

Preparation and Bioactivity Evaluation of Bone like Hydroxyapatite - Bioglass Composite

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Abstract—In this study, hydroxyapatite (HA) composites are prepared on addition of 30%CaO-30%P₂O₅-40%Na₂O based glass to pure HA, in proportion of 2, 5, and 10 wt %. Each composition was sintered over a range of temperatures. The quantitative phase analysis was carried out using XRD and the microstructures were studied using SEM. The density, microhardness, and compressive strength have shown increase with the increasing amount of glass addition. The resulting composites have chemical compositions that are similar to the inorganic constituent of the mineral part of bone, and constitutes trace elements like Na. X-ray diffraction showed no decomposition of HA to secondary phases, however, the glass reinforced-HA composites contained a HA phase and variable amounts of tricalcium phosphate phase, depending on the amount of bioglass added. The HA-composite material exhibited higher compressive strength compared to sintered HA. The HA composite reinforced with 10 wt % bioglass showed highest bioactivity level.

Keywords—Bioactivity, Bioglass, Compressive strength, Hydroxyapatite.

I. INTRODUCTION

BONE substitution is still an unsolved problem. Currently, the best substitutes are the bone grafts provided either by a patient (autograft) or by a donor (allograft) [1]. However, bone grafts have well-known limitations [2, 3]. Due to these drawbacks, several synthetic materials for bone substitution have been developed and characterized during the last few decades. In this framework, bioceramics have raised especial interest due to their bioactivity and the possibility of tailoring their composition, tuning their degradation rate, and adjusting their formulation to compositions close to that of the mineral phase of bone [4].

Bioactive ceramics, such as bioglass (BG) and dense hydroxyapatite (HA), have been developed over the last two decades. Their accomplishments in the field of biomedical applications, especially in prosthetic applications, have attracted wide attention [5]. Hydroxyapatite (Ca₁₀(PO₄)₆(OH)₂) is one of the most well known phosphates in the biologically active phosphate ceramic family by virtue of its similarity to natural bone mineral. Synthetic and biologically harvested hydroxyapatite finds a variety of biological applications and elicits the formation of an apatite

layer at the interface with bone tissue [6-8]. Applications include bone repair in prosthetics [9], and dental applications [10]. Nevertheless, due to the poor mechanical properties of bulk HA ceramics, such materials cannot be used as implant devices for load-bearing applications [11].

Numerous techniques have been investigated in attempts to improve the mechanical properties for particular applications or implant configurations, by formation of HA composites reinforced with polymers, ceramics, etc. Such composites aim to retain their useful bioactive properties whilst providing more suitable mechanical properties for particular applications. In recent years increasing interest has been shown in sintering of HA with glass additions. There are two main motivations for sintering HA with a glassy phase—to enhance the densification and therefore the mechanical strength by acting as a sintering aid, and to enhance bioactivity through the combination of two bioactive phases [12, 13]. Sintering with low glass additions may promote densification through liquid-phase sintering, resulting in composite materials with enhanced mechanical properties. For example, small additions of phosphate glasses have been shown to significantly enhance composite flexural strength (by up to 400%), and fracture toughness (by up to 200%) [14-16]. Glass composition and the level of glass additions have a large effect on both the phase assembly and the mechanical properties of the resulting composites [17].

Glasses within the P₂O₅-CaO-Na₂O system possess enormous potential as biomaterials, because their compositions are similar to that of the inorganic constituent of the mineral part of bone [18]. All constituents of bioactive glasses could potentially be used as additives to HA ceramics to improve properties of the HA ceramic [19]. In experimental studies related to the reinforcement of HA, it is observed that glass-reinforced HA composites exhibit greater biological activities than commercial HA [20].

In this work, the effects of phosphate glass additions, from the system P₂O₅-CaO-Na₂O on the sintering, phase composition, and mechanical properties of HA are analysed. The objective is to provide a more comprehensive understanding of the mechanical and possible biocompatibility of such composites, which should help to determine the optimum composites for use as implant materials.

II MATERIALS AND METHODS

A: Formulation of Hydroxyapatite-Bioglass Composites

Preparation of Bioglass compositions

Phosphate based glass with the chemical compositions listed in Table I was prepared from reagent grade chemicals P₂O₅, CaO, and Na₂O (Merck Ltd.) as described by Santos et al. [21].

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Preparation of HA ceramic reinforced with Bioglass

HA powder was mixed with various proportion of 2 wt %, 5 wt %, and 10 wt % of bioglass to form three HA based reinforced ceramics denoted as C1, C2, and C3 respectively. The above mixture was prepared by mixing the contents for about 5 hours in a mortar and pestle.

Preparation of green pellets

All the above compositions were compacted to prepare the green pellets. For this 1gm of each composition was uniaxial compacted in a cylindrical die of 15 mm diameter using a compaction load of 20 kgf resulting in disc pellets. Compositions of the HA reinforced ceramic pellets are given in Table II.

TABLE I
BIOGLASS COMPOSITION IN WT %

| CaO | P ₂ O ₅ | Na ₂ O |
|-----|-------------------------------|-------------------|
| 30 | 30 | 40 |

TABLE II
PELLETS COMPOSITION IN WT % of HA REINFORCED CERAMIC

| HA reinforced ceramic pellets | Bioglass | HA |
|-------------------------------|----------|-----|
| HA | 0 | 100 |
| C1 | 2 | 98 |
| C2 | 5 | 95 |
| C3 | 10 | 90 |

Sintering

The green pellets prepared were subjected to sintering in a micro-controller temperature furnace following the sintering cycle given in Fig. 1. The samples were heated at a rate of 2°C/min, soaked at 100°C for 10 minutes, followed by further heating to 550°C, where it was soaked for 30 minutes to allow annealing of samples. The temperature was further raised to 1250°C using the same heating rate of 2°C/min. The samples were soaked for 3 hours at 1250°C followed by cooling gradually to 850°C in 3 hours. The samples were then soaked at 850°C for 30min from where they were cooled to room temperature in 2 hours.

B: Material Characterisation

Density Measurement

The densities of the green and sintered bioceramic samples were determined using Archimedes principle. 8 to 10 samples were used to determine the average density for each group.

Mechanical Characterisation

The hardness test and compression test were performed using Vickers Microhardness Tester (at 0.98 N) and Instron Universal Testing Machine (cross speed of 0.5 mm/min) respectively.

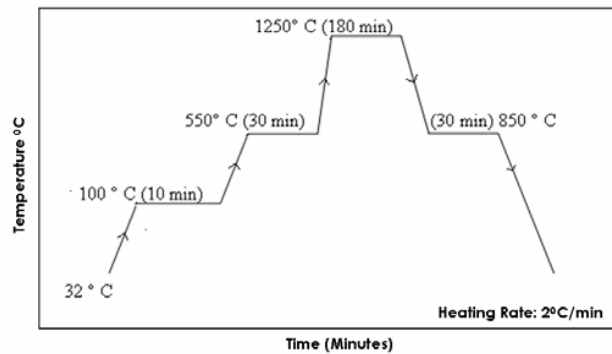


Fig.1. Sintering cycle followed for HA, C1, C2, and C3 compositions

X-Ray Diffraction Analysis

X-Ray diffraction studies of sintered bioglass, hydroxyapatite, and hydroxyapatite-bioglass composites were examined using a Philips Xpert diffractometer with CuK α (1.54Å) radiation. The data were recorded over the 2 θ range of 20° to 70° with a 0.017° step size and scan step time of 20.03 s. The phases present in HA, bioglass, HA reinforced ceramics C1, C2, and C3, were identified using JCPDS file 03-0407. In compositions HA, C1, C2, and C3, the peaks for HA were identified for Ca₁₀(PO₄)₆OH₂.

The peak broadening of XRD pattern was used to estimate the crystallite size in a direction perpendicular to the crystallographic plane based on Scherrer's formula as follows (1):

$$X_s = 0.9\lambda / FWHM \cos \theta \quad (1)$$

where X_s is the crystallite size in nm, λ the wave length of X-ray beam ($\lambda = 0.15406$ nm for CuK α radiation), FWHM the full width at half maximum for the diffraction peak under consideration (rad), and θ is the diffraction angle (°). The diffraction peak at $2\theta = 25.99^\circ$ was chosen for calculation of crystallite size since it was sharper and isolated from others. This peak assigns to (002) Miller's plane family and shows the crystal growth along the c-axis of the hydroxyapatite crystalline structure.

The fraction of crystalline phase (X_c) was evaluated as follows (2):

$$X_c = 1 - V_{112/300} / I_{300} \quad (2)$$

where I_{300} is the intensity of (300) diffraction peak and $V_{112/300}$ is the intensity of the hollow between (112) and (300) diffraction peaks of hydroxyapatite.

The lattice parameters for HA, C1, C2, and C3 are given in Table III.

Scanning Electron Microscopy (SEM)

Micro structural characterization was carried on the sintered HA, C1 to C3 compositions using SEM JSM 6100. All these sintered samples were made conducting by sputter coating with gold in a sputtering machine JFC 1100. The micrographs were used to study the nature of bonding, distribution of bioglass particles and the morphology of the phases in HA, C1, C2, and C3 compositions.

TABLE III
LATTICE PARAMETERS FOR HA/PHOSPHATE GLASS COMPOSITES

| Composition | a (Å) | c (Å) | Crystallite size(nm) | Crystallinity (%) |
|--------------|-------|-------|----------------------|-------------------|
| HA | 9.886 | 6.854 | 71.7 | 79 |
| 2 % BG (C1) | 9.908 | 6.855 | 71.7 | 81 |
| 5 % BG (C2) | 9.908 | 6.855 | 71.7 | 79 |
| 10 % BG (C3) | 9.990 | 6.856 | 71.7 | 77 |

Bioactivity (In Vitro) Characterisation

The dissolution behaviour of HA and C1 to C3 compositions was performed in a simulated body fluid (SBF) medium of pH 7.4 at a ratio of 1mg/ml in a water bath at 37°C. The SBF medium consisted of 9g NaCl, 5g KCl and 0.2g MgHPO₄.3H₂O per litre. The changes in pH were measured at pre-determined time intervals (0-15 days) using pH meter. The dissolution of calcium ions in SBF medium was determined by an atomic absorption spectrometer (AAS).

III. RESULTS AND DISCUSSION

Density, microhardness, and compression test results for HA-bioglass composites are listed in Table IV. Results indicate that HA-glass composites have higher density values compared to HA and the density increases with the increase in the bioglass addition from 2 wt % to 10 wt %. Also, it can be seen from Table IV, that the hardness increases with the increase in reinforcement content.

Compression test results of HA/bioglass composites showed substantially higher strength values compared to HA sintered bodies. While strength has been observed 41±20 MPa for HA, it obtained interesting values for C1, C2, and C3 compositions, as listed in Table IV. It can be observed that increasing reinforcement content increases compressive strength. The reason for high standard deviation values can be attributed to brittleness character of HA.

TABLE IV
DENSITY, HARDNESS, AND COMPRESSIVE STRENGTH OF HA, C1, C2, AND C3 COMPOSITIONS

| Composition | Density, gm/cm ³ | Hardness, VHN | Compressive strength, (Mpa) |
|-------------|-----------------------------|---------------|-----------------------------|
| HA | 2.59 ± 0.12 | 240 ± 15 | 41 ± 20 |
| 2 % BG | 2.76 ± 0.05 | 250 ± 19 | 48 ± 20 |
| 5 % BG | 2.78 ± 0.05 | 285 ± 26 | 64 ± 15 |
| 10 % BG | 2.79 ± 0.05 | 290 ± 12 | 67 ± 17 |

Fig. 2 shows the XRD patterns of the four different HA reinforced ceramics and the analysis of the structure is listed in Table V. Identical patterns were recorded for all these compositions, which suggest that the presence of the sintering additive did not alter the phases of pure HA ceramic. The primary ceramic present when 2, 5, and 10 wt % bioglass was sintered with hydroxyapatite is hydroxyapatite (JCPDF#03-0407) with β-TCP (Ca₃(PO₄)₂) as the secondary phase. No other phases like α - tricalcium phosphate (α-TCP) and calcium oxide (CaO) were detected. This indicates that the

bioglass behaves more as a sintering aid and promotes the conversion of HA to β-TCP.

Various researchers have already reported that same sintering aids promote formation of other phases like α- and β TCP, the amount and the rate being depending on the sintering additive [22, 23]. Formation of small amounts of β TCP is advantageous as it allows ionic substitutions and there may be enhanced bioactivity of the material.

TABLE V
PHASES DEVELOPED DURING SINTERING OF HA WITH 2, 5, AND 10 WT % BIOGLASS

| Bioglass (wt %) | Composition of Phases Present |
|-----------------|--|
| 0 | Synthetic Hydroxyapatite (Ca ₁₀ (PO ₄) ₆ OH ₂) |
| 2 | Synthetic Hydroxyapatite + β-TCP (β-Ca ₃ (PO ₄) ₂) |
| 5 | Synthetic Hydroxyapatite + β-TCP |
| 10 | Synthetic Hydroxyapatite + β-TCP |

The lattice parameters of HA and β-TCP changed as the percentage glass was increased, indicating a change in stoichiometry due to either lattice vacancies or substitutions.

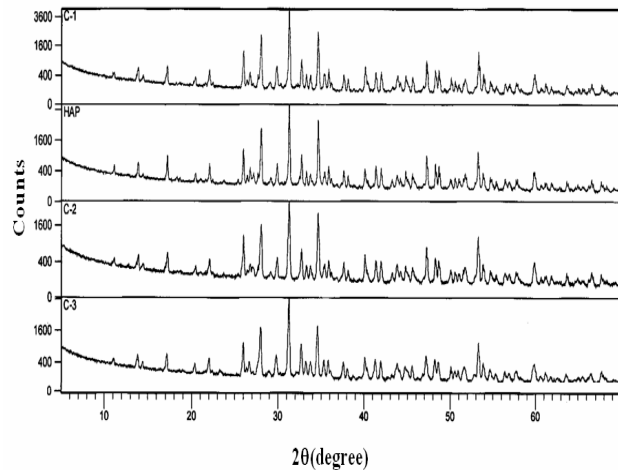


Fig. 2. Comparison of the XRD pattern for HA, C1, C2, and C3

Fig. 3 shows SEM for 100 % HA, HA reinforced with 2 wt %, 5 wt %, and 10 wt % bioglass. SEM for 100% HA shows large agglomerates of HA crystals indicating very good bonding of the HA particles through solid phase sintering. The porosity is well distributed throughout the sample. No liquid phase formation is observed. The SEM picture for HA reinforced with 2 wt % bioglass shows the particulate bonding. The HA particles have agglomerated through solid phase sintering and the bioglass particles are uniformly spread at the network boundary or the grain boundary of agglomerated HA. The porosity is uniform in distribution and size.

It is observed from SEM for C2 composition i.e. HA reinforced with 5 wt % bioglass that the solid phase sintering has occurred to form the HA agglomerate. On comparing this micrograph with that for C1 composition, it is observed that the agglomerate size of HA has relatively increased whereas porosity size distribution is not uniform.

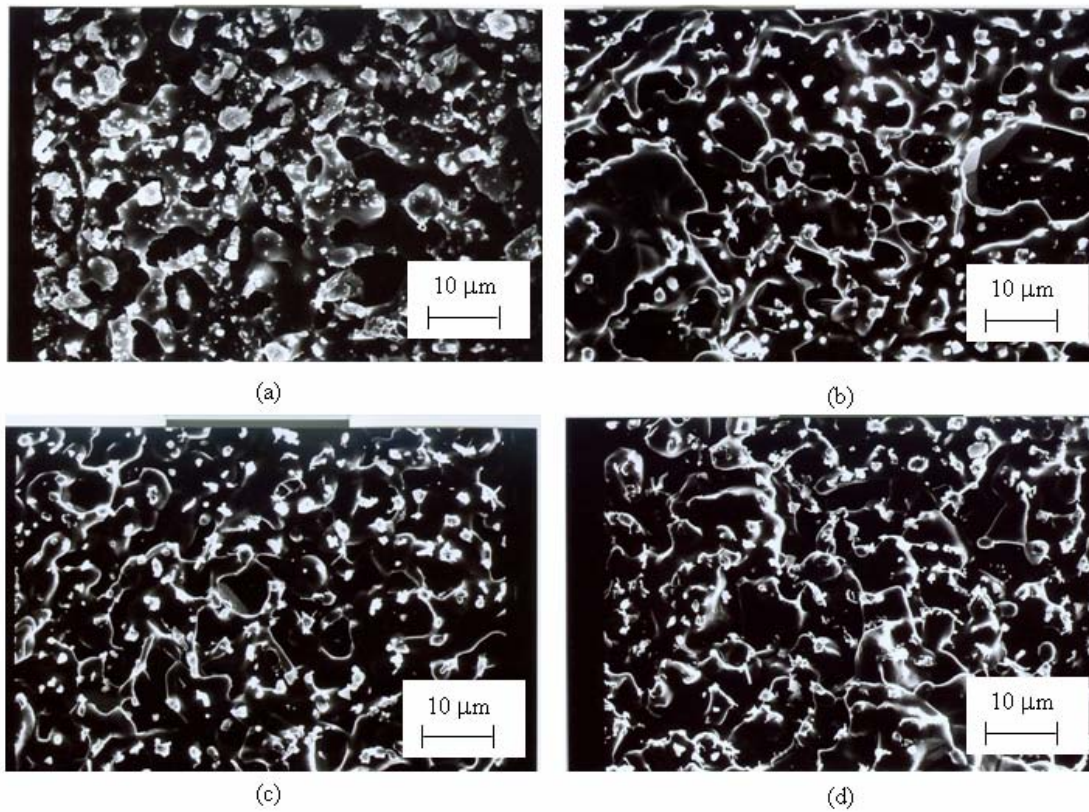


Fig. 3. SEM of (a) HA (b) C1 composition (c) C2 composition and (d) C3 composition

Solid phase sintering is found to be the prominent mechanism for bonding of constituent particles in SEM for C3 composition. As compared to the micrograph of C1 and C2 composition, the C3 composition shows uniform distribution of the bioglass particles within the agglomerate as well as the grain boundary.

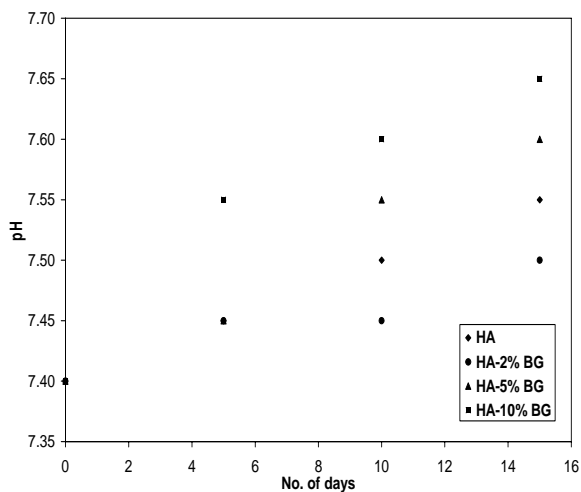


Fig. 4. Change in pH of SBF medium with time

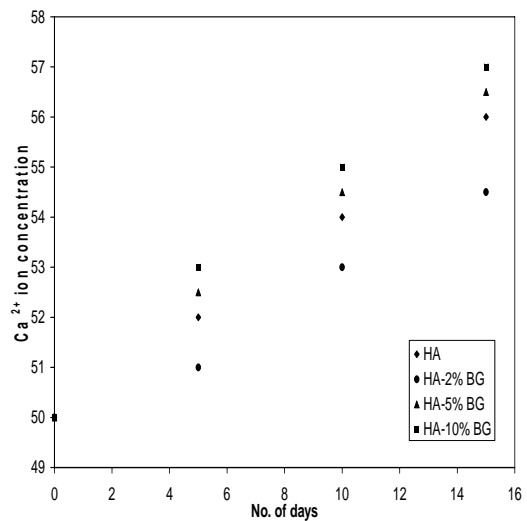


Fig. 5. Change in Ca²⁺ ion concentration in SBF medium with time

The porosity is more uniform in size as well as distribution. In order to determine the mineralization ability and bioactivity of each of these sintered bioceramics, the samples were immersed in SBF for 14 days. All surfaces exhibited

dissolution coupled with mineralization. There are some important distinctions in the nature of dissolution and mineralization on the bioceramic surfaces as can be seen from Fig. 4 and Fig. 5. It has been observed that the pH of SBF medium is higher for 5 wt % and 10 wt % bioglass reinforced HA than 2 wt % bioglass reinforced HA. The pure HA surface exhibited the least level of activity with the surface exhibiting some dissolution, on the other hand the composition with 2 wt % bioglass exhibited a fine layer of mineralization on the surface coupled with some dissolution. The composition with 5 wt % and 10 wt % bioglass exhibited larger levels of mineralization coupled with large dissolution of the glassy phase. The level of mineralization in these three chemistries increases with the amount of glassy phase.

IV. CONCLUSION

The glasses chosen were phosphate-based glasses closely related to the composition of HA. X-ray diffraction of the sintered bioceramics indicates that pure hydroxyapatite retains its structure after sintering with no increase in β -TCP content. In compositions with 2, 5, and 10 wt % Bioglass, β -TCP is the only new phase detected. A strong chemical bond is developed between HA and the phosphate-based glass through solid phase sintering. As the bioglass content is increased, the liquid phase sintering also contributes towards the bonding. It should also be noted that compressive strength, and hardness values of samples containing 10 wt % bioglass is better than for 2 wt %, and 5 wt % reinforced composites. Also HA reinforced with 10 wt % bioglass shows the maximum mineralization and bioactivity in SBF medium.

REFERENCES

- [1] S. Stevenson, "Enhancement of fracture healing with autogenous and allogeneic bone grafts," *Clin Orthop.*, 1998, vol. 355(Suppl), pp. 239-46.
- [2] J. A. Goulet, L. E. Senunas, G. L. DeSilva, and M. L. Greenfield, "Autogenous iliac crest bone graft. Complications and functional assessment," *Clin Orthop.*, 1997, vol. 339, pp.76-81.
- [3] J. M. Segur, S. Suso, S. Garcia, A. Combalia, and R. Ramon, "Bone allograft contamination in multiorgan and tissue donors," *Arch Orthop Trauma Surg.*, 1998, vol. 118, pp.156-158.
- [4] R. Z. LeGeros, "Properties of osteoconductive biomaterials: calcium phosphates," *Clin Orthop.*, 2002, vol. 395, pp.81-98.
- [5] D.-M. Liu, and H.-M. Chou, "Formation of a new bioactive glassceramic," *Journal of Material Science: Materials in Medicine*, 1994, vol. 5, pp. 7-10.
- [6] R. Xin, Y. Leng, J. Chen, and Q. Zhang, "A comparative study of calcium phosphate formation on bioceramics in vitro and in vivo," *Biomaterials*, 2005, vol. 26(33), pp. 6477-6486.
- [7] G. Goller, H. Demirkiran, F. N. Oktar, and E. Demirkesen, "Processing and characterization of bioglass reinforced hydroxyapatite composites," *Ceram Int.*, 2003, vol. 29, pp. 721-724.
- [8] F. N. Oktar, and G. Goller, "Sintering effects on mechanical properties of glass-reinforced hydroxyapatite composites," *Ceram Int.*, 2002, vol. 28(6), pp. 617-621 617.
- [9] M. Akao, H. Aoki, and K. Kato, "Mechanical properties of sintered hydroxyapatite for prosthetic applications," *J Mater Sci.*, 1981, vol. 16, pp. 809-812.
- [10] M. D. Francis, "The inhibition of calcium hydroxyapatite crystal growth by polyphosphonates and polyphosphates," *Calcified Tissue Research*, 1969, vol. 3, pp.151-162.
- [11] F. J. Garcia-Sanz, M. B. Mayor, J. L. Arias, J. Pou, B. Leon, and M. Perez-Amor, "Hydroxyapatite coatings: a comparative study between plasma-spray and pulsed laser deposition techniques," *Journal of Material Science: Materials in Medicine*, 1997, vol. 8, pp. 861-865.
- [12] I. M. O. Kangasniemi, *Development of Ca, P-ceramic containing bioactive glass composites*, Masters Thesis, University of Leiden, The Netherlands, Feb 1993.
- [13] J. H. Chern Lin, M. L. Liu, K. S. Chen, and C. P. Ju, "Plasma-sprayed hydroxyapatite- bioactive glass coatings on Ti6Al4V," *Ceram Engng Sci Proc.*, 1993, vol. 14, pp. 971-981.
- [14] J. C. Knowles, W. Bonfield, "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.*, 1993, vol.27, pp.1591-1598.
- [15] J. D. Santos, J. C. Knowles, R. L. Reis, F. J. Monteiro, and G. W. Hastings, "Microstructural characterisation of glass reinforced hydroxyapatite composites," *Biomaterials*, 1994, vol. 15, pp. 5-10.
- [16] J. D. Santos, P. L. Silva, J. C. Knowles, S. Talal, and F. J. Monteiro, "Reinforcement of hydroxyapatite by adding P₂O₅-CaO glasses with Na₂O, K₂O and MgO," *J Mater Sci: Mater Med*, 1996, vol. 7, pp. 187-189.
- [17] J. C. Knowles, "Development of hydroxyapatite with enhanced mechanical properties: effect of glass addition on mechanical properties," *Biomaterials*, 1993, vol. 25, pp. 20-23.
- [18] J. D. Santos, R. L. Reis, F. J. Monteiro, J. C. Knowles, and G. W. Hastings, "Liquid phase sintering of hydroxyapatite by phosphate and silicate glass additions: structure and properties of the composites," *Journal of Material Science: Materials in Medicine*, 1995, vol. 6, pp. 348-352.
- [19] W. Suchanek, M. Yashima, M. Kakihana, and M. Yoshimura, "Hydroxyapatite ceramics with selected sintering additives," *Biomaterials*, 1997, vol. 18, pp. 923-933.
- [20] A. Afonso, J. D. Santos, M. Vasconcelos, R. Branco, and J. Cavalheiro, "Granules of osteoapatite and glass-reinforced hydroxyapatite implanted in rabbit tibiae," *Journal of Material Science: Materials in Medicine*, 1996, vol. 7, pp. 507-510.
- [21] J. D. Santos, R. L. Reis, F. J. Monteiro, J. C. Knowles, G. W. Hastings, "Liquid phase sintering of hydroxyapatite by phosphate and silicate glass additions: structure and properties of the composites," *Journal of Material Science: Materials in Medicine*, 1995, vol. 6, pp. 348-352.
- [22] M. Jarcho, "Calcium Phosphate ceramics as a hard tissue prosthetics," *Biomaterials*, 1981, vol. 12, pp. 157-168.
- [23] Jae-Man Cho, "Formation and characterization of Hydroxyapatite coating layer by electron beam deposition," *Journal of Material Research*, 1999, vol. 14, pp. 145-158.