Characterization,Mechanical, and In Vitro Bioactivity Properties of Hydroxyapatite/Bioactive Glass Composite

Israa Kahatan Sabree **Ola Salih Mahdi**
University of Babylon ,College of Materials Engineering
israaks@yahoo.com engoulasaleh.alsaffar@yahoo.com

Abstract

Bioactive ceramic materials can help bone reparation and regeneration by offering support to bone growth. Biological hydroxyapatite powder was prepared by burning animal bone followed by studying the mechanical properties of hydroxyapatite (HA)/ (20wt.%, and 40wt.%) of binary bioactive glass (70% SiO_2- 30% CaO) in order to evaluate the influence of composition on the compressive strength and hardness. HA-composite material exhibited increasing density, microhardness, and compressive strength with increasing amount of glass addition. X-ray diffraction after sintering at 1200°C showed no alter of HA to secondary phases while the hydroxyapatite/ bioactive glass composites contained a HA phase and different amounts of wollastonite phase, depending on the amount of bioglass added.

In vitro tests, the samples were soaked in simulated body fluid (SBF) for ten days in order to evaluate the change in compression strength, weight loss, and pH. The HA composite reinforced with 40 wt % bioglass showed highest compression strength, and lowest weight loss.

Key wards: Bioactive ceramic, Hydroxyapatite, composite bioceramic, bone regeneration

الخلاصة

تساعد مواد السيراميك الحياتية في ترميم واعادة بناء العظم بكونها تدعم نموالعظم. تم تحضيرمسحوق مادة الهيدروكسي ابتايت من عملية حرق عظام الحيوانات واستخدمت كمادة اساس لتحضيرمادة حياتية مركبة تتكون من الهيدروكسي ابتايت/الزجاج النشط بيولوجيا بنسبة (٢٠%و٤٠%) وذلك لتقييم تاثيراضافة الزجاج الحياتي على مقاومة الانضغاط والصلادة ومقارنتها مع مثيلاتها لمادة الهيدروكسي ابتايت.

اظهرت اختبارات الاشعة السينية ان عملية التلبيد بدرجة ١٢٠٠°م لاتسبب تحلل لطور الهيدروكسي ابتايت في حين اظهرت وجود طور wollastonite في المادة المركبة بكميات تختلف باختلاف نسبة الزجاج المضافة رافقها زيادة في الكثافة والصلادة ومقاومة الانضغاط للمادة المركبة .اجريت الاختبارات البايولوجية بتحضير SBF ثم اجراء عملية الغمر لمدة عشرة ايام تم خلالها قياس pH يوميا للوسط وقياس التغير بالوزن ومقاومة الانضغاط في نهاية فترة الغمر ثم اجراء الاشعة السينية للنماذج الثلاث حيث وجد ان العينات الحاوية على ٤٠% من الزجاج النشط بيولوجيا تمتلك اعلى مقاومة انضغاط واقل فقدان بالوزن.

الكلمات المفتاحية: السيراميك الحياتي، الهيدروكسي ابتايت، سيراميك حياتي مركب، تولد النسيج العظمي.

1. Introduction

Bone defects that are generated by tumor resection, trauma, and congenital abnormality have been clinically treated by the implantation of bioceramics or autogenous and allogenous bone grafts. Although autografting is the gold standard procedure for reconstructive surgery, it has several disadvantages, such as the limitation of donor supply, chronic residual pain, the nerve damage, and cosmetic disability at the donor site. On the other hand, there are no donor site problems for allografting, while all ografting have some clinical risks including disease transmission and immune-rejection and inflammation [Fu *et. al*., 2013; Jones *et. al*., 2010]. HA has been incorporated into a wide variety of biological applications including dental implants, coatings on Ti substrates, bioactive scaffolds, and other types of orthopedic implants. HA has similar composition and structure to the inorganic phase of bone therefore, it has been considered to be the ideal material to build bone tissue engineering scaffold due to its osteoconductivity and osteoinductivity [Dorozhkin,2010;Kokubo, 1998].However, poor mechanical

properties mean that hydroxyapatite cannot be used in bulk form for load bearing applications such as orthopedics.

Bioglasses (BG) are a bioactive material which can facilitate direct interfacial bond to soft and hard tissue by forming a carbonated hydroxyapatite layer (HCA) when exposed to biological fluid [Kokubo, 1998]. It was found that dissolution ions released from bioactive glass in biological fluid activate gene expression and enhance osteogenesis process resulting higher rate of bone formation comparing with hydroxyapatite [Xynos *et. al*., 2000; Patel *et. al*., 2002].

Many attempts have been made to appropriate mechanical performance for specific applications or implant configurations, by formation of HA composites reinforced with metals, organic, and inorganic biomaterials. Sintering HA reinforced with bioactive glass has been interested in order to improve the mechanical strength by densification through sintering, and to enhance bioactivity for resulting composite materials through the assembly of two bioactive phases [Kangasniem, 1993; Chern *et. al*., 1993]. Previous researchers [Knowles *et. al*., 1993; Santos *et. al*., 1994; Santos *et. al*., 1996] reported a significant increase in flexural strength and fracture toughness of composite HA/glass comparing with HA, and better biological activities than HA [Afonso *et. al*.,1996]. However, the resulting structure and mechanical properties of HA reinforced glass depend on the glass composition and the addition rate of glass [Knowles, 1993].In present work, the effects of different amount of binary bioactive glass(70% SiO_2- 30% CaO) adding to biological hydroxyapatite on mechanical properties and biocompatibility were investigated for such composite material in order to determine optimum bioglass addition for specific application.

2. Materials and Methods

2.1 Synthesis of Materials

Hydroxyapatite used in this study was prepared from calcinated cow bones. Firstly,a clean cow bone was cut into small pieces and treated with acetone $(CH_3)_2CO$ in a beaker to remove organics until the color of bone turn into white. After neutralized with distilled water the bones were dried in sun rays for 48 hours and heat treated at 900^{o}C for 3 hours to completely remove organics part. Proteins free HA bone were then ground into fine powder by using planetary ball milling for several hour.

2.2 Preparation of Hydroxyapatite-Bioglass Biocomposite

To produce the hydroxyapatite-bioglass biocomposites, sol-gel bioactive glass powder with a composition of 70% SiO_2-30% CaO (Imperial College, London) has been added in the rate of (20 wt.%, and 40 wt.%.) to hydroxyapatite powder (base material). The batch materials were mixed at room temperature by electric mixer for 15 hours, using alumina container. After that, cylindrical tablets were produced by uniaxial pressing of powders (3.2 g) into steel die of 13 mm in diameter and 26 mm in height. 2% of PVA was added as a binder in an uniaxial pressing device (CT340-CT440) at pressure of 200 MPa for a 2 min dwell time. Drying is carried out before firing process, all compacted samples were dried using electric blast dry box (WG43) at 100 $^{\circ}$C for 24 hours to remove moisture. The sintering procedure as shown in figure 1 using protherm electric furnace (PLF 160/15, made in Turkey).

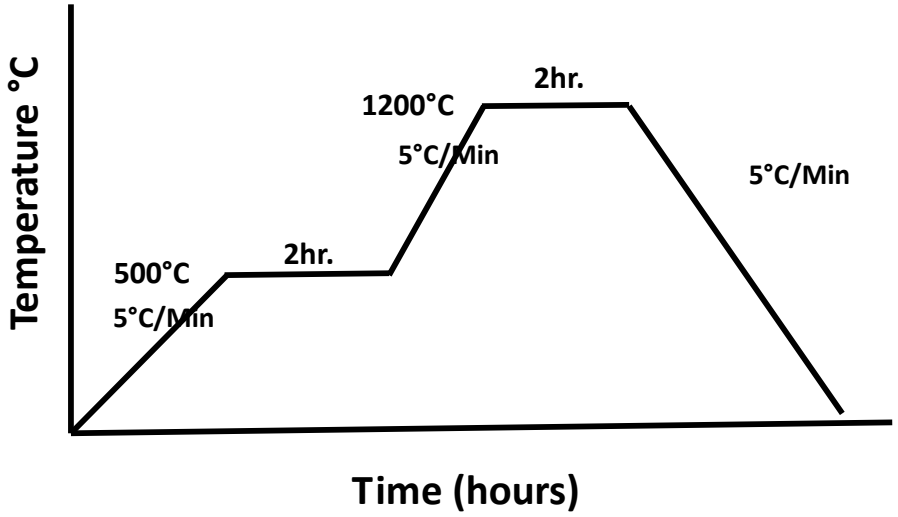

Figure 1.The sintering procedure

2.3 Samples Characterization

2.3.1 X-ray Characterization.

XRD analysis was carried out to identify the phases using x-ray diffractometer [Shimadzo, 6000] at room temperature with Cukα radiation (λ = 1.5405 Å), and a scanning speed of 5°/min from 10° to 60° of 2Θ (Bragg angle) and 40 Kv/30 mA an applied power.

2.3.2 Physical and Mechanical Characterization

Apparent Porosity for the specimens were tested according to ASTM (373-88) by Archimedes method. Dry weight (D) to the nearest 0.01g was recorded. samples were placed in a glass beaker contained distilled water and boiled for 5 hours, then soaked for an additional 24 hours to record the suspended weight (S).After that cotton cloth was used to remove the excess water from the surface, and determined the saturated weight (M)[Meyers *et. al*.,2009] . The apparent porosity was calculated as follow:

$$P = [(M - D)/ V] \times 100 \ldots\ldots (1)$$

Where V is the exterior volume, which calculated as shown below:

$$V = M - S \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Bulk density (B), in grams per cubic centimeter of a specimen is the quotient of its dry weight divided by the exterior volume; including pores according to ASTM (373-88) using digital display hydrostatic dynamics balance (DSJ-5) and calculated as follow :

$$B = D/V \ldots\ldots\ldots\ldots\ldots\ldots (3)$$

Compression strength for samples were tested using computerized universal testing machine with a test speed of 0.5 mm/min, the test was made according to ASTM standard C-773-88. The compression strength was calculated using the following equation.

$$(\sigma c) = P_f / A_0 \ldots\ldots\ldots\ldots\ldots\ldots (4)$$

Where σc , is the compression strength (MPa), p_f, fracture load (N),and A_0 is a cross section area of cylinder sample (mm^2).

Hardness usually measured on microhardness machines with Vickers diamond indenters. The test was made according to ASTM standard C1327-90, using digital microvickers hardness tester (TH-717) at 9.8 N with a dwelling time of 15 seconds. Vickers hardness was calculated using the following equation.

$$H_v=1.854(p/d^2) \quad \text{..... (5)}$$

Where, H_v, is the Vickers hardness (MPa), p, load (N), D, average diagonal length of the indentation impression (μm) [Roy *et. al*., 2010].

For all mechanical and physical tests, five samples were selected to obtain an average value.

2.3.3 Biological Characterization

Simulated Body Fluid (SBF) was prepared by the procedure reported elsewhere by dissolving NaCl, $NaHCO_3$, KCl, $K_2HPO_4.3H_2O$, $MgCl_2.6H_2O$, $CaCl_2$, Na_2SO_4, $(CH_2OH)(NH_2)$ in deionized water, the HCl solution was added to adjust the pH 7.0 [Liu *et. al*., 2004]. The prepared simulated body fluid has the ionic concentration similar to human blood plasma. In vitro bioactivity of composite was investigated by immersing the samples in SBF at 37 ºC with replacing SBF every 4 days to provide constant chemical composition of solution. After 10 days the samples were removed from SBF, washed by deionized water, and dried at 80 ºC for 15 minutes[Encinas-Romero, 2008].Samples analyzed by XRD to identify the formation of hydroxyapatite layer on the surface. Maximum compression strength values were measured for all ceramics samples which immersed in SBF for10 days in order to evaluate the mechanical behavior of Hydroxyapatite/ bioglass composite material in biological solution. The pH measurements for SBF were carried out every day using a Mettler Toledo pH-meter with combination polymer electrode. Prior to each measurement, the pH-meter was calibrated with the standard buffer solutions pH = 7.4. The weight loss of compact samples after 10 days soaking in SBF solution were recorded using analytical electric balance (M254A) with weighing sensitivity of about ± 0.0001 g .

3. Results and Discussion

3.1 X-ray Diffraction

Figure 2 presents the XRD analysis for raw cow bone after firing at 900°C for 3 hours. The result showed sharp peaks with high intensity of crystalline pattern after firing. All the XRD peaks match with the standard ICDD file no.(09-0432) of pure HA and no impurity other than HA was detected. This result indicates that all organic substance completely removed. [Sri Asliza, 2009].From the XRD spectrum of the Hydroxyapatite/bioglass composite sintered at 1200 °C reported in figure 3, it can be seen that the samples were composed only of pseudo-wollastonite (α- $CaSiO_3$), and hydroxyapatite. All these phases are highly biocompatible in human physiological environment[Encinas-Romero *et. al*.,2013].

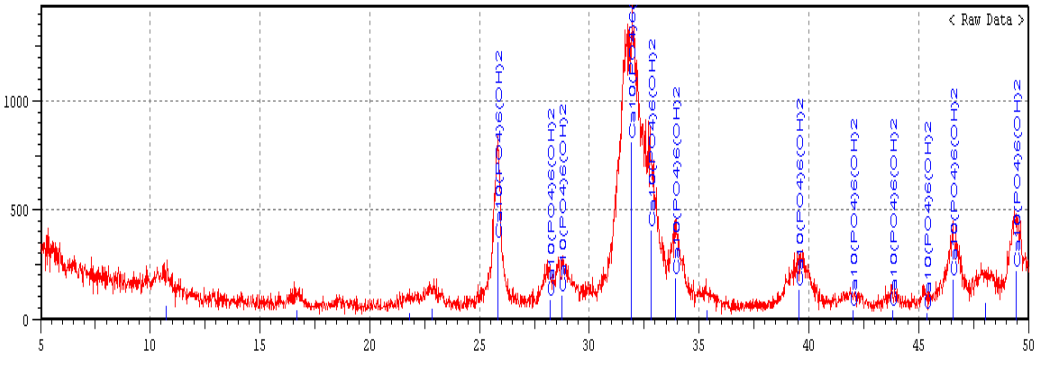

Figure 2. XRD analysis for raw cow bone after firing at 900°C.

3.2 Density and Porosity

Figure 4 shows the relation between the compact samples with different weight percent of bioglass and porosity. It is evident that the increasing of bioglass contents in the compact sample cause decrement in porosity value of the compact sample. It will be interesting to know that the liquid phase formed due to the additions of bioglass fill more pores of hydroxyapatite, the maximum porosity value for hydroxyapatite was(46.41%),while for sample contain 20%bioglass was(44.41%),and for sample contain 40%bioglass was (42.83%). Figure 5 shows the relation between the compact samples with different weight percent of bioglass and bulk density. It is evident that the increasing of bioglass content in the compact sample will cause increase in bulk density value of the compact sample. It will be interesting to know that the density varies as a function of porosity in these samples in which higher porosity showed a lower amount of density. The results shown in table 1.

Table 1 Summary of the physical properties for composite samples.

	HA%	BG%	Density(g/cm^3)	Porosity%
Sample 1	100	0	3.6	45.5
Sample2	80	20	3.9	43.2
Sample3	60	40	4.5	42.2

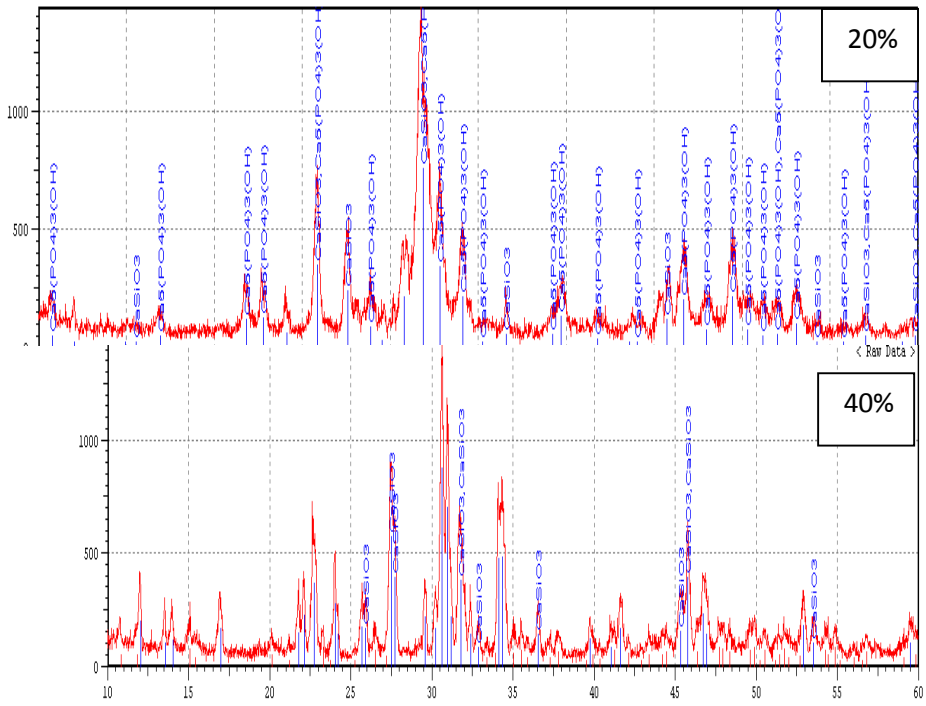

Figure 3. XRD analysis for the HA/bioglass composite sintered at 1200 ^{o}C.

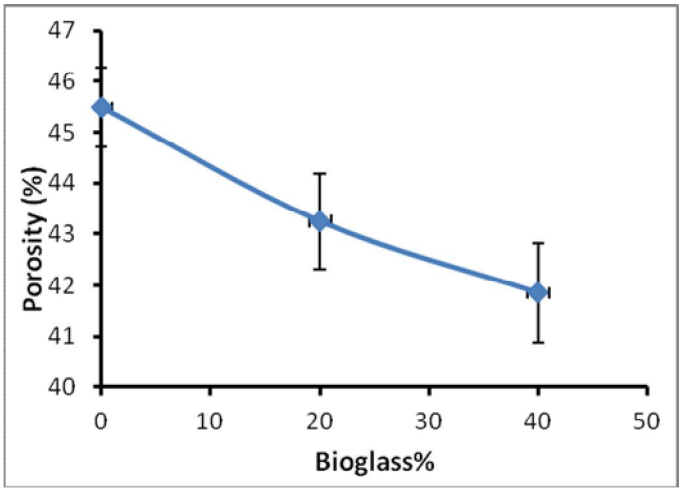

Figure 4. Variation of apparent porosity of the composite samples with bioglass percentage.

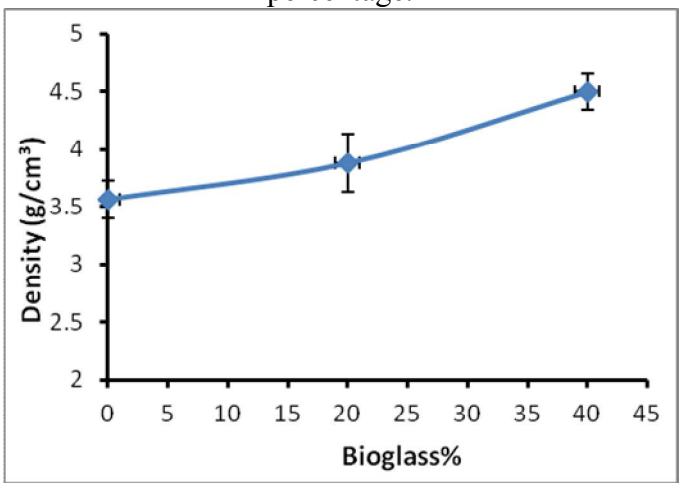

Figure 5. Variation of bulk density of the composite samples with bioglass percentage.

3.2 Compression Strength and Vickers Hardness

Figures 6,and 7 show the relation between the compact samples with different weight percent of bioglass and compression strength, and Vickers hardness. A trend in the increase in hardness and compression strength is observed as the bioglass content in the samples increases. This is a clear indication of how the addition of bioglass to the composites enhances their robustness[Teixeira,2007].The results shown in table 2.

Table 2 Summary of the mechanical properties for composite samples.

	HA%	BG%	compression strength(MPa)	Vickers hardness(MPa)
Sample 1	100	0	33.6	3.5
Sample2	80	20	36.6	4.2
Sample3	60	40	38.0	5.3

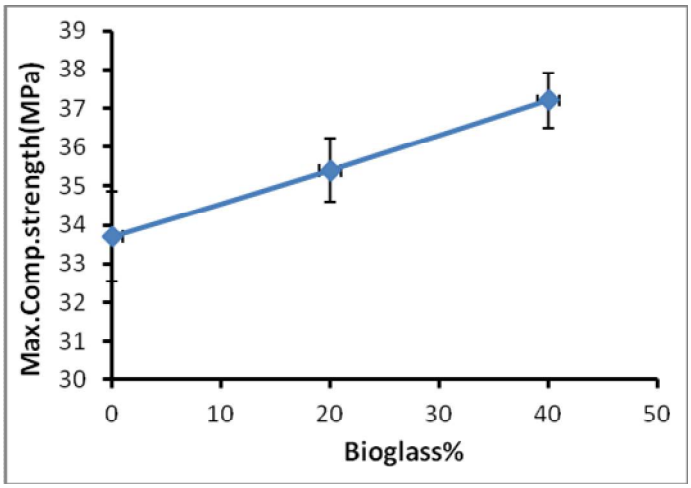

Figure 6. Variation of compression strength of composite samples with bioglass percentage.

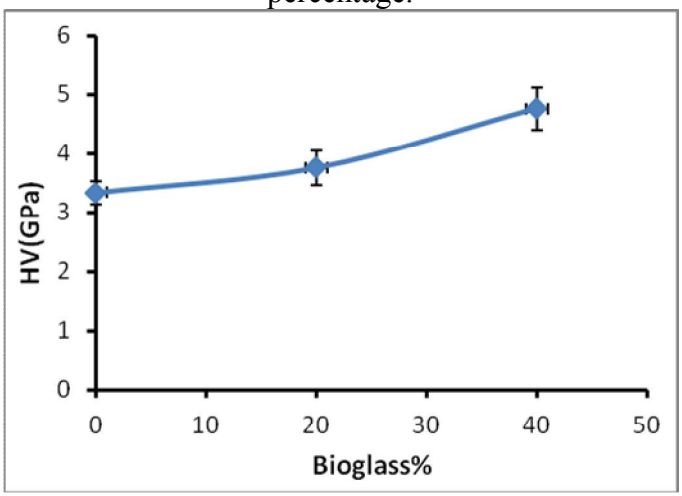

Figure 7. Variation of Vickers hardness of composite samples with bioglass percentage.

3.3 Bioactivity Assessment of Composite in Simulated Body Fluid (SBF)

3.3.1 XRD of Composite Samples after immersing in SBF Solution.

After 10 days soaking in SBF, the phase composition of the sample surface was examined by the XRD in order to check the deposition of hydroxyapatite. The XRD pattern Figure 8. disclosed that the crystalline apatite peak can be observed in both samples surface indicating that the Ca-P-rich layer formed on the surface [Alexandru Ro ,2011]. All peaks belong to hydroxyapatite formation on the surface of composite material at the end of 10 days of immersion in SBF.

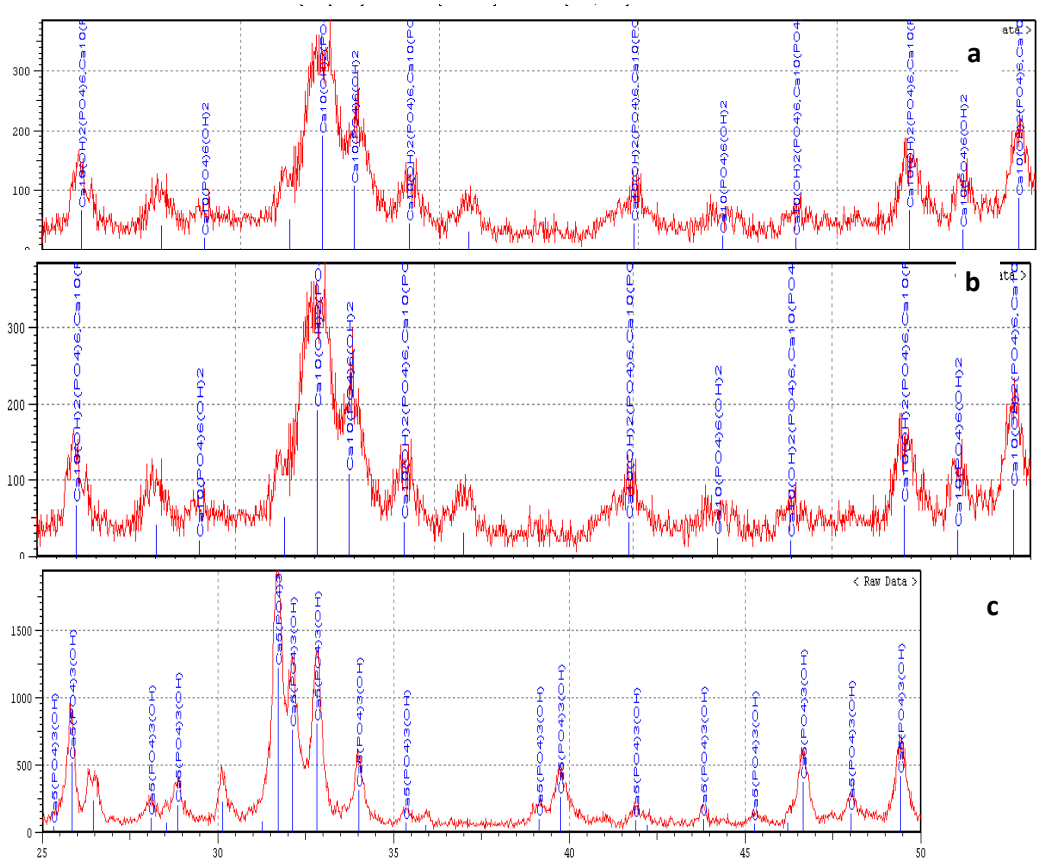

Figure 8. XRD analysis for the HA/bioglass composite surface after 10 days of immersion in SBF a- 100%HA ,b-20% bioglass,c- 40% bioglass .

3.3.2 pH of the Solution

PH of the solution was recorded as shown in figure 9. The data were collected for 10 days .The starting pH value of the solution was 7.6 for each composite samples.Up to 4 days, the pH values for all the samples fluctuate in between 8.5 for pure HA,8.8 for 20% bioglass, and, 8.7 for 40% bioglass. The surface reactions of the prepared ceramics in the SBF medium are believed to cause an increase in the concentration of cations and media alkalization leading to an increase in pH due to exchanges between Ca^{2+} and H^{+} in the medium [S. K. Padmanabhan,2012]. The pH values then stabilize until 8 days. After 8 days, sharp decrease in pH value of the solution irrespective of samples is noticed. The pH values then stabilize and the values fluctuate in between 7.2 for pure HA,7.4 for 20% bioglass, and, 7.5 for 40% bioglass till 10 days due to precipitation of apatite into the surface of the samples occurs by consuming the Ca^{2+}, P^{+}, and OH^{-} ions in the SBF solution[De Aza *et. al*.,2007].

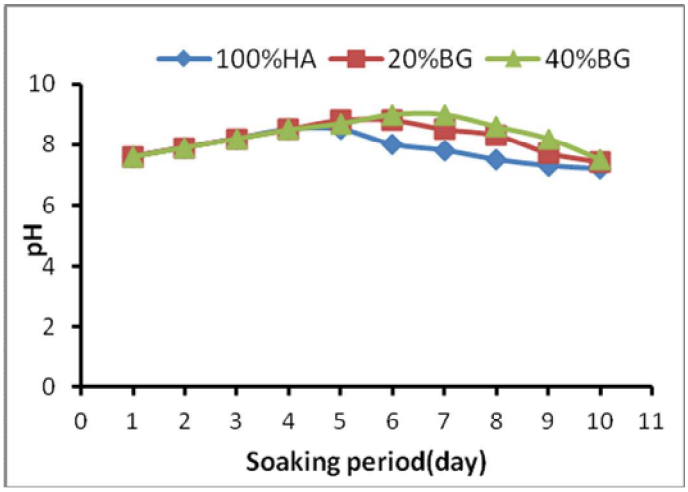

Figure 9. Variation of pH media value for composite samples with soaking time .

3.3.3 Weight Loss for Composite Samples after Soaking

Figure 10 shows the relation between the compact samples with different weight percent of bioglass and weight loss after 10 days soaking in SBF.A trend in the increase in weight loss is observed as the bioglass content in the samples increases with respect to pure HA. The high degradation rate of composite samples is due to the release of Ca and Si ions to the solution. This shows that the composite samples were more biodegradable with respect to pure HA scaffold. This difference in bioactivity and biodegradation attributed to the differences in the microstructure, surface area, and the phases present in the materials [Siriphannon *et. al*., 2002].

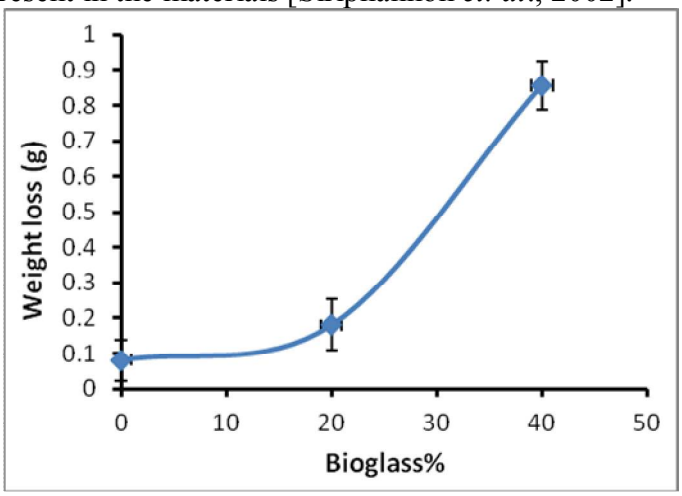

Figure 10.Variation of weight loss of composite samples with bioglass percentage after soaking in SBF .

3.3.4 Compression Strength after 10 Days Soaking in SBF

Figure 11 shows the relation between the compact samples with different weight percent of bioglass and compression strength after 10 days soaking in SBF. A trend in the increase in compression strength is observed as the bioglass content increases. The increase in compressive strength of composite samples was mostly due to the reinforcement of bioglass acicular crystals into the hydroxyapatite matrix.[Jin ,2011] has already reported same kind of reinforcement mechanism in reinforced calcium phosphate pours materials.

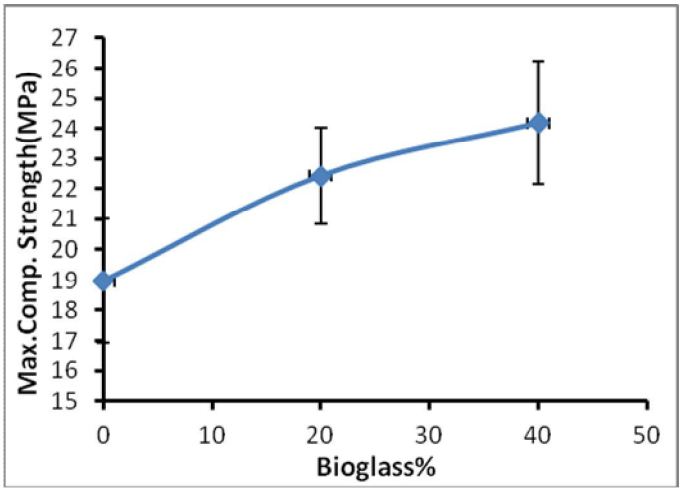

Figure 11. Variation of compression strength of composite samples with bioglass percentage after soaking in SBF .

4. Conclusions

1- The hydroxyapatite/ bioactive glass composite material was characterized in terms of physical and mechanical properties. It was found that addition of bioactive glass to the hydroxyapatite matrix in the rate of (20,40)wt% lead to increase samples density, decrease porosity and improve the mechanical properties (hardness & compressive strength).

2- After sintering process for hydroxyapatite/ bioactive glass samples at 1200ºC, X-Ray results displayed present of pseudo-wollastonite (α-$CaSiO_3$), and hydroxyapatite. All these phases are highly biocompatible in human physiological environment.

3- In vitro, X-Ray result showed the ability of hydroxyapatite/bioactive glass samples to form Ca-P-rich layer on the surface after soaking in SBF for 10 days.

4- There is a comparable change in pH during the 10 soaking days in SBF for both of hydroxyapatite and hydroxyapatite/bioactive glass, this could be attributed to the identical behavior to form Ca-P-rich layer on the surfaces.

5- The hydroxyapatite/40% bioactive glass samples displayed the largest degradation rate represented by highest weight loss after 10 soaking days in SBF.

5. References:

Afonso, A., Santos J. D., Vasconcelos M., Branco R., and Cavalheiro J., Granules of osteoapatite and glass-reinforced hydroxyapatite implanted in rabbit tibiae, Journal of Material Science: Materials in Medicine, vol. 7, 507–510,1996.

Chern, Lin J. H., Liu M. L., Chen K. S., and Ju C. P., Plasma-sprayed hydroxyapatite-bioactive glass coatings on Ti6Al4V, Ceram Engng Sci Proc, vol. 14, 971-981, 1993.

Composite Powders by Sol-Gel Processing," Int J. Appl. Ceram. Technol. Vol 5, pp. 401–411.

Dorozhkin, SV., Bioceramics of calcium orthophosphates, Biomaterials, 31 (7): 1465e85, 2010.

Encinas-Romero, M. A., Jesús Peralta-Haley, Jesús Valenzuela-García L., Felipe F. Castillón-Barraza,"Synthesis and Structural Characterization of Hydroxyapatite-Wollastonite Biocomposite, Produced by an Alternative Sol-Gel Route", Journal of Biomaterials and Nano biotechnology, Vol 4,pp. 327-333,2013.

Encinas-Romero, M.A., Aguayo-Salinas S., Castillo S.J., F.F. Castillon-Barraza, and Castano V.M., Synthesis and Characterization of Hydroxyapatite-Wollastonite, 2008.

Fu, Q., Saiz E., Rahaman M., and Tomsia A.P., Toward strong and tough glass and ceramic scaffolds for bone repair, Advanced Functional Materials, Vol.23, 5461-5476, 2013.

Jin, Y.H.B., Oktar F.N., Dorozhkin S., Agathopoulos S., "Sintering behavior and properties of reinforced hydroxyapatite/TCP biphasic bioceramics with ZnO-whiskers". J. Compos. Mater, Vol 45, pp. 1435-1345, 2011.

Jones, J.R., Lin S., Yue S., Lee P.D., Hanna J.V., Smith M.E., and Newport R.J., Bioactive glass scaffolds for bone regeneration and their hierarchical characterization, J. Engineering in Medicine, Vol. 224, Part H, 2010.

Kangasniemi, I. M. O., Development of Ca, P-ceramic containing bioactive glass composites, Master Thesis, University of Leiden, The Netherlands, Feb 1993.

Knowles, J. C., Bonfield W., Development of a glass-reinforced hydroxyapatite with enhanced mechanical properties:the effect of glass composition and its relationship to phase changes, J. Biomed. Mater. Res., vol.27, 1591-1598, 1993 .

Knowles, J. C., Development of hydroxyapatite with enhanced mechanical properties: effect of glass addition on mechanical properties, Biomaterials, vol. 25, 20-23, 1993.

Kokubo, T. Apatite formation on surfaces of ceramics, metals and polymers in body environment, Acta Mater, 46(7):2519e27,1998.

Marc Andre,´ Meyers, Po-Yu Chen, Albert Yu-Min Lin, Yasuaki eki,"Biological materials: Structure and mechanical properties", Progress in Materials Science, Vol 53 ,pp. 1–206, 2009.

Park, J., Bioceramics: Properties, characterizations, and Applications, 2008.

Patel, N., Best S.M., Bonfield W., Gibson I. R., Hing K. A., Damien E., and Revell P.A., A comparative study on the in vivo behavior of hydroxyapatite and silicon substituted hydroxyapatite granules, J. Materials Science: Materials in Medicine, 13, 1199-1206, 2002.

Radu Alexandru Ro şu, Ibolyka Bran, Mihaela Po pesc u, Carmen Opriş, ," In vitro characterization of hydroxyapatite layers deposited by AP S and HVOF thermal spraying methods", Ceramics – Silikáty ,Vol 56,pp.25-31, 2011.

Roy, M.,"Vickers Hardness Measurements: Practices and Calibration Check" The University of British Columbia, 2010.

Sanosh Kunjalukkal Padmanabhan , Marina Carrozzo , Francesca Gervaso , Francesca Scalera , Alessandro Sannino and Antonio Licciulli," Mechanical Performance and In Vitro Studies of Hydroxyapatite/ Wollastonite Scaffold for Bone Tissue Engineering", Key Engineering Materials ,Vol 493-494, pp.855-860, 2012.

Santos, J. D., Knowles J. C., Reis R. L., Monteiro F. J., and Hastings G. W., Microstructural characterization of glass reinforced hydroxyapatite composites, Biomaterials, vol. 15, 5-10, 1994 .

Santos, J. D., Silva P. L., Knowles J. C., Talal S., and Monteiro , F. J., Reinforcement of hydroxyapatite by adding P2O5-CaO glasses with Na2O, K2O and MgO, J Mater Sci: Mater Med, vol. 7, 187-189, 1996.

Siriphannon, P., Kameshima Y., Yasumori A., Okada K., Hayashi S., " Formation of hydroxyapatite on CaSiO3 powders in simulated body fluid" J. Eur. Ceram. Soc. Vol 22 ,pp. 511–520, 2002.

Sobczak, A., Kowalski, Wzorek Z.," Preparation of hydroxyapatite from animal bones", Acta of Bioengineering and Biomechanics ,Vol 11,No 4,2009.

Sri Asliza M. A, Zaheruddin K, Shahrizal H,"Study the Properties of Dense Hydroxyapatiteextract From COW Bone", Journal Of Nuclear And Related the Chnologies,Vol 6,No1,2009.

Teixeira, S., Magallanes M., de Aza A. Mateus A.Y.,Rodriguez M.A., Peña P.,Ferraz M.P., de Aza S.,Monteiro F.J.,"Cell culture studies on hydroxyapatite and wollastonite-tricalcium phosphate based materials for tissue engineering", European Cells and Materials Vol. 14, No 1,pp.95,2007.

Liu, X., Ding, C., Chu , P. K. ,cc Mechanism of apatite formation on wollastonite coatings in simulated body fluids. Biomaterials. 25, 1755–176, 2004.

Xynos, I.D., Hukkanen M.V.J., Batten J.J., Buttery L.D., Hench L.L., and Polak J.M., Bioglass 45S5 stimulates osteoblast turnover and enhances bone formation in vitro: Implication and applications for bone tissue engineering, Calcified Tissue International, 67, 321-329, 2000.