MICROSTRUCTURE AND IN-VITRO TEST BIOACTIVITY BEHAVIOR OF Co-Cr-Mo (F-75)/HYDROXYAPATITE IN PHOSPHATE BUFFERED SALINE SOLUTION

Shamsul Baharin Jamaludin[1)], Nur Maizatul Shima Adzali[1)], Mohd Nazree Derman[1)]*
[1)] Sustainable Engineering Research Cluster, Schools of Materials Engineering, University Malaysia Perlis, Perlis, Malaysia

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[*]*Corresponding author: e-mail: sbaharin@unimap.edu.my , Tel.0604 9798154, Sustainable Engineering Research Cluster, Schools of Materials Engineering, University Malaysia Perlis, Kompleks Pusat Pengajian 2, Taman Muhibah, 02600, Arau, Perlis, Malaysia*

Abstract
Cobalt based alloys were the first metallic materials successfully used in orthopedic applications. However, these alloys are bio-inert, it is necessary to make them bioactive in the human body to improve their application as biomaterials. This research reported the microstructure and in-vitro bioactivity behavior of the novel Co-Cr-Mo alloy (ASTM F-75) filled with different amounts of hydroxyapatite (HAP). F-75 powder was mixed with 2, 6 and 10 wt. % of HAP before being cold compacted at 550 MPa using a uni-axial press machine. The composites then were sintered at 1100°C for 2 h. In-vitro bioactivity behavior of the composites was evaluated by immersing the composites into simulated body fluids for up to 18 days. Results showed that the nucleus of apatite, identified as the apatite layer formed on the surface of the prepared F-75/HAP composite after 18 days of immersion in the phosphate buffered solution (PBS). The Co-Cr-Mo alloy was successfully converted into a bioactive composite by adding 2, 6 and 10 wt. % of HAP particles. It was proposed that the formation of the apatite layer on the surface of F-75/HAP can contribute to the improved biocompatibility and osteoconductivity of F-75/HAP.

Keywords: powder metallurgy, in-vitro, biomaterials, Co-Cr-Mo alloy, composite

1 Introduction

In general, as implant materials, metals need to enhance their corrosion resistance as well as their bioactivity behavior [1]. Bioactivity behavior is a specific biological response between implants and human body, which results in the formation of a bond between tissue and implant materials [2]. Metallic materials play a very predominant role in fulfilling almost every difficult factor that arises in implant applications [3]. In dental applications, biomaterials can be fabricated by stainless steels, Co-Cr-Mo alloys, Ti and Ti alloys, bio-glass and polymeric materials. These materials can replace diseased damaged or loosened teeth [4, 16]. For cardiovascular implants, stainless steels and silicone rubber are commonly used to maintain heart rhythm, and a Co-Cr alloy is used to replace diseased heart valves. However, metallic biomaterials are categorized as bio-inert materials since they do not bond with the bone material during implantation. The second very important group of materials used in implantology is bioactive materials such as ceramic and bio-glass. This group is able to rebuild a bone tissue [5, 6, 7]. Several bioactive materials, such as bio-glass, fluorapatite and wollastonite have already been studied with metals and alloys [7, 8, 9, 10]. An interest has been taken in hydroxyapatite

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(HAP) as an addition to Co-Cr-Mo alloys for biomedical applications. HAP was reported as a suitable reinforcing material for the development of metal matrix composites like Ti-HAP, Ti-6Al-4V-HAP and AZ91-HAP to control the corrosion rate [1, 11]. When implanted in the human body, HAP spontaneously bonds to living bone via apatite layer deposited on the surface without forming fibrous tissue [12]. This paper reports on the microstructure and the bioactivity behavior of the composite F-75 filled with HAP. The composite was produced by powder metallurgy method.

2 Experimental procedure

In this research, commercially available Co-Cr-Mo (F-75) alloy powders and hydroxyapatite (HAP) powders were used as the starting materials. Co-Cr-Mo powders (average size 10.35 μm) were supplied by the Sandvik Osprey Ltd. Hydroxyapatite (HAP) powders (average size 11.05 μm) were supplied by the Merck Company. Figure 1 shows the SEM micrographs of these two starting powders. F-75 powder exhibits spherical particles, while hydroxyapatite powder has a flaky-acicular shape. The composite was fabricated by blending, pressing and sintering. The F-75 alloy powder with 2, 6, and 10 wt. % of HAP particles were mixed in a rotary milling machine for 20 minutes at 154 RPM. The cylindrical green compacts of 13 mm in diameter and 14 mm in height were cold compacted under pressure of 550 MPa. The samples were sintered at 1100°C for 2 hours in a tube furnace under argon atmosphere. Microstructures were observed using a scanning electron microscope (SEM) (model JEOL, JSM-6420LA), equipped with energy dispersive spectrum (EDS). The sample was prepared by the standard metallographic methods of wet rotary grinding on a series of SiC papers, followed by polishing on the soft napped cloth (BUEHLER, USA). X-ray diffraction (XRD) was carried out on a Shimadzu (model XRD 6000), Japan with CuK_{α} radiation for phase analysis. The recorded spectrums were matched in accordance to the data from the JCPDF. To evaluate the bioactivity of the F-75/HAP composites, immersion tests were carried out in a simulated body fluid (SBF) according to the BS ISO 23317:2007 [2]. This SBF solution was prepared from phosphate buffered saline (PBS) tablet that have been supplied by the Sigma Aldrich Company. One tablet was dissolved in 200 mL of deionized water yields 0.01 M phosphate buffer, 0.0027 M potassium chloride and 0.137 M sodium chloride, with pH 7.4. The samples were immersed in phosphate buffer saline solution (PBS, Aldrich, USA) for 18 days at 37°C. Afterwards, the samples were gently cleaned with deionized water and dried at 37°C prior to analysis [15].

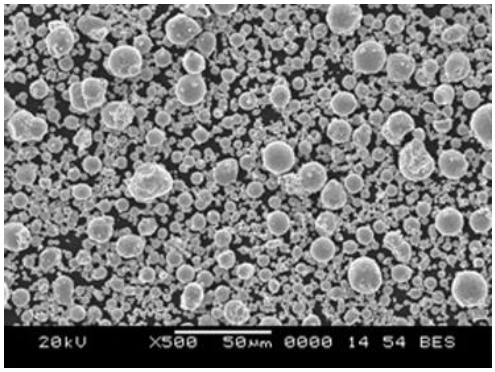

Fig. 1 a) Particles Co-Cr-Mo (F-75)

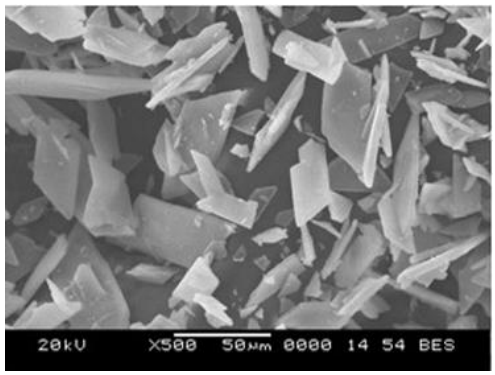

Fig. 1 b) Particle hydroxyapatite

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3 Results and discussions

3.1 Microstructure

Figure 2 shows SEM micrographs of the sintered F-75 without HAP, and with 2, 6, and 10 wt. % of HAP. It reveals a relatively uniform distribution of the HAP particles (gray area) in the F-75 matrix (lighter color is Co-Cr-Mo particles, while black area presents pores). As the percentage of HAP increased, more pores could be seen in the F-75/HAP composites. Figure 2(d) shows an agglomeration of HAP (indicated as circle), which occurred after sintering. Figure 2(e) shows the XRD patterns of the surface of the sintered samples. No phases other than the constituent were developed in the composites. The peaks have been identified as belonging to the phases of Co-Cr-Mo (JCPDS:260425) and HAP (JCPDS: 9432) for the F-75/10HAP composite. However, it is difficult to determine the phases with high confidence due to the very low intensity of the peaks. These weak peaks probably indicate the decomposition of HAP at higher temperature [23]. It was reported that, synthetic HAP decomposed to β-tricalcium phosphate ($Ca_3P_2O_8$) (β-TCP) when sintered at 800°C, 900C and 1000°C. However, XRD patterns of synthetic HAP sintered at 1100°C revealed that more β-TCP was formed. It was accompanied by the formation of α-TCP as minor phase [24]. The absence of β-TCP peaks in our study was believed to be due to the small amount of HAP addition (10 wt. %) in the composite.

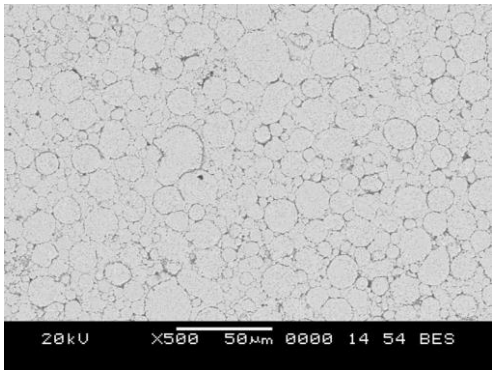

Fig. 2 a) SEM of sample without HAP

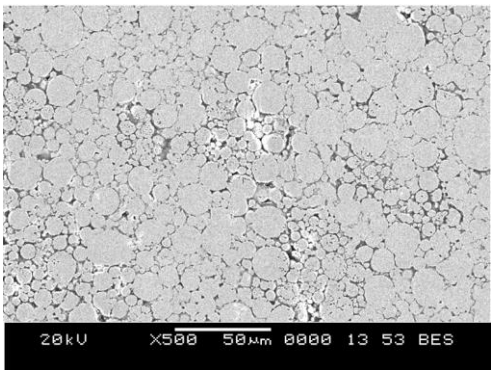

Fig. 2 b) SEM of F-75/2 wt. % HAP

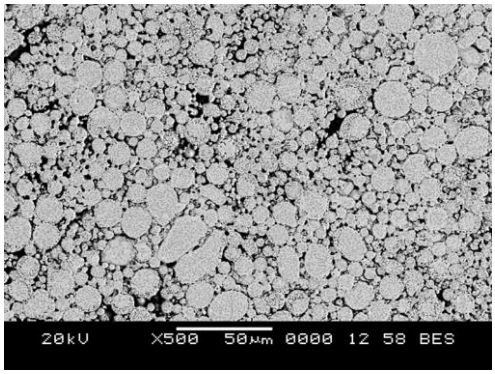

Fig. 3 c) SEM of samples F-75/6 wt. % HAP

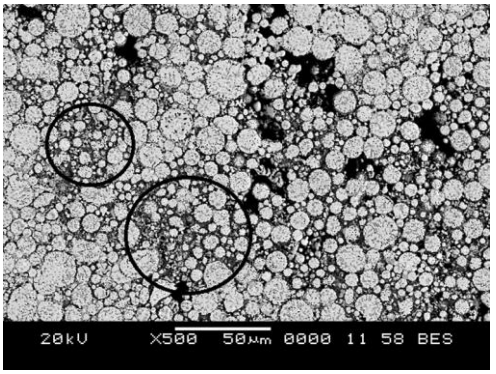

Fig. 2 d) SEM of samples F-75/10wt. % HAP respectively

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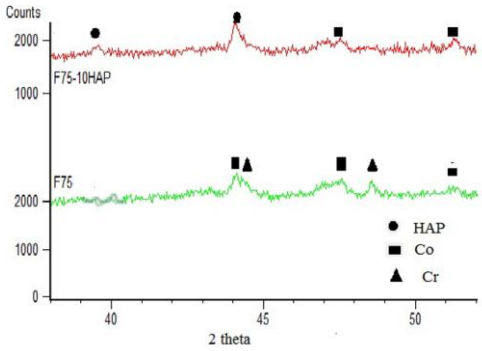

Fig. 2 e) XRD patterns for F75 and F75-10HAP after sintered

3.2 Bioactivity behavior

Previously, simulated body fluid (SBF) has been recommended for investigating the bioactivity behavior of ceramic based biomaterials by assessing the potential of apatite formation. Silicate-based bio-ceramics such as bio-glass have been reported to have excellent apatite forming abilities in SBF [18]. However, by soaking phosphate and sulphate-based ceramics in SBF, obvious apatite formation has not been remarkable, although they do exhibit superior in-vivo bone formation abilities [19]. Consequently, SBF has been appropriate for investigating apatite formation of silicate-based ceramics, but not for phosphate and sulphate-based ceramics. Phosphate buffered saline (PBS) is another physiologic solution commonly used in biochemistry to imitate human extracellular fluid [15]. In comparison to SBF, ionic species such as Mg^{2+} and Ca^{2+} are absent in PBS. In the present investigation, HAP was used as bioactive filler in the F-75 alloy, therefore PBS was used instead of SBF to determine the ability of apatite formation during in-vitro bioactivity test. **Figure 3** shows the morphologies of the sintered samples surface after immersion in the PBS for 18 days. Figure 3a shows that the sample without HAP exhibited the corrosion at grain boundaries without the formation of white particles/granular on the corroded surfaces and grain boundaries. For samples with HAP addition between 2 and 10 wt. % HAP (Figure 3b to 3d), the formation of needle like particles could be clearly seen as shown in figure 3c. More white particles (spherical shape) precipitated on the surface of the samples as more HAP is added; most pores are invisible due to the coverage of the newly formed deposition. When 10 wt. % of HAP is added, the entire surface of the sample (Figure 3d) was covered by this precipitate. The microstructural and EDS results (Figures 3d and 4 respectively) indicate that the precipitate layer composed of calcium (Ca), phosphorus (P), Co, Cr and Mo [1, 15]. This result indicates that calcium concentration increases obviously compared to phosphorus. It can be confirmed that the increase in calcium concentration is mainly due to a dissolve of the CaO phase in the as-sintered composite [1]. However, the presence of NaCl and Si in the EDS analysis is due to insufficient washing and sample grinding during sample preparation respectively. Previous researches showed that the deposition of needle-like crystals of HAP was occurred when phosphate bio-ceramic soaked in PBS [15, 20]. According to previous findings, the formation of apatite particles and transformation into the apatite layer as more HAP is added can be divided into two stages: nucleus formation and nucleus growth [13, 14]. Certainly, the nature and crystallinity of apatite phase phases depend on various parameters, including concentrations of phosphate/carbonate sources, ionic strength and pH of the soaked solution, and the kinetics of the nucleation and growth processes [15]. In general, the formation mechanism of

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apatite is dissolution-precipitation reaction. The apatite formation mechanism starts with the partial dissolution of calcium ions from HAP and then reacts with phosphate ions in PBS to form apatite [21, 22]

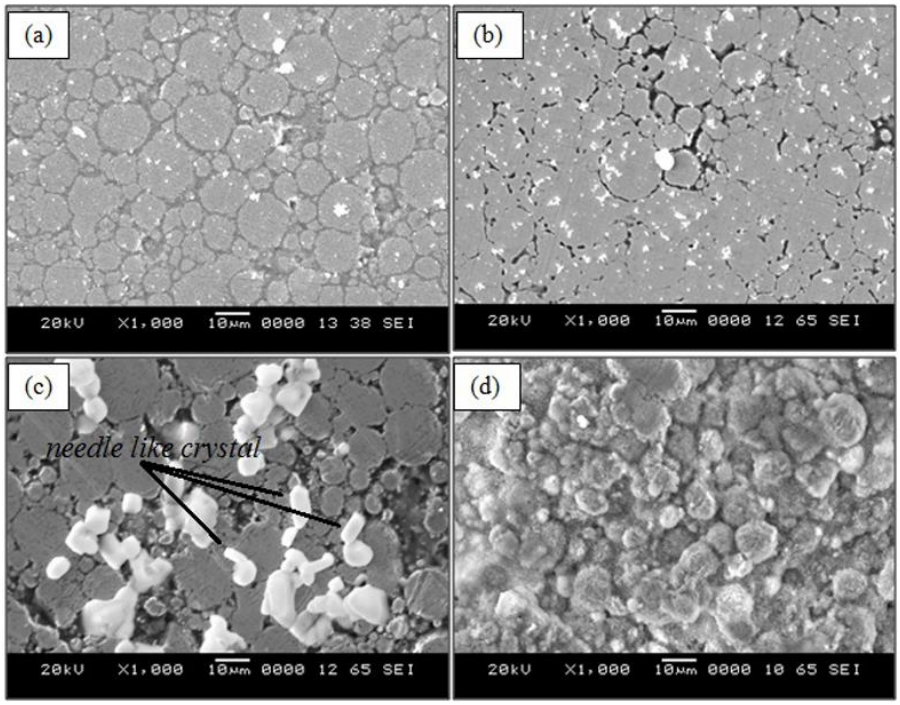

Fig. 3 SEM microphotographs of samples F-75/HAP after immersion in PBS for 18 days with (a) no HAP (b) 2 wt. % HAP (c) 6 wt. % HAP and (d) 10 wt.% HAP

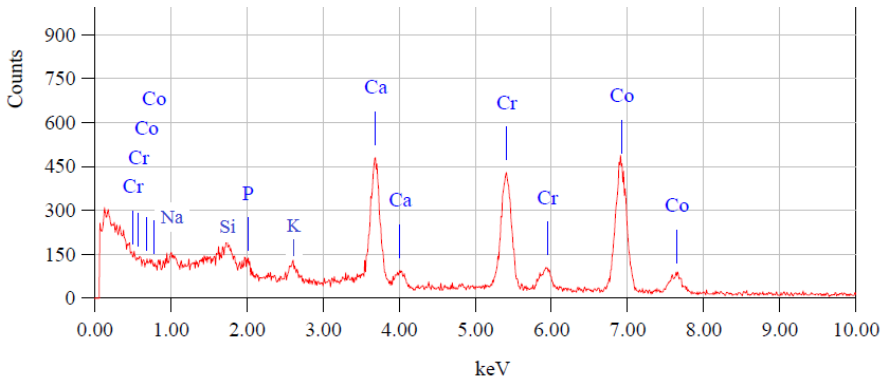

Fig. 4 EDS results for sample F-75/10HAP after immersion in PBS for 18 days

4 Conclusion

This research shows that it is possible to bioactivate the surface of F-75 alloy with HAP additions to produce the F-75/HAP composite. The bioactivity behavior of F-75/HAP composites was evaluated by immersing the composites in PBS and the result shows apatite

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formation on the surface of the material considered as the mark of bioactivity. The result showed that formation of apatite layer increases as the content of HAP increases. Thus, we conclude that this composite can be good alternative materials for biomedical application.

References

[1] C. Q. Ning, Y. Zhou: Biomaterials, Vol. 23, 2002, p. 2909-2915, DOI:10.1016/S0142-9612(01)00419-7
[2] British standard (BS ISO 23317), 2009
[3] U. Kamachimudali, T. M. Sridhar, Baldev Raj: Sadhana, Vol. 28, 2003, p. 601-637, DOI: 10.1007/BF02706450
[4] D. Shi: Biomaterials and Tissue Engineering, Springer, Berlin, 2004
[5] R. Singh, N. B. Dahotre: Journal of Materials Science Medicine, Vol. 18, 2007, p. 725-751, DOI: 10.1007/s10856-006-0016-y
[6] M. Gradzka-Dahlke, J. R. Dabrowski, B. Dabrowski: Journal of Materials Processing Technology, Vol. 204, 2008, p. 199-205, DOI:10.1016/j.jmatprotec.2007.11.034
[7] Z. Oksiuta, J. R. Dabrowski, A. Olszyna: Journal of Materials Processing Technology, Vol. 209, 2009, p. 978-985, DOI:10.1016/j.jmatprotec.2008.03.060
[8] X. Liu, C. Ding, Z. Wang: Biomaterials, Vol. 22, 2001, p. 2007-2012, DOI:10.1016/S0142-9612(00)00386-0
[9] M. Razavi, M. H. Fathi, M. Meratian: Materials Characterization, Vol. 61, 2010, p. 1363-1370, DOI:10.1016/j.matchar.2010.09.008
[10] M. Fathi, M. Ahmadian, M. Bahrami: Dental Research Journal, Vol. 9, 2012, p. 173-179
[11] F. Witte et al: Biomaterials, Vol. 28, 2007, p. 2163-2174, DOI:10.1016/j.biomaterials. 2006.12.027
[12] I. B. Leonor, A. Ito, K. Onuma, N. Kanzaki, R. L. Reis: Biomaterials, Vol. 24, 2003, p. 579-585, DOI:10.1016/S0142-9612(02)00371-X
[13] D. Cortes, A. Medina, S. Escobedo, M. A. Lopez: Journal of Materials Science, Vol. 40, 2005, p. 3509-3515, DOI: 10.1007/s10853-005-2856-0
[14] Y. P. Guo, Y. Zhou, D. C. Jia, C. Q. Ning, Y. J. Guo: Materials Science and Engineering C, Vol. 30, 2010, p. 472-479, DOI: 10.1016/j.msec.2010.01.008
[15] C. Wu, J. Chang: Materials Letters, Vol. 61, 2007, p. 2502-2505, DOI:10.1016/j.matlet.2006.09.045
[16] L. Kaewsichana, D. Riyapana, P. Prommajana, J. Kaewsrichan: ScienceAsia, Vol. 37, 2011, p. 240-246, DOI: 10.2306/scienceasia1513-1874.2011.37.240
[17] E. Battistella, E. Varoni, A. Cochis, B. Palazzo, L. Rimondini: Journal Applied Biomaterials Biomechanic, Vol. 9, 2011, p. 223-231, DOI: 10.5301/JABB.2011.8867
[18] L. L. Hench: Journal American Ceramic Society, Vol. 74, 1991, p. 1487-1510, DOI: 10.1111/j.1151-2916.1991.tb07132.x
[19] G. Liu, L. Zhao, L. Cui, W. Liu, Y. Cao: Biomedical Materials, Vol. 2, 2007, p. 78-86, DOI:10.1088/1748-6041/2/2/004
[20] X. Yao, H. Yao, G. Li, Y. Li: Journal Materials Science, Vol. 45, 2010, p. 1930-1936, DOI: 10.1007/s10853-009-4182-4
[21] G. Y. Ping: PhD Thesis, Harbin Institute of Technology, China, 2009
[22] P. Wongwitwichot, J. Kaewsrichan, K. H. Chua, B. H. I. Ruszymah: Open Biomedical Engineering Journal, Vol. 4, 2010, p. 279-285, DOI: 10.2174/1874120701004010279

[23] H. Ye, X. Y. Liu, H. Hong: Materials Science and Engineering C, Vol. 29, 2009, p. 2036-2044, DOI:10.1016/j.msec.2009.03.021
[24] F.Z. Mezahi, H. Oudadesse, A. Harabi, Y. le Gal, G. Cathelineau: Journal of the Australian Ceramic Society, Vol. 47, 2011, p. 23-27

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