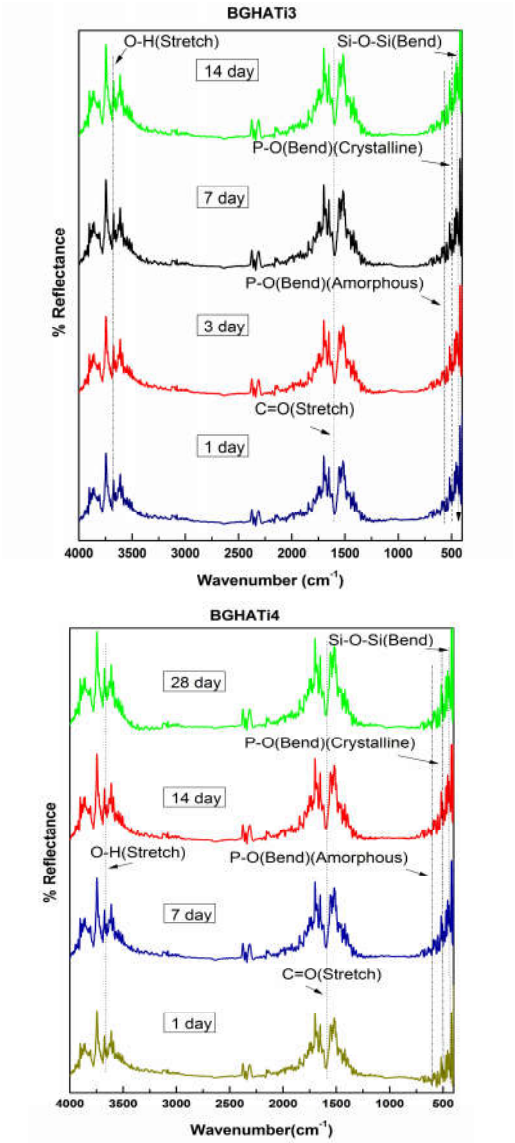
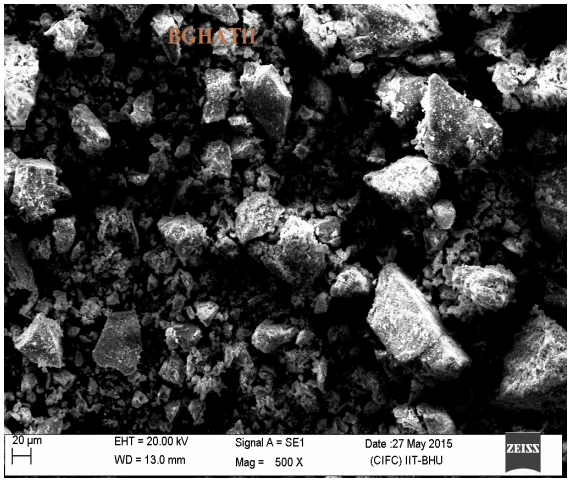


Fig. 4 The FTIR of BGHATi3, BGHATi4 composite post immersion in SBF after 1,3,7,14,28 days.

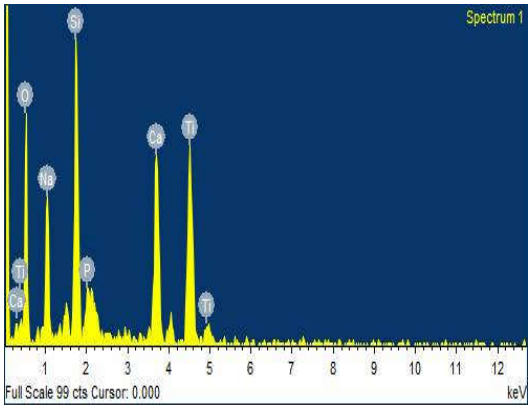
Surface morphology

Post-immersion in SBF the glass releases Ca^{2+} and Na^{+} ions from its surface via an exchange with the H_3O^{+} ion in the SBF to form Si–OH or Ti–OH groups on their surfaces [20]. Water molecules in the SBF simultaneously react with the Si–O–Si or Ti–O–Ti bond to form additional Si–OH or Ti–OH groups, the formed Si–OH and Ti–OH groups induce apatite nucleation, and the released Ca^{2+} and Na^{+} ions accelerate apatite nucleation by increasing the ionic activity product of apatite in the fluid [21]. As a result, the apatite layer forms onto the composite surface after soaking in SBF in a short period (3days) and this phenomenon is confirmed by SEM of BG/HA/titania composites post-immersion as shown in Fig.5(A-D) for BGHATi1 biocomposites, shows that this composite has many particles on its surface proving slight formation of apatite layer due to the composite contains high content of silica characterizing melted and dense structure that reduced nucleation of apatite layer compared to other composites. In this domain, the simultaneous dissolution of silicates results in the formation of silanol groups on material's surface, which are essential for nucleation sites resulting in HA formation [22]. Once the apatite nuclei formed, they can grow spontaneously by consuming the calcium phosphate ions in the surrounding fluid [23].

For BGHATi2,BGHATi3 and BGHATi4 composites, SEM at the same magnification indicates the presence of rich spherical shapes build up on each other to form a bone-like apatite layer for both composites especially BGHATi4 composites. This result is due to BGHATi4 composites contains high content of titania which leads to increase of Ti–OH groups at the expense of Si–OH groups resulting in high nucleation of apatite (Fig. 5D). In this study, we noted that the rutile form of titania is the main phase in four composites and is essential for improvement of apatite nucleation especially BGHATi3 and BGHATi4 composites compared to BGHATi1 composites containing low content of titania. The catalytic effect of the Si–OH groups and Ti–OH groups for the apatite nucleation has proven by the observation that silica and titanium will form apatite on their surfaces in SBF and are abundant on the composite surfaces [22, 24].



A



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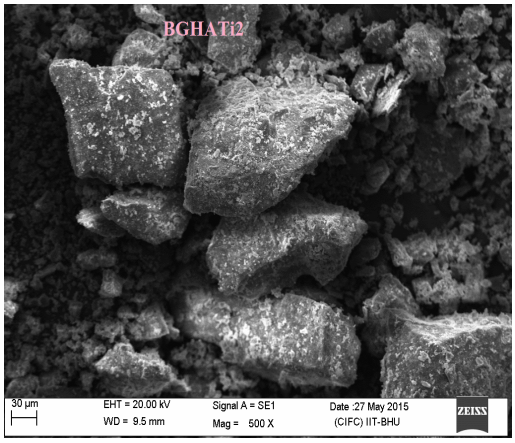

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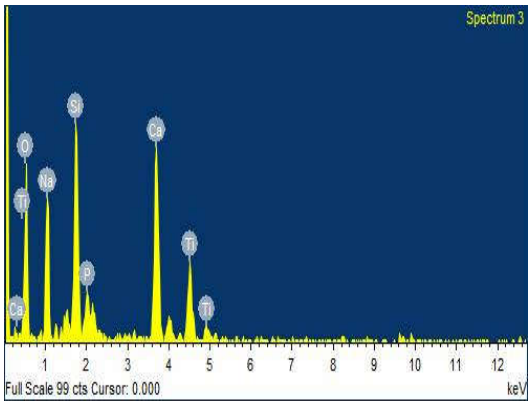

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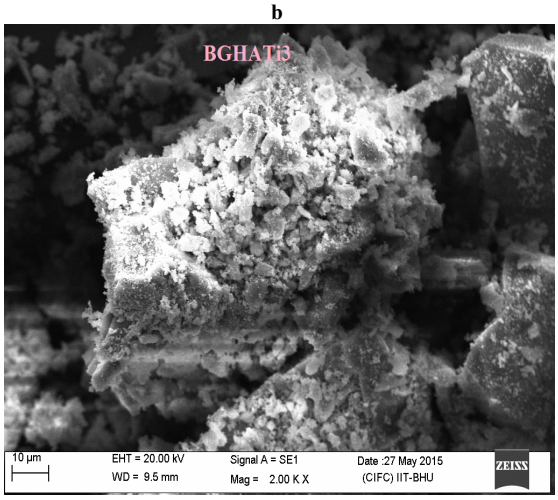

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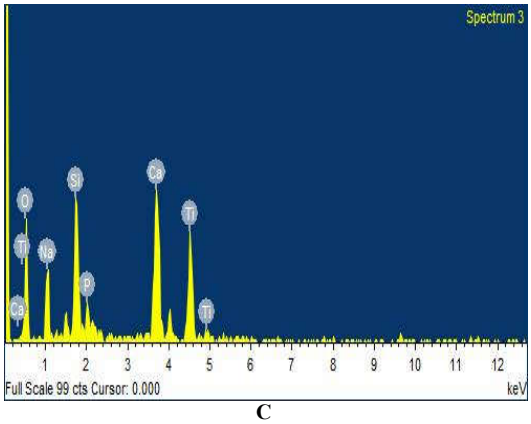

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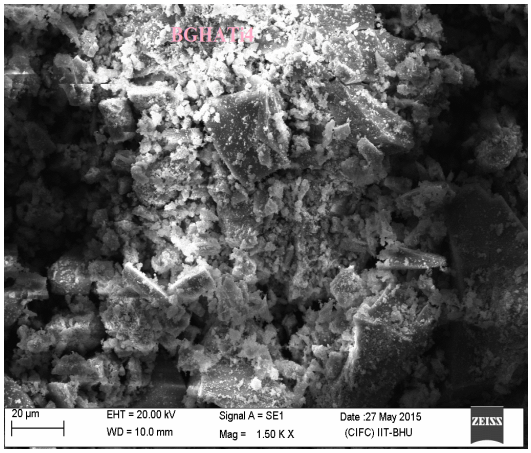

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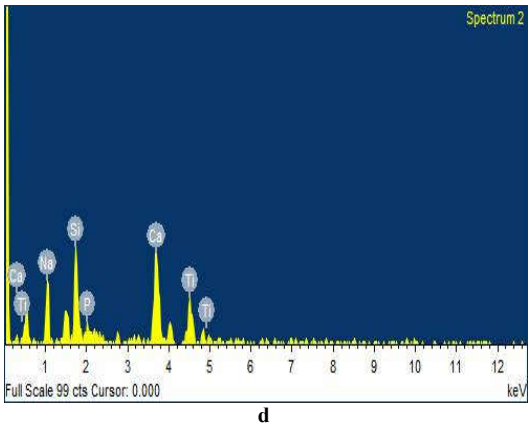

d

Fig.5 SEM (A-D) and EDAX (a-d) of the BG/HA/TiO_2 composites for BGHATi1, BGHATi2, BGHATi3 and BGHATi4 composites post-immersion in SBF

EDAX point analyses that Ca, P, and Ti coexist in different properties of sintered pellet as shown in Fig. 5 (a-d) conferming the interfusion between HA and TiO_2 particles before their impinging into the substrate.

pH behavior in SBF

The variation in pH values of simulated body fluid (SBF) after soaking of biocomposite for various time periods is shown in Fig. 6. It was observed that the pH of all samples showed the similar tendency of behavior [25]. The maximum pH values was recorded on 1 day of immersion. The change of pH of SBF solution can be explained by ion exchange process on the glass surface. Cations such as Na^+ or Ca^{2+} near the glass surface can go into solution in exchange for H^+ or H_3O^+ ions from the solutions which results in a pH increase, after certain point

decrease in pH can be explained by considering the precipitation of calcium phosphates and carbonates. The update of carbonate and phosphate ions shifts the equilibriums towards the products side, thus causing a decrease in the pH [26]. It was observed that addition of HA in base bioactive glass (45S5), sequence of reactions occurred in SBF after immersion of biocomposite for various time periods are in favour of formation of hydroxyapatite layer on the surface of the samples[27][28].

$$HCO_3^{3-} \longrightarrow CO_3^{2-} + H^+ \text{ and } HPO_4^{-2} \longrightarrow PO_4^{-3} + H^+ \quad \text{---------(7)}$$

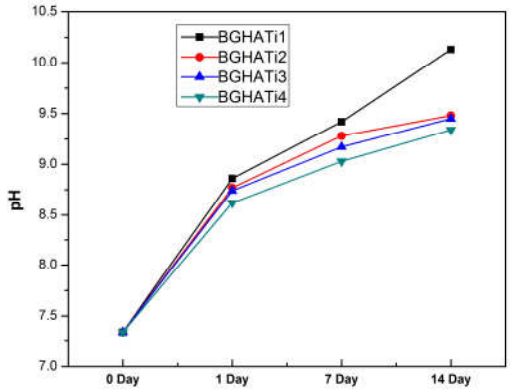

Fig. 6 pH of different biocomposites (BGHATi1, BGHATi2, BGHATi3,BGHATi4).

Mechanical testing

The density increased rapidly when the pellet samples were sintered at 1200˚C and 1300˚C due to the partial HA decomposition into α-TCP and TTCP. Density of biocomposite sintered at 1200˚C increased with increasing HA and TiO_2 content as shown in Fig.7 because density of titanium is more than 45S5 bioglass and HA.

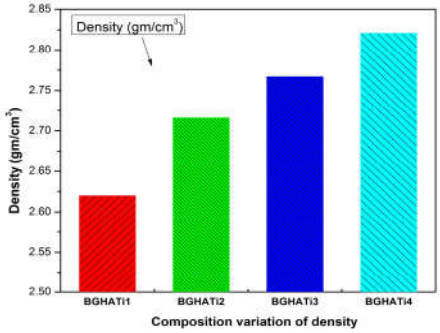

Fig.7 Variations of density in different HA and titania reinforced with bioglass composites.

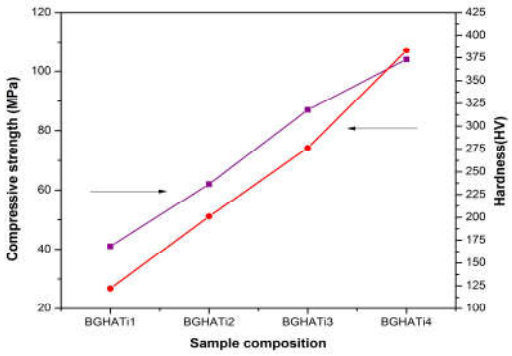

Fig.8 Variation of compressive strength depending on reinforcement content of pellet samples.

Variation of compressive strength depending on reinforcement content compostion for both sintering temperature is given in Fig.8. The figure shows that increasing reinforcement content from 5, 10, 15 and 20 wt.% increased the compressive strength from 41 to 104 MPa for sintering at 1150˚C. Such a phenomenon can be attributed to the occurrence of a new phase among bioglass, HA and TiO_2 for higher sintering temperatures.

CONCLUSION

In the present investigation, a comparative study was made on physical, bioactive and mechanical properties of biocomposite. The following conclusions are obtained from this analysis:

Composite reactions between Ti and hydroxyapatite occurred during the sintering of Ti/HA/BG composites. The phase components of the composite varied with the initial TiO_2 content and the sintering temperature. After sintered at 1200˚C, the composite with a lower TiO_2 content had main crystalline phases of $CaTiO_3$, CaO and TixPy. Increasing the initial TiO_2 content to 50 vol%, Ti_2O and residual α-Ti also observed. Additional increase of the initial TiO_2 content resulted in a composite with only α -Ti as its main crystalline phase. Moreover, the addition of Ti/HA to the bioactive glass showed more effect on the main phase components of the composites.

The phase and FTIR analyses confirmed the interaction between BG, HA and titania (anatase) forming calcium titanium silicate and rutile compounds. The data proved that the increase of TiO_2 content into the composite improved the mechanical properties as in BGHATi4 composite having the highest content of titania powder. It is noted that BGHATi4 composite has compressive strength (104 MPa) comparable to that of the cortical bone that is located in the range of 100–230 MPa.

Bioactivity results indicated that BGHATi3 and BGHATi4 composites including high content of TiO_2 resulting in the abundance of Ti–OH groups on the composite surface had an enhanced capability to form the bonelike apatite layer compared to BGHATi1 composite having high content of BG characterizing dense structure. Additionally, the formed rutile titania in these biocomposites had the capability to induce the formation of carbonated apatite layer comparable to anatase form. Thus, these biocomposite materials are gifted for medical applications such as bone substitutes especially in load-bearing.

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References

1. R.M. Nerem, Ann. Biomed. Eng. 19 (1991) 529–545.
2. L.G. Griffith, G. Naughton, Science 295 (2002) 1009–1014.
3. R. Lanza, R. Langer, J. Vacanti, Principles of Tissue Engineering, third ed. Academic Press, San Diego, CA, USA, 2007.
4. R. Langer, J.P. Vacanti, Science 260 (1993) 920–926.
5. M. JARCHO, C. H. BOLEN, M.B. THOMAS, J. BOBICK, J. F. KAY and R. H. DOREMUS, *J. Mater. Sci.* **11** (1976) 2027.

6. K. DE GROOT, *Biomater.* **1** (1980) 47.
7. G. DE WITT, H. VAN DIJK, N. HATTU and K. PAIJS,*J. Mater. Sci.* **16** (1981) 1592.
8. L. L. HENCH, *Bioceramics* **81** (1998) 1705.
9. D.-M. Liu, H.-M. Chou, Formation of a new bioactive glassceramic, *Journal of Material Science: Materials in Medicine* 5 (1994) 7–10.
10. F.J. Garcı´a-Sanz, M.B. Mayor, J.L. Arias, J. Pou, B. Leo´ n, M. Pe´rez-Amor, Hydroxyapatite coatings: a comparative study between plasma-spray and pulsed laser deposition techniques, *Journal of Material Science: Materials in Medicine* 8 (1997) 861–865.
11. D.J. Berry, W.D. Harmsen, M.E. Cabanela, M.F. Morrey, J. Bone Joint Surg. (Am) 84A (2) (2002) 171–177.
12. H.M. Kim, F. Miyaji, T. Kokubo, M. Kobayashi, T. Nakamura, Bull. Chem. Soc. Jpn. 69 (1996) 2387–2394.
13. K P Santosh, Min-Cheol Chu, A Balakrishnan, T N Kim and Seong-Jai Cho. Preparation and characterization of nano-hydroxyapatite powder using sol-gel technique. Bull Mater. Sci. October2009; Vol.32 No.5:465-470.
14. Kokubo T, Takadama H, Biomaterials 2006; 27:2907–15.
15. C.C. Wang, J.Y. Ying, Chem. Mater. 11 (11) (1999) 3113–3120.
16. H.M. Kim, F. Miyaji, T. Kokubo, M. Kobayashi, T. Nakamura, Bull. Chem. Soc. Jpn. 69 (1996) 2387–2394.
17. B.N. Çetiner, Z.E. Erkmen, Production of Hydroxylapatite Based Titania Biocomposite as Porous Bone-Like Scaffold, Archives of material science and engineering 65(2014) No.2:066-71.
18. M.M. Perira, A.E.C. Hench, Calcium phosphate formation on sol–gel galsses in vitro., Studies Inorganic Chemistry, vol. 18, Elsevier, Amsterdam,1994, p. 59.
19. J.C. Elliott, Structure and chemistry of the apatite and other calcium orthophosphate., Studies Inorganic Chemistry, vol. 18, Elsevier, Amsterdam,1994, p. 59.
20. L.L. Hench, The discovery of bioactive glasses, in: Science Faith and Ethics, Imperial College Press, London, 2001.
21. T. Kokubo, H. Kim, M. Kawashita, Biomaterials 24 (2003) 2161–2175.
22. K.H. Karlsson, Glass Technol. 45 (2004) 157–161.
23. Y. Ebisawa, T. Kokubo, K. Ohura, T. Yamamuro, J. Mater. Sci. Mater. Med. 1 (1993)239- 244.
24. P. Li, C. Ohtsuki, T. Kokubo, K. Nakanishi, N. Soga, T. Nakamura, K.A. de Groot, J. Biomed. Mater. Res. 28 (1994) 7–15.
25. Marta Cerrutia, David Greenspanb, Kevin Powers, Biomaterials 26 (2005) 1665–1674.
26. Delia S. Brauer, Natalia Karpukhina, Matthew D. O'Donnell, Robert V. Law, Robert G. Hill, Fluoride-containing bioactive glasses: Effect of glass design and structure on degradation, pH and apatite formation in simulated body fluid. Acta Biomaterialia 6 (2010) 3275-3282.
27. V.R. Mastelaro, E.D. Zanotto N.Lequeux, RCortes, J.Non-Cryst. Solids 262 (2000) 191–199.
28. P. Ducheyne, Q. Qiu, Biomaterials 20 (1999) 2287 – 2303.

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