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Effect of Sodium Alginate on the Properties of Calcium Phosphate for Bone Implant Application

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ABSTRACT

Calcium (CaP) is a bioceramic material which is limited in mechanical strength. Due to the limitation, Sodium Alginate (SA) was reinforced in CaP matrix composite with a different ratio of CaP/SA weight percentage (100/0, 99/1, 97/3, 95/5, 90/10 and 80/20). The composite was prepared by using the precipitation method and the compacted powder was sintered at 1000°C. The sintered samples are characterized through x-ray diffraction (XRD). The physical and mechanical properties were determined by density, shrinkage and hardness test. Microstructure and grain size of the samples were examined under scanning electron microscope (SEM). Tricalcium phosphate (TCP) was detected in XRD analysis after sintered at 1000°C. The grain size and shrinkage of the CP were increased with SA as well as hardness and density. SA reinforcement has improved the density and hardness of CaP with by 2.90g/cm^3 and 4.71GPa respectively. The grain size and the crystallite size also increased with SA. Due to the improved properties, CaP/SA composites can be proposed to be one of the biomaterials for a bone implant application.

Keywords: Calcium Phosphate, Bioceramic, Hardness, Sodium Alginate.

1. INTRODUCTION

Calcium phosphate (CaP) is an inorganic material that has mostly been used in bone treatment or bone replacement due to its chemical structure being close to the natural bone. CaP shows a positive effect for bone substitution as it has a highly developed bonding with bone tissues that exhibit a good osteo-conductive and bio-resorption behavior [1]. CaP is also known as an excellent material for biocompatibility and bioactivity [2]. Although, the composition is suitable for human bone, the mechanical strength of the CaP is not suitable for natural bones. As such, the application of CaP is limited to artificial bone implants due to its lower mechanical strength [3,4,5]. In order to improve the mechanical properties of sintered CaP, composites of SA/CaP were prepared. The SA was used as a reinforcement that could improve biodegradation of the CaP without releasing any toxicity in the human body. [6,7]. SA is a natural polymer extracted from brown algae [8].

The properties of SA that is biocompatible, low toxicity and low cost have led this material in many biomedical applications such as in wound healing drugs and protein delivery[9]. Among the biopolymer materials, alginate is one of the biopolymer that has usually been used in bone tissue engineering as it easily modifies in any form [10]. Based on previous studies, the addition of SA can improve the properties of CaP. Some studies have shown that the formation of

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tricalcium phosphate (TCP) in hydroxyapatite (HA) improved the mechanical properties of the HA sample [11]. Formation of α-TCP after sintering was observed by Coelho (2012) where the intensity decreased with the addition of SA [12]. Mechanical strength of the CaP cement improved with the addition of SA compared to the pure CaP cement [12]. Rajkumar *et al.,* (2011) observed that the addition of SA in HA improved the hardness and density of the green body sample [13]. In this study, SA is chosen as a reinforecemnet in CaP and sintered at 1000°C in order to enhance the mechanical and physical properties of CaP.

2. MATERIAL & METHODS

2.1 Powder Preparation

Calcium phosphate ($CaHPO_4$) (Fisher Scientific) was mixed with sodium alginate (SA) (R&M Chemical) in different weight percentages (1.0, 3.0, 5.0, 10 and 20 wt%) by using a precipitation method. CaP was stirred for 2 hours at 80°C with distilled water by using a magnetic stirrer. After that, SA was added slowly with vigorous stirring. The solution was further stirred for another 2 hours at a similar temperature until a paste-like solution was formed. The paste aged at ambient room temperature for another 24 hours. Thereafter, the paste was oven dried for another 24 hours at 80°C and the powder formed was ground by using mortar and pestle to produce fine powders.

2.2 Samples Preparation

The fine powders were compacted into 13 mm diameter of disc shape. The green bodies were then sintered at 1000°C with 2°C/min for 2 hours. Samples were grinded and polished with nylon cloth by using diamond paste prior to the hardness test and SEM examination [14].

2.3 Samples Characterization and Testing

Sintered samples were characterized by using x-ray diffraction (XRD) (Bruker D8 Advance). The analysis was set at 40 kV and 40 mA with diffraction angles (2θ) between 25° and 60° with step size of 0.02° per second [15]. The crystallite size was calculated based on the Scherrer's equation. The microstructure and grain size were examined under SEM (Hitachi, SU 1510) with 15kV. In order to get a better microstructure, the samples were polished and thermal etched at 950°C for 30 minutes. The grain sizes were revealed by using the intercept method. Straight lines were drawn diagonally to the microstructure and the intercept lines between grain boundaries were measured. The measurement must be taken at the two lines that were drawn diagonally and the average values were recorded [14]. The Archimede's principle was applied to measure the bulk density of the sintered samples. The hardness of the sample was performed by using the Vicker's hardness (Shimadzu) according to the ASTM C1327-15. At least 10 indentations with constant load 0.2Hv (1.961N) for 10s loading time was used. The diameter of the samples was recorded before and after the sintering process to calculate the shrinkage.

3. RESULTS AND DISCUSSION

XRD analysis of different ratio of CaP/SA is shown in Figure 1. Based on the analysis, two phases were observed after sintered that belongs to calcium phosphate ($Ca_2P_2O_7$) (JPDS no. 71-2123) and whitlockite ($Ca_3(PO_4)_2$) (JPDS no. 03-0713) peaks. SA reduced the intensity of CaP ($Ca_2P_2O_7$) peak. However, the intensity of whitlockite ($Ca_3(PO_4)_2$) increases with SA. Whitlockite also known as tricalcium phosphate (TCP) [16] was observed on the sample probably due to the dehydroxylation where it occurred during sintering process between 800 to 1350°C [17]. This is

in line with Ou *et al.,* (2013), the β-TCP and tetracalcium phosphate (TTCP) phases appeared after the sintering process at 1100°C [18]. TCP peak was also observed in the HA sample after sintered at 1250°C [11]. Some other studies on HA/TCP have shown that the samples completely transformed β-TCP to α-TCP when sintered above 1200°C [19]. Dehydroxylation that occurred due to sintering process can influence the density and strength of the sample [17]. Slosarcyk *et al.,* (1998) have proven that dehydroxylation phenomenon that formed HA into TCP resulted the best mechanical property compared to the pure HA [11]. According to Rajkumar *et al.,* (2011), the absence and the presence of (SA) in HA has provided a significant effect on the crystal growth of HA [14]. The formation of sharp peaks of HA was due to the increase of the sintering temperature and crystallinity of the sample [20, 21].

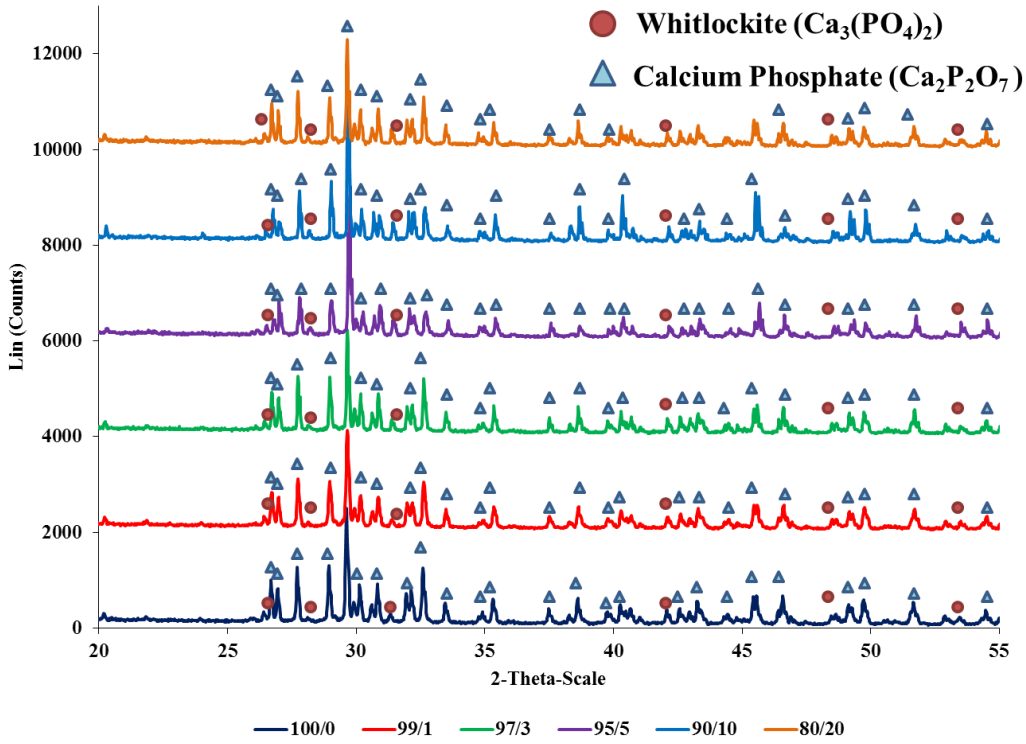

Figure 1. XRD analysis of CaP/SA at different ratio.

Average crystallite size of the sample taken at 32° at 2θ scale is shown in Figure 2. Maximum crystallite size was observed at 3wt% SA with 143.53nm. Based on the result, it shows that the addition of (SA) increased the crystallite size of CaP. This is in agreement with Rajkumar et al. (2011) where the average crystallite size increased with (SA) until 1.5wt% [13]. Based on the result, it proves that the presence of (SA) in CaP can increase the crystallite size of the CaP.

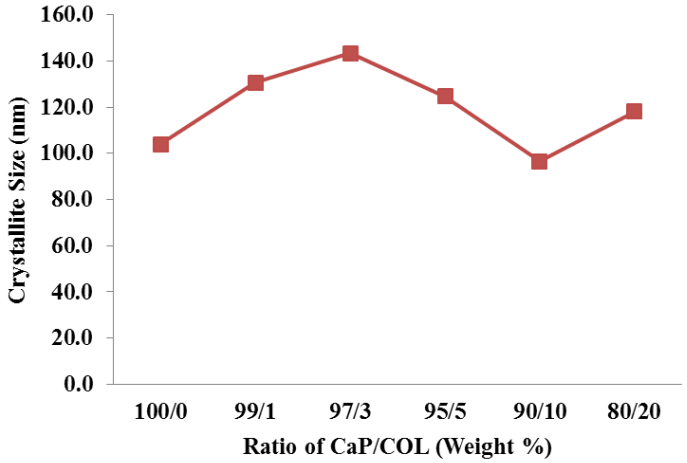

Figure 2. Crystallite size of CaP/SA at different ratio of weight percentages

The density and hardness can be observed in Figure 3 (a) and Figure 3 (b). Both curves reveal that the hardness and density increased with the SA content. Maximum density and hardness were observed at 5wt% of SA with 2.90g/cm^3 and 4.71GPa respectively. While the minimum density and hardness were found in pure CaP with 2.05g/cm^3 and 0.06 GPa respectively. This result proves that the addition of SA in CaP improved the hardness and density which is similar to the results reported by Rajkumar (2011) [13]. Based on these results, the hardness is relatively related to the density of sintered sample [22], where the hardness gradually increased with increasing the density of the sample. Gibson et al. (2001) reported that the density of the sintered sample clearly influenced the hardness of sample [23]. The density increased to 5wt% of SA but further addition led to the decrease of density. According to Gibson et al. (2001), increasing the density of the sample influenced large grain growth and low porosity [23]. Meanwhile the decrease in the density occurred due to larger pores presented [24]. This can be proven in this study, based on Fig that shows the addition of SA has led to less porosity to 5wt% of SA, further addition of SA showed the formation of pores that leads to decreasing density. Hoepfner *et al.,* (2003) observed that the grain size is one of the factors that influences the HA hardness [22]. This can be seen in Figure 5 that shows that the increase of the grain size is in line with the hardness to 5wt% of SA. However, further increase in grain size led to a decrease in the hardness [25]. Thangamani *et al.,* (2002) also reported that the decrease in hardness occurred due to the retarded densification of the HA [25].

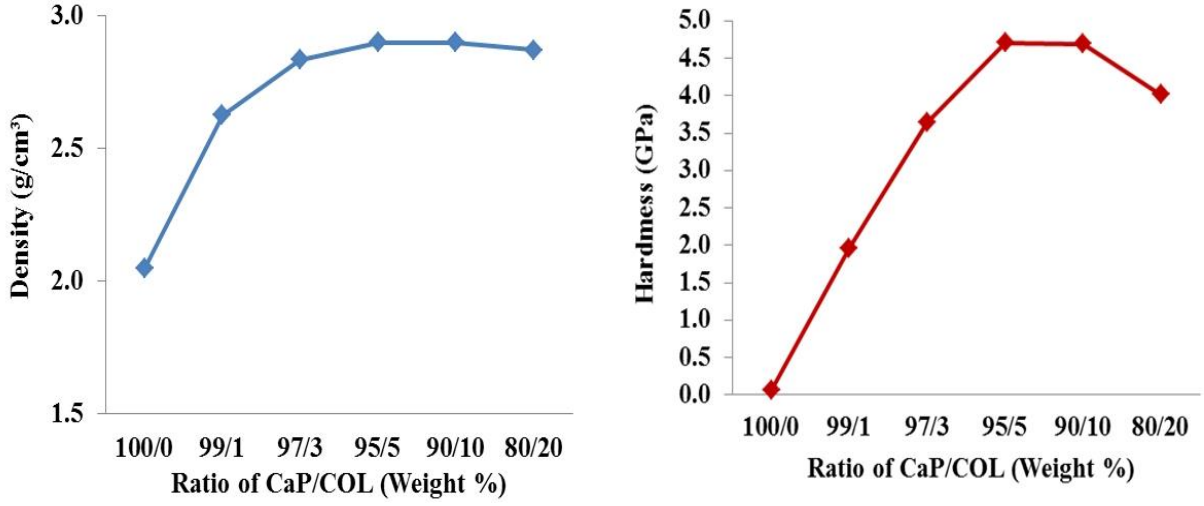

Figure 3. (a) Density and (b) Hardness of CaP/SA at different ratio of weight percentages.

Figure 4 shows that the shrinkage took place after the sintering process. The diameter of the sample shrinks rapidly when increasing the addition of SA. Maximum shrinkage occurred at 20wt% of SA with the addition of 17.36%, while the minimum shrinkage took place at pure CaP with 4.27%. Shrinkage was directly influenced by the dense structure of the composite. This result can be observed in the microstructure of the composite in Figure 6, where the formation of dense structure occurred with the addition of SA. The shrinkage occurred due to the reduction in porosity and where the particle extended to be too large to fill the porosity between the particle that caused the reduction size of sample [26].

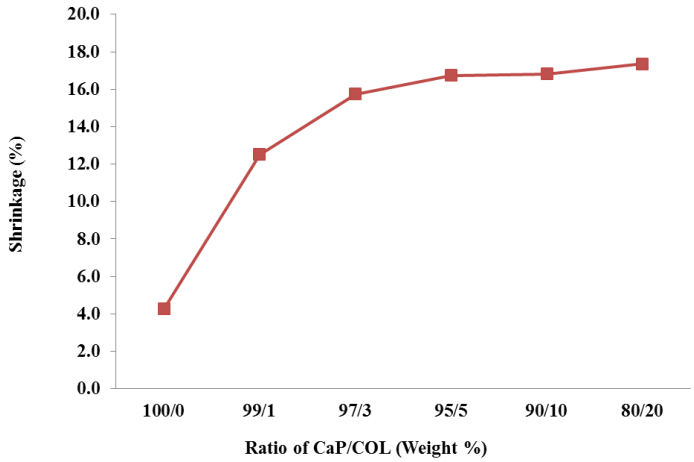

Figure 4. Shrinkage of CaP/SA at different ratio weight percentages.

The grain size and the surface microstructure of the CaP/SA composite are illustrated in **Error! Reference source not found.** and Figure 6 respectively. Figure 5 shows the maximum grain size obtained at 80/20 ratio of CaP/SA with 1.82µm. While the minimum grain size was observed at 100/0 sample with 0.84µm. Based on Muralithran *et al.,* (2000), the grain size influenced the density and hardness of HA sample [24]. Based on Figure 6, the porosity of the sample reduced when increasing the addition of SA and the formation of larger grains was observed. Formation of large and small grains that were observed at 95/5 sample formed the highest density and hardness of the CaP sample. This is in agreement with Adzila *et al.,* (2016) combination of small and large grain that formed strong bonding between the grain size which enhanced the density and hardness of CaP sample [27]. However, closed-porosity and the larger the grain size led to the decrease in the density as well as the hardness of the CaP sample [23, 27]. The grain size also linearly increased with the crystallite size from the XRD analysis with equiaxed hexagonal and cubic structure.

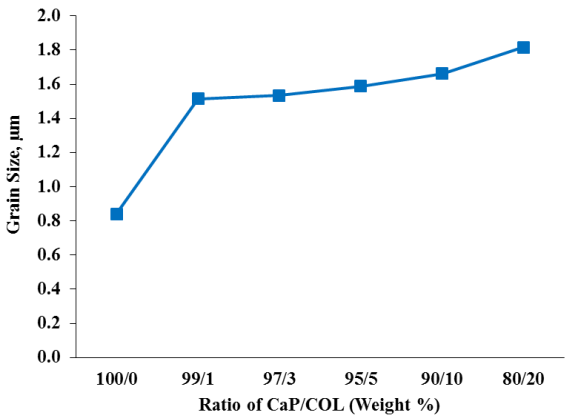

Figure 5. Grain Size of CaP/SA at Different Ratio of Weight Percentages

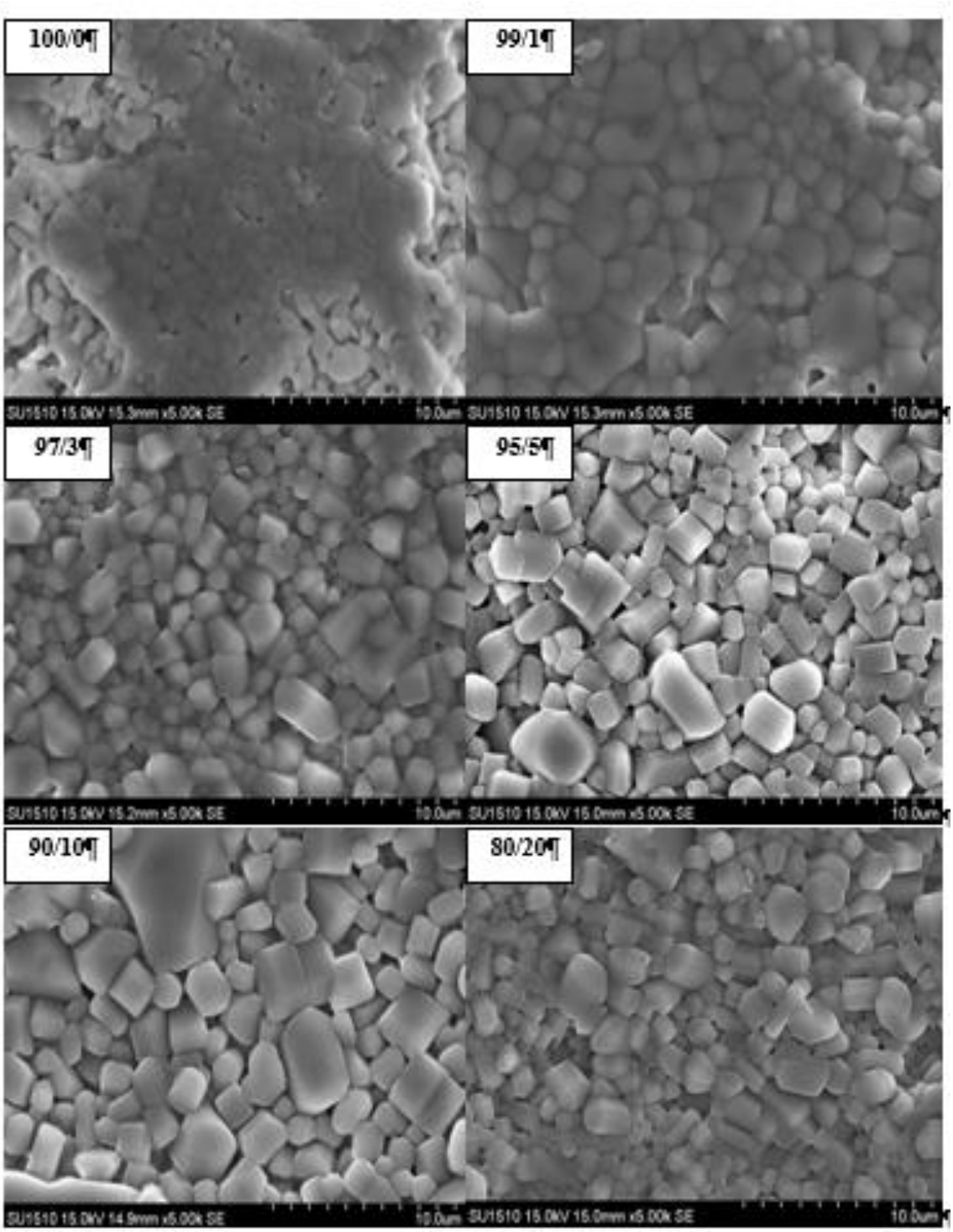

Figure 6. Microstructure of CaP/SA at Different Ratio of Weight Percentages

4. CONCLUSION

From this study, the XRD analysis has shown the formation of the whitlockite phase (TCP) after sintering process and the intensity increased with the addition of SA. CaP/SA biocomposite has improved the densification and the hardness of CaP with 2.90g/cm^3 and 4.71GPa respectively. The presence of SA in CaP affects the crystallite size, morphology as well as the grain size of the

CaP sample. Further characterization such as in vitro analysis is required to validate the biocompatibility of CaP/SA biocomposite as one of the potential materials for the bone implant application.

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REFERENCE

[1] Laasri, S., Taha, M., Hajjaji, A., Laghzizil, A., & Hlil, E., K. Mechanical Properties of Calcium Phosphate Biomaterials. Mol. Cryst. Liq. Cryst. **628** (2016) 198–203.

[2] Lertcumfu, N., Jaita, P., Manotham, S., Jarupoom, P. Eitssayeam, S., & Pengpat, K. Properties of Calcium Phosphates Ceramic Composites Derived from Natural Materials **42** (2016) 10638–10644.

[3] Zhou, K., Zhang, X., Chen, Z., Shi, L., & Li, W. Preparation and Characterization of Hydroxyapatite-Sodium Alginate Scaffolds by Extrusion Freeforming. Ceram. Int. **41**, 10 (2015) 14029–14034.

[4] Boilet, L., Moreau, G., Tricoteaux, A., Lu, J., Leriche, A., Lardot, V., Cambier, F., & Descamps, M. Processing and Properties of Biphasic Calcium Phosphates Bioceramics Obtained by Pressureless Sintering and Hot Isostatic Pressing. J. Eur. Ceram. Soc. **33**, 7 (2013) 1263–1270.

[5] Ghosal, S. Densification and Mechanical Characterization of Pure and Doped Hydroxyapatite. National Institute of Technology, Rourkela, (2013).

[6] Turco, G., Marsich, E., Bellomo, F., Semeraro, S., Donati, I., Brun, F., Grandolfo, M., Accardo, A., & Paoletti, S. Alginate. Hydroxyapatite Biocomposite For Bone Ingrowth : A Trabecular Structure with High and Isotropic Connectivity. Biomacromolecules **i**, 10 (2009) 1575–1583.

[7] Zhensheng, L., Ramay, H. R., Hauch, K., D., Xiao, D., & Zhang, M. Chitosan-Alginate Hybrid Scaffolds for Bone Tissue Engineering. Biomaterials **26**, 18 (2005) 3919–3928.

[8] Kuen, Y. L., & Mooney, D. J. (2012) Alginate: Properties and Biomedical Applications. Prog. Polym. Sci. **37**, 1 (2012) 106–126.

[9] Comaposada, J., Gou, P., Marcos, B., & Arnau, J. Physical Properties of Sodium Alginate Solutions and Edible Wet Calcium Alginate Coatings. Food Sci. Technol., (2012) 212-219.

[10] Venkatesan, J., Bhatnagar, I., Manivasagan, P., Kang, K., and Kim, S. Alginate Composites for Bone Tissue Engineering : A Review. Int. J. Biol. Macromol. **72** (2015) 269–281.

[11] Slosarczyk, A. & Bialoskorski, J. Hardness and Fracture Toughness of Dense Calcium – Phosphate-Based Materials. J. Mater. Sci. Mater. Med. **9** (1998) 103–108.

[12] Coelho, W. T., Fernandes, J. M., Vieira, R. S., Thurmer, M. B., & Santos, L., A. Modification on the Properties of a Calcium Phosphate Cement by Addition of Sodium Alginate. Brazilian Symposium on Glass and Related Materials, 1822–1827.

[13] Rajkumar, M., Meenakshisundaram, N., & Rajendran, V. Development of Nanocomposites Based on Hydroxyapatite/Sodium Alginate: Synthesis and Characterisation. Mater. Charact. **62**, 5 (2011) 469–479.

[14] Kanasan, N., Adzila, S., Koh, C. T., Rahman, H. A., & Panerselvan, G. Effects of magnesium doping on the properties of hydroxyapatite/sodium alginate biocomposite. Adv. in App. Ceram. **118**, 7 (2019) 381-386.

[15] Adzila, S., Mustaffa N. A. & Kanasan, N. Magnesium-doped calcium phosphate/sodium alginate biocomposite for bone implant application. J. Aust. Ceram. Soc., (2020) 1-7.

[16] Rusy, A., J., Wei, M., Sorrell, C. C., Dickson, M. R., Brandwood, A., & Milthorpe, B. K. Sintering Effects on the Strength of Hydroxyapatite. Biomaterials, **16**, 5 (1995) 409–415.
[17] Ou, S. F., Chiou, S. Y., & Ou, K. L. Phase Transformation on Hydroxyapatite Decomposition. Ceram. Int. **39**, 4 (2013) 3809–3816.
[18] Ryu, H., Sun, K., Lee, J., Joong, D., Hyup, J., Chang, B., Lee, D., Lee, C., and Chung, S. Magnesia-Doped HA / β -TCP Ceramics and Evaluation of their Biocompatibility. Biomaterials **25** (2004) 393–401.
[19] Shi, Z., Huang, X., Cai, Y., Tang, R., & Yang, D. Size Effect of Hydroxyapatite Nanoparticles on Proliferation and Apoptosis of Osteoblast-Like Cells. Acta Biomater. **5** (2009) 338–345.
[20] Pang, Y. X. & Bao, X. Influence of Temperature, Ripening Time and Calcination on The Morphology and Crystallinity of Hydroxyapatite Nanoparticles. J. Eur. Ceram. Soc. **23** (2003) 1697–1704.
[21] Hoepfner, T. P., & Case, E. D. The Influence of the Microstructure on the Hardness of Sintered Hydroxyapatite. Ceram. Int. **29** (2003) 699–706.
[22] Gibson, I. R., Ke, S., Best, S. M., Bonfield, W., Materials, B., Mary, Q., College, W., & Road, M. E. Effect of powder characteristics on the sinterability of hydroxyapatite powders. J. Mater. Sci. Mater. Med. **2** (2001) 163–171.
[23] Muralithran, G., & Ramesh, S. The Effects of Sintering Temperature on The Properties of Hydroxyapatite, Ceram. Int. **26** (2000) 221–230.
[24] Thangamani, N., Chinnakali, K., & Gnanam, F. D. The Effect of Powder Processing on Densification, Microstructure and Mechanical Properties of Hydroxyapatite," Ceram. Int. **28**, 4 (2002) 355–362.
[25] Rossi, A. L., Barreto, I. C., Maciel, W. Q., Rosa, F. P., Rocha-Leão, M. H., Werckmann, J., Rossi, A. M., Borojevic, R., & Farina, M. Ultrastructure of Regenerated Bone Mineral Surrounding Hydroxyapatite-Alginate Composite and Sintered Hydroxyapatite. Bone **50**, 1 (2012) 301–310.
[26] Adzila, S., Kanasan, N., Hassan, M. F., Ariffin, A. M. T., Yunos, M. Z., Nasrull, M., Haq, A.. Synthesis and Characterization of Magnesium doped Calcium Phosphate for Bone Implant Application. ARPN J. Eng. Appl. Sci. **11**, 14 (2016) 8694–8697.
[27] Landi, E., Tampieri, A., Celotti, G., & Sprio, S. Densification Behaviour and Mechanisms of Synthetic Hydroxyapatites. J. Eur. Ceram. Soc. **20** (2000) 2377–2387.