Fabrication, Characterization and Osteoblast Response of Cobalt-Based Alloy/Nano Bioactive Glass Composites

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ABSTRACT

In this work, cobalt-based alloy/ nano bioactive glass (NBG) composites with 10, 15 and 20 wt% NBG were prepared and their bioactivity after immersion in simulated body fluid (SBF) for 1 to 4 weeks was studied. The scanning electron microscopy images of two- step sintered composites revealed a relatively dense microstructure the density of which decreased with the increase in the NBG amount. Microstructural analysis as well as energy dispersive X-ray analysis (EDX) revealed that after 1 week of immersion in SBF, a small amount of calcium phosphate phases precipitates on the surface of the composite samples. After 2 weeks of immersion, a considerable amount of cauliflower-like shaped precipitations was observed on the surface of composites. The observed peaks in the Fourier transform infrared (FTIR) spectroscopy of the composite samples in SBF immersed for 4 weeks were assigned to hydroxyapatite. Therefore, a hydroxyapatite layer has been possibly formed on the surface of the composite samples during immersion in SBF. Cell culture results indicate that unlike the Co-based alloy, the Co-based/NBG composites are bioactive and bone cells could be vivid and grow on their surfaces, promising their possibility for implant applications.

1. Introduction

Metals and metallic alloys have many applications in dentistry, orthopedic and bone fractures as artificial implants or restored materials. Orthopedic implants are mainly made of metals to suffer mechanical stresses in service. Titanium and its alloys, cobalt-based alloys, and stainless steel are the most common metals that could be used for orthopedic implants applications. Their main characteristic is proper mechanical properties but their corrosion resistance in physiological fluids and their weak bioactivity are the two concerns regarding their use as implants [1]. In general, metallic alloys are not bioactive, i.e. they are not able to bond to the living tissue without cementation or external fixation devices [2-4]. On the contrary, certain glasses and ceramics are bioactive. In the other words, when they are implanted into body, an apatite layer could be formed on the surface of materials and the newly formed bone tissue fills up the gap between the surrounding bone and the apatite layer [5-7]. A tight chemical bonding is then formed between the bony apatite and the surface apatite which prevents implant loosening. This is the essential requirement for an artificial material to be implanted in a living body. The bone-bonding ability of bioceramics as well as bioactive glasses is a result of their capability to be coated

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with an apatite-like layer when they are in contact with physiological fluids. they are in contact with physiological fluids.

Irrespective of their bioactivity, bioglasses are not strong enough to be used for load-bearing applications [8-11]. Therefore, bioglasses cannot be used in load-bearing applications, where metallic alloys are still the materials of choice. Hence, it could be inferred that in order to benefit from both good mechanical properties and bioactivity, it is reasonable to fabricate the composite from a metallic biomaterial and a bioactive ceramic. Cobalt-based alloys in the Co-Cr-Mo system have been widely used as implant components due to their mechanical properties, good wear and corrosion resistances as well as biocompatibility [12-14]. One of the most important bioactive ceramics is bioglass and the most representative bioactive glass is the so-called 45S5 Bioglass, whose composition is particularly rich in sodium and calcium oxides and characterized by a high calcium-to-phosphorous ratio which makes the glass surface very reactive in aqueous media, both in vitro and in vivo [15].

Based on the literature [16-18], compared to micro bioactive glass, nano bioactive glass differs in properties such as specific surface area, pores size, wettability and surface energy which promote reactions with vivid texture. Therefore, it is expected that using nano bioglass as the reinforcement phase accelerates apatite formation on the composite surface and strengthen its bonding to bone. As a result, the implant would be fixed at its position. Therefore, in this study, cobalt-based alloy/ nano bioactive glass composites were prepared with different amounts of bioglass and their microstructure, before and after immersion in simulated body fluid (SBF), were investigated. The SBF solution was also analyzed after immersion by inductively coupled plasma optical emission spectroscopy (ICP-OES) in order to study the variation of calcium, phosphor and silicon ions concentration.

2. Materials and methods

Cobalt-based alloy powder was purchased from CARPENTER Powder Products. Its composition is reported in Table 1. Nano bioactive glass (NBG) particles with chemical composition close to 45S5 Bioglasss containing 45% SiO_2, 24.5% Na_2O, 24.5 CaO and 6% P_2O_5 in weight percentages were prepared by sol-gel technique as described in detail by Fathi and Doostmohammadi [19]. The particle size of the purchased metallic alloy powder and the synthesized NBG powder was in the range 75-180 μm and less than 100 nm, respectively. In order to fabricate the Co-based alloy/NBG composites, three different compositions containing 10, 15 and %20 NBG powder were weighed. The homogenous mixture of the metallic alloy and the ceramic component was obtained by mixing in a planetary ball mill for 1 h under argon atmosphere to avoid oxidation. The mixed powders were uniaxially pressed under 700 MPa to prepare green pellets of composites. Because of the advantages of two-step sintering compared to conventional sintering (lower sintering temperature as well as higher obtainable density) [20], the green samples were sintered in a two-step process. The studied temperature range for the first step and the second step was 900-1100°C and 700-900°C, respectively, and their corresponding soaking time was 10 min and 2-14 h, respectively. For each sintering cycle, five samples were used and after measuring the density of sintered ceramics via Archimedes method, the optimum sintering temperature of each composition was determined.

Simulated body fluid (SBF) soaking was used to evaluate the in vitro bioactivity of the prepared composite samples. As described by Kokubo et al. [21], this buffer has an ionic composition similar to that of human blood plasma. The sintered ceramics were immersed in the SBF solution and stored in plastic flasks, maintained at 37 °C in an incubator for 7, 14, 21, and 28 days. The formation of the apatite layer on the composite samples were recognized, analyzed and confirmed using Fourier transform infrared spectroscopy (Bomem, MB-100) in the range 4000-600cm^{-1} with a scan rate of 2 cm^{-1}. Microstructure of the optimally sintered composite samples before and after immersion in SBF solution was investigated by scanning electron microscopy (Phillips XL 30). The SBF solution was also analyzed after immersion by inductively coupled plasma optical emission spectroscopy (ICP-OES) in order to study the variation of calcium, phosphor, and silicon ions concentration.

Standard protocols of MTT tests [22, 23] were conducted on the samples to determine the cell proliferation, adhesion, and cytotoxicity behaviors. Human osteoblast-like MG63 cells were used as the cell culture model. At first, the samples were sterilized using dry heat at 140 °C for 2 h and preconditioned for 24 h with Dulbecco's modified Eagle's medium (DMEM). These media were removed and the cells were plated at 9300 cells/cm2 in DMEM containing 10% fetal bovine serum (FBS) and 0.5% antibiotics (diluted from a stock solution containing 5000 U/ml penicillin and 5000 U/ml streptomycin; GIBCO, Grand Island, NY) and cultured at 37°C in an atmosphere of 100% humidity and 5% CO_2. The media were changed at 24 h and then every 48 h until the cells reached confluence (7 days). The cells densities on the samples and control (polystyrene plate) were measured using a UV spectrometer by viable color change in the cells. The absorbance of 250 µl of blue colored solution was measured at 570 nm using Mikroplate Reader EL800 (BIO-TEK, Vermont USA).

Table 1. Composition of cobalt-based alloy.

Element	Co	Cr	Mo	Ni	Fe	Si	Mn	Others
Weight percent	60.80	27.60	5.30	2.76	1.40	0.88	0.87	0.39

3. Results and discussion

3.1. Determination of optimum sintering temperature

Based on the density measured by Archimedes method, the optimum sintering time and temperature for all compositions were found 1000°C for the first step and 800°C for the second step with 10 h soaking times. These sintering conditions resulted in a relative density of the sintered composite samples higher than 96, 94 and 91% for samples containing 10, 15 and 20% NBG, respectively. That is, the higher the amount of NBG, the lower the density of the sintered composite. A possible explanation of this behavior is that with increasing the volume fraction of bioglass particles, internal friction of the composite powder increases which hinder consolidation of the material. It was described by Dai et al. [24] that a small amount of the reinforcing phase fills easier the inter particles voids of metallic matrix and therefore can be compacted more easily due to a lower friction. However, higher volume fraction intensifies the bridging effects during single-action pressing and effectively suppresses densification.

3.2. SEM images of the sintered composites

SEM images of optimally sintered composite samples containing 10, 15 and 20% NBG are compared in Fig. 1. As can be seen, all compositions are relatively dense and with increasing the amount of NBG the number and volume of the pores in the microstructure increase, which is due to the increase in internal friction of the composite powder which hinders consolidation of the material.

The homogenous distribution of dark zones which are agglomerated NBG in a relatively light metallic matrix could be seen in the SEM images of all composite samples with different amounts of NBG. The presence of coarser pores in the composite containing %20 NBG than those in other composites is in good agreement with its lower relative density. Moreover, increase in the amount of NBG enhances agglomeration of its particles, leading to the reduction in surface area and consequently reduction in free energy of the system.

3.3. SEM images of the composites immersed in SBF solution

The composite samples were immersed in SBF solution and soaked for 1 to 4 weeks. Microstructural analysis as well as the EDS results (not shown in this paper) revealed that after 1 week, a small amount of calcium phosphate phases precipitates on the surface of the composite samples. Moreover, higher amount of NBG in the composite resulted in more calcium phosphate precipitations. SEM images of the composite samples containing different amounts of NBG after 2 and 4 weeks of immersion in SBF solution are compared in Fig. 2. After 2 weeks of immersion, a considerable amount of cauliflower-like shaped calcium phosphate precipitations is observed on the surface of the composites.

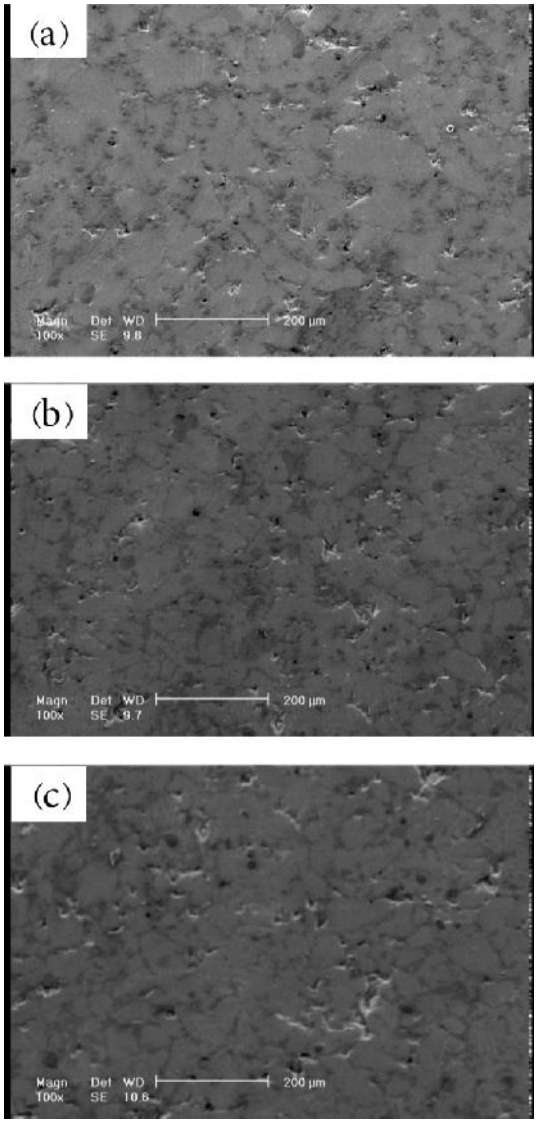

Fig. 1. SEM images of optimally sintered composite samples containing (a) 10, (b) 15 and (c) 20% NBG.

Moreover, with increasing the immersion time, the amount and compactness of calcium phosphate precipitations will increase as well.

Ohtsuki et al. [25] proposed the mechanism of apatite formation on the surface of bioglass during immersion in SBF solution. Based on their suggestion, chemical reaction of the Ca (II) and Si (IV) ions dissolved from the bioglass with the P (V) ion in the surrounding body fluid leads to the apatite formation on the surface of the bioglass. In this reaction, the Ca (II) ion increases the degree of supersaturation of the surrounding body fluid with respect to apatite, which is already supersaturated even in the normal condition, and the Si (IV) ion provides favorable sites for nucleation of the apatite on the surface of the bioglass.

The formation ability of apatite in laboratory tests is a measure of bond formation ability between the implant and the bone. In the other words, apatite formation on the surface of the composite samples indicates bioactivity of the samples. Bone adhesion with a bioactive implant is stronger than a bioinert one. In the case of bioactive implants, if fracture occurred the interface between the implant and the bone would be sound, but in the case of bioinert implants, separation at the interface could be seen. According to SEM images of the composite samples containing 10 and 15% bioactive glass, after 3 weeks of immersion in SBF, some cracks are seen on the surface of the samples (Fig. 3). This observation is in good agreement with the reported results by Kashyap et al. [26] for 45S5 bioactive glass. The observed cracks could be attributed to stress release of the substrate. During immersion in SBF, mineralization of the formed apatite on the composite surface occurs. After removing the samples from SBF and drying them, the formed layer shrinks and, as a result, tension stresses are accumulated under the formed apatite layer. Stress release accompanied by cracking of the apatite layer can be seen in Fig. 3.

3.4. FTIR analysis of the formed layer on the samples after immersion in SBF

FTIR analysis of the composite samples which were immersed for 4 weeks in SBF is shown in Fig. 4. The observed peaks in this figure are characteristic peaks of hydroxyapatite. According to this analysis, the observed calcium phosphate precipitations on the surface of the immersed samples are the hydroxyapatite phase. Therefore, reinforced composites with NBG have apatite formation ability in SBF, i.e. unlike the cobalt-based alloy, the composite samples are bioactive.

It has been proved that bioactivity of bioactive glass is higher than that of hydroxyapatite and that the amount of bone cells formation on the bioactive glass surface is higher [27]. The main advantage of the composites containing bioactive glass is bond formation ability with soft and hard tissues.

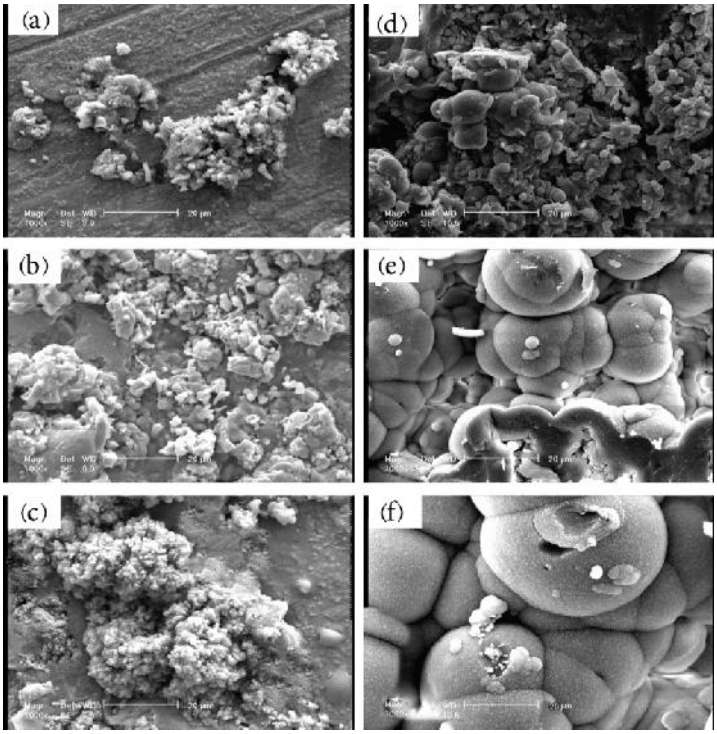

Fig. 2. SEM images of the composite samples containing (a) 10, (b) 15 and (c) 20% NBG after 2 weeks and (d) 10, (e) 15 and (f) 20% NBG after 4 weeks of immersion in SBF.

3.5. Variation of the Ca, P and Si ions concentration in SBF

The study of calcium ions release in SBF is important since these ions play an effective role in activation of osteoblast cells [28]. Moreover, calcium ions enhance cellular interaction [29] and act as an intermediate for cell to cell bonding in cell culture. Fig. 5 shows the calcium ion release rate of the composite samples for different immersion times in SBF. As can be seen in this figure, up to 14 days, the calcium ions release increases and after that decreases. This behavior is in good agreement with the reported results by Cannillo et al. [30] regarding the calcium ions release of immersed 45S5 bioactive glass in SBF. In fact, by increase in the immersion time, the precipitation rate predominates over the dissolution rate. This observation could be attributed to the development of a released ions supersaturated environment in SBF. This condition facilitates apatite nucleation and the increase in calcium ions concentration in the solution induces apatite formation on the composite samples surfaces [31].

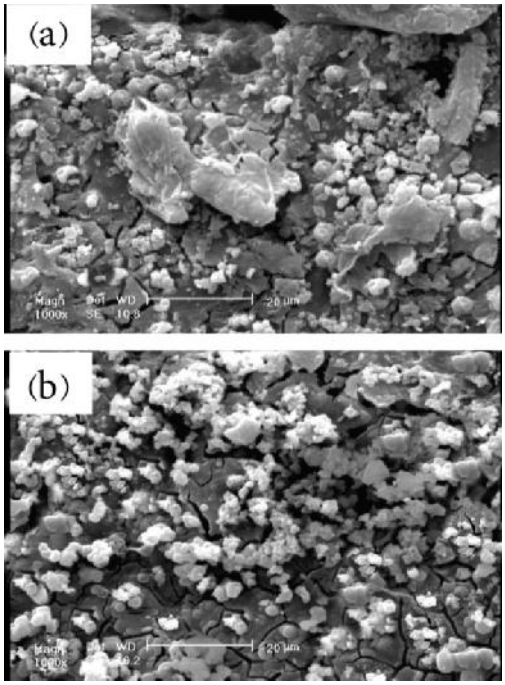

Fig. 3. SEM images of the composite samples containing (a) 10 and (b) 15% bioactive glass, after 3 weeks of immersion in SBF.

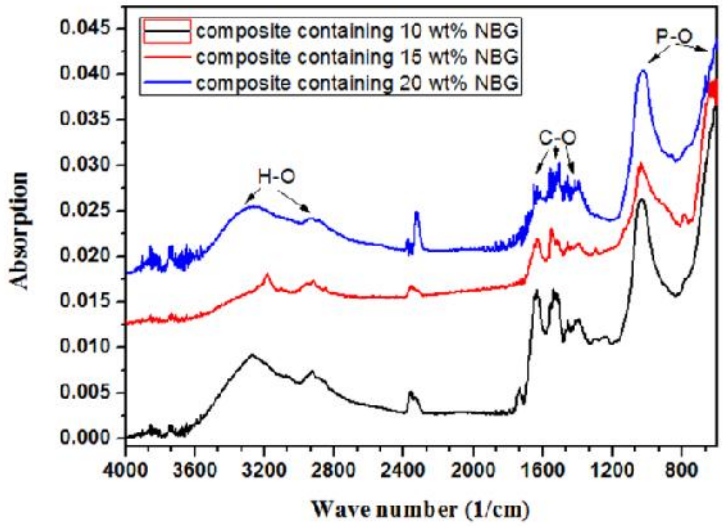

Fig. 4. FTIR analysis of the composite samples immersed for 4 weeks in SBF.

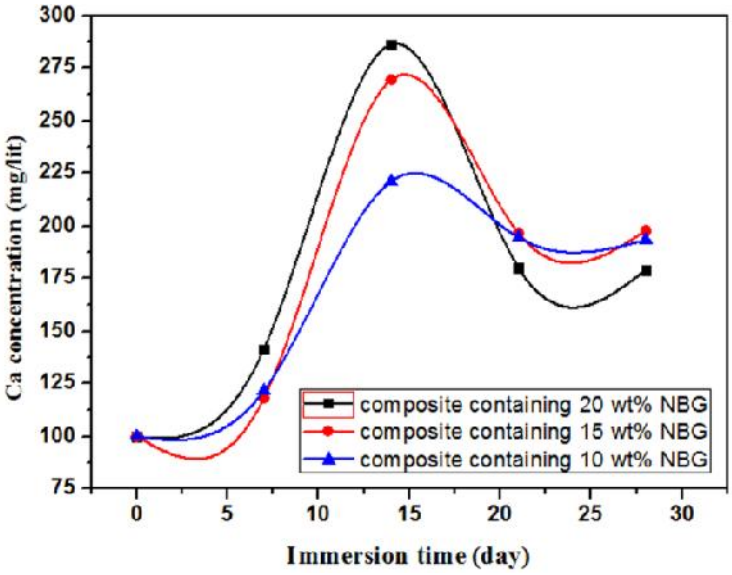

Fig. 5. Calcium ion release rate of the composite samples for different immersion times in SBF.

Variation of phosphor ions concentration after different immersion times is plotted in Fig. 6. As can be seen, the P ions concentration decreases during immersion in SBF. In general, concentration variations in SBF during the initial days are significant and after that the concentration variation diminishes.

The continuous reduction in phosphor ions concentration indicates that the amount of released phosphor from bioactive glass could not compete with the amount of the consumed phosphor ions for apatite formation. A similar trend has been observed by other researchers, too [27, 32, 33].

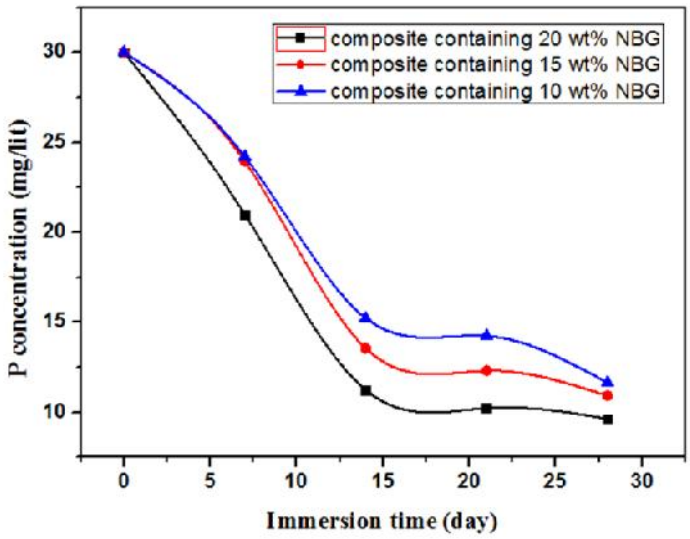

Fig. 6. Variation of phosphor ions concentration after different immersion times.

Variation of calcium and phosphor ions concentrations in SBF implies the formation of calcium phosphate on the sample surface and formation of this phase is proved more than before. According to the results, the composite containing 20% NBG has the highest ability of absorption of the Ca2+ ions.

Variation of silicon ions concentration versus the immersion time is shown in Fig. 7. As can be seen, by increase in the immersion time up to 14 days, ion concentration increases and reaches to a maximum and for longer time, concentration remains unchanged. The more the silicon ions release, the more the sites for apatite nucleation [32, 34].

Beherei et al. [33] investigated itania/bioactive glass composites and found that the Si-OH and Ti-OH groups affect apatite layer formation and ionic reactions after immersion in SBF. In the other words, these groups have a catalytic effect on calcium phosphates formation on the composite samples surfaces. Moreover, it was found that the Ca2+ and Na+ ions release from bioactive glass and their substitution with H3O+ ions in SBF accelerates apatite nucleation on titania/bioactive glass composite samples surfaces. At higher amounts of bioactive glass, due to higher number of Si-OH groups, reactions with phosphor ions on the surface occur more completely and faster.

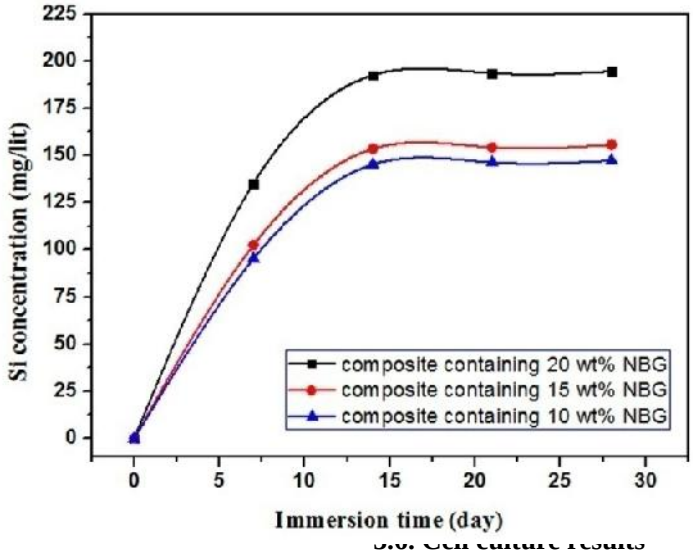

Fig. 7. Variation of silicon ions concentration versus immersion time in SBF.

Cell culture test was carried out in order to evaluate cell adhesion and growth on the surface of the samples. For this purpose, MG63 cells were used. SEM images of Co-based alloy and the composite samples containing 10, 15 and 20% NBG after 7 days cell culture are compared in Fig. 8. As can be seen, cell adhesion to the surface of Co-based alloy is very weak and there is no cell-to-cell bond in this sample. In the case of the composite samples, the cells are bonded to the surface and there is proper contact between the cells and the sample. In the other words, the cells can propagate on the composite samples, indicating full adhesion of the cells to the samples.

The presence of bioglass in the composites increases adhesion, bone cell differentiation and mineral phase precipitations on the samples.
On the other hand, the occurred reactions with bioglass dissolves Si, Ca, P and Na ions, which promotes reactions between the cells and the composite.

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According to Fig. 8, for all composite samples, the cells propagated on the surface and good adhesion with widespread microtrabecular network could be formed, indicating bioactivity of the composite samples. Cell proliferation of the composite samples after 7 days of incubation was studied. Cell density indicates vivid cells in contact with the sample. Percentage of the vivid cells after 7 days of cell culture on the composite samples containing 10, 15 and 20 % NBG are compared in Fig. 9. There is a meaningful difference between the percentage of vivid cell on the reference sample and all the composite ones. Increase in NBG was accompanied by increase in cell proliferation and vivid cells percentage. Cell culture results indicate that Co-based/NBG composites unlike Co-based alloy are bioactive and that the bone cells could be vivid and grow on their surfaces.

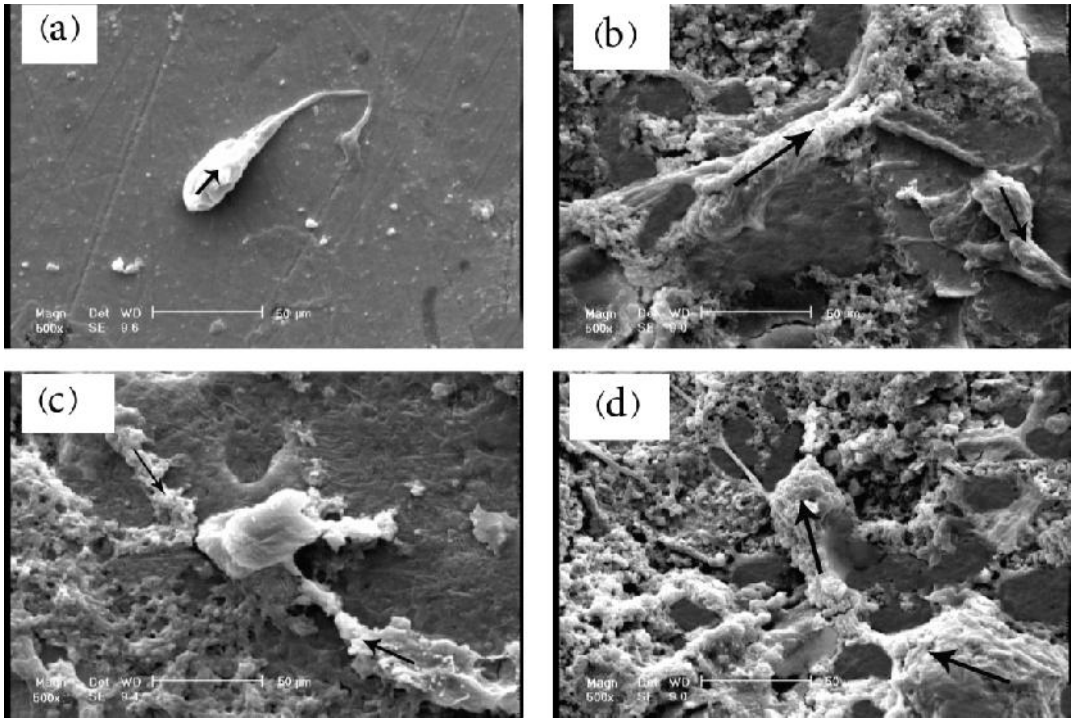

Fig. 8. SEM images of 7 days cell cultured (a) Co-based alloy and composite samples containing (b) 10, (c) 15 and (d) 20 % NBG (The remarked arrows show the formed cells.)

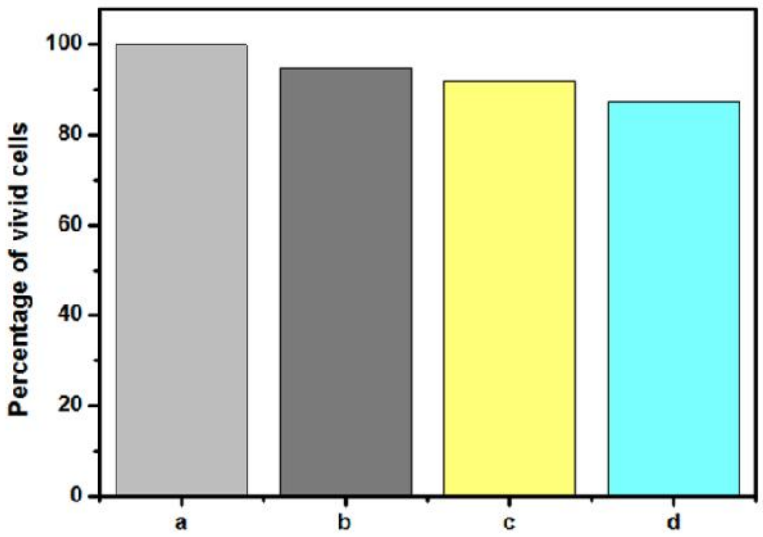

Fig. 9. Percentage of the vivid cells after 7 days of cell culture on (a) the controlling sample and the composite samples containing (b) 20, (c) 15 and (d) 10 % NBG.

4. Conclusions

In this study, the sintering behavior and bioactivity of cobalt-based alloy/ NBG composites were investigated. SEM images showed that the two-step sintering is a suitable method to achieve a relatively dense microstructure. Immersion in SBF is accompanied by formation of cauliflower-like shaped precipitations on the composite surfaces. EDS and FTIR analyses proved these precipitations are hydroxyapatite, indicating bioactivity of the prepared composites. Cell culture results indicate that unlike the Co-based alloy, the Co-based NBG composites are bioactive and that the bone cells could be vivid and grow on their surfaces. The results promise the possibility of cobalt-based/ NBG composites for implant applications.

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